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Superior de Ensenada, Baja California**



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**Doctorado en Ciencias  
en Ciencias de la Vida  
con orientación en Biología Ambiental**

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**Genética de la conservación de *Yucca schidigera*  
(Asparagaceae) en Baja California: aspectos evolutivos y  
reproductivos**

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para cubrir parcialmente los requisitos para obtener el grado de  
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**Genética de la conservación de *Yucca schidigera* (Asparagaceae) en Baja California: aspectos evolutivos y reproductivos**

Resumen aprobado por:

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El mutualismo obligado entre las plantas del género *Yucca* (Asparagaceae) y sus polinizadores, las polillas del género *Tegeticula* (Lepidoptera: Prodoxidae) es un excelente modelo de estudio para entender la coevolución en interacciones insecto-planta. En este trabajo, se analizaron distintos aspectos de la variación poblacional en *Yucca schidigera*, una especie que se distribuye desde los desiertos del suroeste de EUA hasta el paralelo 29.5 ° N en Baja California. Éste estudio se enfocó en las poblaciones ubicadas en el sur del rango de distribución de la especie y como objetivo se planteó evaluar la variación y la diferenciación genética, la estructuración geográfica de la variación fenotípica en rasgos vegetativos y reproductivos, y los costos de la depredación de semillas derivados del mutualismo. Este conjunto de atributos es fundamental para la interacción entre las plantas de *Yucca* y sus polinizadores. Se evaluaron siete microsatélites en 240 individuos pertenecientes a 13 poblaciones de Baja California. Las poblaciones de *Y. schidigera* mostraron alta variación genética ( $H_{exp}=0.79$ ) y ésta variación decreció en función de la latitud. Al combinar los resultados del análisis genético con modelos de nicho climático proyectados al pasado, se observó un patrón de expansión del rango geográfico de la especie durante el Pleistoceno. Los datos genéticos apoyan la hipótesis de que la especie colonizó su rango de distribución actual en la península de Baja California desplazándose hacia el sur. Basándose en el análisis de siete rasgos vegetativos y diez rasgos florales evaluados en 423 individuos pertenecientes a 15 poblaciones, el nivel de variación fenotípica fue relativamente alto, y la mayoría de ésta variación se concentró dentro de las poblaciones. La poca estructura geográfica de los rasgos fenotípicos coincide con los niveles bajos de diferenciación genética ( $F_{ST}=0.067$ ;  $P<0.001$ ). La producción de semillas, la masa de la semilla y su tasa de germinación fueron significativamente diferentes entre poblaciones, sin embargo, estas diferencias no estuvieron asociadas con la intensidad de infestación de las larvas de *Tegeticula mojavella*. La variación en costos asociados al mutualismo no mostró asociación con variables geográficas (e.g., elevación, latitud) lo que sugiere que el costo asociado al mutualismo es regulado por factores locales. Nuestros resultados indican que es importante incluir el mayor número de poblaciones en los esquemas de manejo y conservación de ésta especie puesto que la mayoría de su diversidad genética y fenotípica se extiende homogéneamente a lo largo de su distribución.

**Palabras clave:** Baja California, Modelos de Nicho Climático, Diferenciación Genética, Microsatélites, Mutualismo, Variación Genética, Variación Fenotípica

Abstract of the thesis presented by **Leonardo Mario De la Rosa Conroy** as a partial requirement to obtain the Doctor of Science degree in Life Sciences with orientation in Environmental Biology

**Conservation genetics of *Yucca schidigera* (Asparagaceae) in Baja California: a focus on evolutionary and reproductive features**

Abstract approved by:

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The obligate mutualism between *Yucca* plants (Asparagaceae) and yucca moths (Lepidoptera: Prodoxidae) has been used successfully as a model to study the co-evolution of insect-plant interactions. We use this approach to study biological variation among populations of Mojave Yucca (*Yucca schidigera*), a woody monocot which distribution includes the southwestern deserts of USA to latitude 29.5 °N in Baja California. Populations located in the southern range of *Y. schidigera* distribution were sampled to evaluate the distribution of genetic variation and genetic differentiation, the geographic structure of phenotypic variation in vegetative and reproductive traits; and the geographic differentiation in the cost of mutualism. All of which are key features of the interaction between *Yucca* plants and their obligate pollinators. Using a set of seven microsatellite loci evaluated in 240 individuals that were sampled from 13 populations, we detected high genetic diversity across *Y. schidigera* populations ( $H_{exp}=0.79$ ) with genetic variation decreasing significantly with latitude. Analysis with Environmental Niche Models support a range expansion of *Y. schidigera* into northwestern Baja California during the Pleistocene. Genetic data concur that the species' colonized Baja California from North to South as suitable climatic conditions became widely available during the Last Glacial Maximum. The analysis of eight vegetative traits and ten floral traits in 423 individuals sampled in 15 populations resulted in substantial variation across *Y. schidigera* populations with most of the variance distributed within populations. Low geographic structure for phenotypic traits was consistent with low levels of genetic differentiation registered ( $F_{ST}=0.067$ ,  $P<0.001$ ). Seed production, seed mass and germination rate of were also significantly variable among populations but were not related to intensity of infestation by the *Tegeticula* moths. We registered population-level differences in the cost of mutualism which were not associated with geographic variables (e.g., latitude, elevation), suggesting that variation in the cost of mutualism in *Y. schidigera* populations is subject to local ecological factors. Our results support that management efforts should consider most populations in this species distribution because a large fraction of its genetic and phenotypic diversity is found within each population across its geographical range.

**Keywords:** Baja California, Climatic Niche Models, Genetic Differentiation, Microsatellites, Mutualism, Genetic Variation, Phenotypic Variation

## Dedication

A la vida, y al gran misterio que guarda la naturaleza.

A éste grandioso e incomparable hogar que compartimos con las demás especies.

A quienes dedican sus vidas a la interminable búsqueda del conocimiento, porque gracias a ellas y a ellos es posible entender, imaginar y aplicar las soluciones para el rescate de nuestro planeta.

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## Chapter 1. General introduction

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### 1.1 Introduction

Mutualistic interactions are particularly important to ecology and evolution because the role they may play in promoting species diversification. Species interactions are considered mutualistic if each involved species experiences higher net benefit from the biotic association than when they are alone (Herre et al., 1999). Mutualistic interactions are fundamental for biodiversity because they may promote speciation by allowing species to increase their realized niche, expand their distribution range, and potentially diversify through adaptive radiation (Chomicki et al., 2019). Mutualistic interactions also contribute to biological communities by providing ecosystem services such as nutrient cycling, nitrogen fixation and pollination (Kato and Kawakita, 2017).

Plants are the base on which communities and ecosystems assemble, and insect species that interact with plants influence how community networks are shaped. Insect-plant interactions have been studied from an evolutionary perspective since Darwin (1859), and their importance for plant and animal diversification has been demonstrated from different perspectives (Ehrlich and Raven, 1964; Thompson, 2005). In terrestrial ecosystems, flowering plants outnumber most other macro organisms in terms of their species richness (Smith, 2010), this is, of course with the exception of arthropods and microbe species. Angiosperms have diversified as a consequence of selective pressures from their pollinators, becoming a very numerous group, with over 350,000 species estimated (Kearns et al., 1998; Ollerton et al., 2011). Such a successful diversification is in part, the result of the modulation of species interactions between plants and insects (Chomicki et al., 2019). Flowering plants engaged in obligate pollination mutualisms depend on anthophilous animals, which are species adapted to obtain floral rewards. These plants have what may be called zoophilous flowers, which are floral structures adapted to be pollinated by animals. Obligate pollination mutualisms represent one of the most specialized interactions in terrestrial ecosystems (Kato and Kawakita, 2017).

In particular, obligate pollination mutualisms consist of highly-specific relationships between a host plant and a pollinator. Frequently in this type of interaction, the pollinator species is a seed predator, using its host plant as a source of food, shelter and mating grounds (Bronstein, 2001). The two most iconic systems of obligate pollination mutualisms where the pollinator is also a seed predator, happen between *Ficus* trees (Moraceae), which are pollinated by fig wasps of the family Agaonidae (Janzen, 1979), and *Yucca* plants (Asparagaceae), which are pollinated by yucca moths (Lepidoptera: Prodoxidae Pellmyr,

2003). These fascinating systems were first described by Cunningham (1888), and Riley (1892) respectively, and have been extensively studied ever since. In both of these systems, adult females actively pollinate and oviposit in the plant's ovary, where a subset of the developing seeds will be used as a resource to feed the pollinator's progeny (Pellmyr and Leebens-Mack, 1999). Because some fertile seeds escape the pollinator's larvae, the interaction has a beneficial effect on the host plant and the pollinator/seed predator.

The study of obligate pollination systems has led to key findings about the evolutionary processes of insect-plant associations. Probably the main advantage of obligate pollination systems is that its components can be quantified with relative ease, in part because the number of species that can be held accountable for pollination and seed predation is usually one and the same, and thus, costs and benefits that result from the interaction can be compared by measuring the number of seeds produced by the plant (Bronstein, 2001). This obligate interaction involves two species with very different life cycles and phylogenetic origins, which have co-evolved in a tight relationship where matching phenotypes are promoted by strong reciprocal selection (Kato and Kawakita, 2017). Studies on the *Yucca*-yucca moth interaction have expanded our understanding of trait specialization (Yoder et al., 2013), species diversification (Pellmyr, 2003; Smith et al., 2008), origins of opportunistic species (Segraves et al., 2005), co-evolution of the interacting species (Yoder and Nuismer, 2010), or the influence of abiotic factors on mutualistic interactions (Segraves, 2003; St Clair and Hoines, 2018).

It has been suggested that the colonization of *Yucca* plants by ancestral lineages of Prodoxidae moths perhaps occurred  $41.5 \pm 9.8$  mA (Pellmyr and Leebens-Mack, 1999), however, recent genomic studies of the Agavoidea subfamily indicate a more recent origin for the divergence of the *Yucca* lineage (20 mA, Flores-Abreu et al., 2019). Genomic analyses have shown that the origin of yucca moths as pollinators could have occurred twice: once in the lineage leading to *Yucca*, and once within the *Hesperoyucca* lineage (McKain et al., 2016). *Yucca* moths have evolved features that allow them to use *Yucca* plants as nurseries for their offspring. In particular, yucca moths collect pollen from an anther by using modified appendices (i.e., maxillary palps), fly to another flower, and then oviposit in the flower's ovary using a specialized, blade-like ovipositor. After laying her clutch, the female moth walks up to the ovary, and actively pollinates the stigma before leaving the flower (Aker and Udovic, 1981; Pellmyr, 2003). *Yucca* moths profit from this nursery pollination mutualism by feeding on a subset of seeds that develop in one of the three locules in a fertilized flower. Once the larvae reach the third instar, they bore a hole through the fruit's exocarp and exit to pupate in the ground (Pellmyr, 1999, 2003; Powell, 1992). *Yucca* flowers are also modified: ovaries are particularly large, and the stamens are bent away from the pistil. Flowers

become receptive during a short period of time (12-48 h), which reduces the probability of passive pollen transfer by generalist insects (Powell, 1992). Evidence supports that for the last 20 Myr, this obligate pollination system has remained common within species in the *Yucca* clade (Rentsch and Leebens-Mack, 2014). The fact that practically no *Yucca* species has escaped this highly specialized mutualism shows that the interaction has been advantageous for both counterparts through evolutionary time.

The genus *Yucca* comprises over 40 species of long-lived perennials native to North America. Species of the genus *Yucca* are among the oldest species in desert ecosystems of North America. Three phylogenetic sections have been defined within *Yucca*: two sections produce indehiscent fruit (Sarcocarpa and Clistocarpa), and fruits in species within section Chaenocarpa are dehiscent (Pellmyr et al., 2007). While seeds in capsular-fruited species (Chaenocarpa) are wind dispersed, seeds in sections Clistocarpa and Sarcocarpa (which are spongy and fleshy-fruited *Yucca* species respectively) were presumably dispersed by megafauna from the Pleistocene that has now gone extinct (Cole et al., 2011; Laudermilk and Munz, 1934). Long-distance seed dispersal (e.g., distances >100 m) by large birds and introduced cattle has been reported in Sarcocarpa and Clistocarpa (Lenz, 2001), and studies on Mojave desert populations of the Joshua Tree (*Y. brevifolia*, section Clistocarpa) have found that ongoing climate change and the loss of seed dispersers could be constraining population connectivity across *Y. brevifolia* distribution (Cole et al., 2011).

*Yucca* plants are long-lived and are also capable of vegetative reproduction. In caulescent species, individuals produce several stalks throughout their life, showing different branching patterns depending on the species. Because *Yucca* species are polycarpic and mainly outcrossed, it is expected that *Yucca* populations maintain high levels of genetic diversity. Despite the wide number of investigations that have focused on this mutualism model, few studies have quantified variation in traits associated from the mutualism among several *Yucca* populations. Additionally, only few studies in *Yucca* have focused in studying the geographic structure of genetic and phenotypic variation.

A species within the section Sarcocarpa, *Yucca schidigera* is distributed in southwestern US and northern Baja California. Its distribution range overlaps with *Y. brevifolia* across the southern fringes of the Mojave desert in California and Arizona, and with *Y. baccata* in southern Nevada (Turner et al., 2005) where the existence of hybrids with *Y. schidigera* has been reported (Leebens-Mack et al., 1998). However, *Y. schidigera* populations across Baja California do not co-occur with other *Yucca* species (Turner et al., 2005).

Mainly because chemicals in *Y. schidigera* have a series of agronomic, industrial and biomedical applications (Patel, 2012), its wild populations have endured pressure from industrial harvesting in Baja California since the 1970s (Castellon-Olivares et al., 2002). Pharmacological industries have recently discovered novel medicinal applications for the phenolic-rich compounds that are obtained from *Y. schidigera* extracts (Piacente et al., 2005; Sastre et al., 2015; Tamura and Miyakoshi, 2012), which has increased the market price for *Y. schidigera* extracts, potentially threatening its populations. Mexican law on the management of this species does not consider demographic or genetic aspects of the population. The scale of extraction in Mexico is not documented by environmental authorities despite the fact that wild populations of *Y. schidigera* currently represent the main non-timber plant resource in the Baja California chaparral (Castellon-Olivares et al., 2002).

Despite the scale of extraction, management and conservation policies available for *Y. schidigera* are still minimal. Habitat fragmentation associated with extraction activity will usually precede reduction of gene flow, which in turn can potentially increase the loss of genetic variation through drift because of poor replacement of alleles in populations (Frankham, 1995; Frankham et al., 2002; Reed et al., 2003). Extraction of individuals will usually decrease population reproductive success by removing vigorous individuals first, and modify the age structure, processes that can lead to inbreeding which will further reduce reproductive capacity (Ouborg et al., 2006).

In consequence, I consider that the use and management of *Y. schidigera* should include baseline information on the ecology and genetics of its populations. In particular, conservation genetics unites population genetics concepts with the goal of preserving biodiversity in threatened or endangered populations. In particular, the field of conservation genetics is concerned with maintaining the highest possible levels of genetic variation, allowing future adaptation and evolution, and minimizing the damaging effects of genetic drift and inbreeding. In other words, the genetics-oriented approach in conservation biology is oriented to exploring, evaluating and protecting the evolutionary potential of species (Frankham et al., 2002).

## 1.2 Goals and structure of this thesis

This thesis describes population-level variation of three key ecological and evolutionary features in *Y. schidigera* populations in Baja California. The first chapter is focused on the description and analysis of the geographic distribution of genetic variation, and its relationship with a possible range expansion of *Y.*

*schidigera* populations during the Pleistocene, which could explain the colonization of the species distribution in the Baja California peninsula.

In the second chapter of this thesis, we analyze variation of phenotypic traits across populations. Because *Yucca* plants maintain a highly specific interaction with their pollinators, we hypothesize that phenotypic traits related to the plant's reproductive structures should be less variable than traits associated with the vegetative structure.

The third and last chapter describes the amount of population-level variation in key traits associated with the mutualism between *Y. schidigera* and its obligate pollinator/seed predator, the yucca moth *Tegeticula mojavella*. We ask if the interaction is geographically structured and if the strength of the mutualism is variable across populations. Based on the results obtained in each chapter, we discuss how the three different scales of biological variation (genetic, phenotypic and ecological) are related for this particular model. This work also provides baseline ecological information of *Y. schidigera* populations in the Baja California peninsula. We consider this to be an important contribution to the conservation of *Y. schidigera* considering that only little information about the evolutionary ecology of this species is currently available.



## Chapter 2. Genetic patterns and changes in availability of suitable habitat support a colonization history of a North American perennial plant

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### 2.1 Introduction

Range expansions driven by climatic change are common in nature (Chen et al., 2011; Excoffier et al., 2008; Kropf et al., 2003) and can have profound consequences over the amount and distribution of genetic variation of a species (Hewitt, 2000, 2004; Klopstein et al., 2006). During range expansion, populations at the leading limit of the species distribution may experience cycles of local extinction and re-colonization more frequently than the rest of the populations across the complete geographical distribution (Eckert et al., 2008; McInerney et al., 2009). Recurrent colonization events can result in a series of founder effects that reduce genetic variation through drift. Assuming a linear stepping-stone colonization model, genetic diversity is expected to diminish steadily along a species' colonization axis (Excoffier et al., 2008). Moreover, the genetic composition of populations associated with the colonization front of a species deviates from what is theoretically expected for populations under equilibrium (Slatkin and Excoffier, 2012). These populations usually show more fragmented distributions and receive a fewer number of migrants. As a result, peripheral populations are characterized by having lower within-population genetic diversity than populations located near the center of the distribution (Lira-Noriega and Manthey, 2014).

Distribution shifts caused by climatic fluctuation often have a latitudinal dimension (Hewitt, 2004). During the climatic oscillations of the Quaternary, many temperate plant species of North America experienced migration and local extinction which resulted in episodes of geographic range expansion and contraction in rather short periods of time (Hewitt, 2000; Stewart et al., 2010). Evidence gathered from genetic studies suggests that during the Last Glacial Maximum (LGM; 26.5 – 19 ka; Clark et al., 2009), numerous plant species shifted their distribution towards southern locations, where more suitable climatic habitat was available (Ramírez-Barahona and Eguiarte, 2014; Rodríguez-Banderas et al., 2009). Incorporating genetic data analyses and Environmental Niche Models (ENMs) has been an advantageous strategy in studies aiming to understand the impact of climate-induced distribution shifts on patterns of genetic differentiation (Knowles et al., 2010). By providing different sources of evidence, this type of approach can be used to identify the influence of historic processes on a number of evolutionary attributes of populations, potentially explaining how different taxa respond to historical climate changes (e.g., Klimova et al., 2017).

Located in western North America, the Baja California (BC) Peninsula stretches nearly 1,300 km south of California from 32° 46' N to 22° 53' N. It is a relatively narrow peninsula (45 -250 km). Steep mountain ranges that run north to south, together with large extensions of desert shape the heterogeneous landscape across this region. Such high environmental variation has promoted a rich assemblage of species that we observe today across the BC Peninsula. Tectonic and climatic processes have strongly influenced the natural history of the peninsular biota. The separation of the peninsula from mainland Mexico occurred during the Tertiary, when prolonged northwest motion of the Pacific plate allowed the formation of the Gulf of California (10-7 mA). Subsequently, during the Plio-Pleistocene, a series of marine incursions may have inundated the peninsula across two regions: the central desert region and the isthmus of La Paz, which is located north of the cape region (Dolby et al., 2015). Finally, climatic oscillations during the Quaternary changed the distribution of species across the region. These processes have shaped the current peninsular biota, resulting in significant differences between the distribution of vegetation during glaciation events and what we observe in current time (Dolby et al., 2015).

Over the last decades, the biota of the BC Peninsula has been subject to numerous studies, with many of them focusing on the phylogenetic history of different native taxa (Riddle et al., 2000). Genetic data support the idea that range expansions during the late Quaternary influenced current patterns of genetic diversity in plant species distributed across the region. For example, genetic evidence for northward post-glacial range expansions has been found for different columnar cactus species in the BC Peninsula (Clark-Tapia and Molina-Freaner, 2003; Gutiérrez-Flores et al., 2016; Nason et al., 2002). On the other hand, latitudinal variation in genetic diversity for the desert succulent *Euphorbia lomelii* (Euphorbiaceae) supports a recent southward range expansion across the peninsula (Garrick et al., 2009).

Evidence from paleoecological records of floristic assemblages changing during the Pleistocene in the BC Peninsula suggests that chaparral (e.g. California sclerophyll vegetation) reached lower latitudes (~27.5 °N – 30 °N) than the extent of its current distribution (Rhode, 2002; Wells, 2000). The presence of woodland and chaparral vegetation across central latitudes of the peninsula during the Pleistocene suggests that climatic conditions during the LGM were similar to the montane habitat that currently occurs in the northwestern region of the peninsula. Consistent with this, fossil records found in the Mojave, Colorado and Sonoran deserts indicate that the distribution of a distinctive Mojave desert perennial, the Joshua tree (*Yucca brevifolia*), expanded significantly during the LGM (Cole et al., 2011).

Mojave yucca, *Yucca schidigera* Roezl (Asparagaceae), is a long-lived, arborescent monocot, native of southwestern US and the BC Peninsula. Its distribution extends from 36 °N to 29.5 °N and its populations

occur in arid and semiarid habitats at elevations ranging from 100 to 1,800 m above sea level (Rebman et al., 2016; Turner et al., 2005). This species is polycarpic, and widespread flowering events occur every three to five years (*pers. obs.*). Pollination depends on two yucca moth species of the family Prodoxidae (*Tegeticula californica* and *T. mojavella*; Pellmyr, 2003; Pellmyr et al., 2008). Presumably, *Y. schidigera* colonized its current distribution in the BC Peninsula during the late Quaternary together with other plant species of the North American deserts (Axelrod, 1978).

To investigate the distribution of genetic variation across the southern populations of *Y. schidigera*, we sampled 13 sites along a 350 km transect in the BC Peninsula (29.9 °N to 32.5 °N approximately). We integrated results from the analysis of nuclear microsatellite data with ENMs projected to three different times during the Quaternary. This allowed us to assess the scenario where *Y. schidigera* colonized its current distribution during the Quaternary following a north-south direction. If this were the case, we would expect:

- i) genetic variation to decrease towards southern populations,
- ii) that populations associated with the historic colonization front will be more genetically differentiated than central populations,
- iii) the ENMs to support an increase in suitable habitat conditions along the BC peninsula during the late Quaternary.

## 2.2 Methods

### 2.2.1 Study area

Our sampled populations are distributed within a 350 km latitudinal transect in the northwestern part of the BC Peninsula. This region is characterized by two mountainous formations (namely, Juárez and San Pedro Mártir sierras) that extend latitudinally, resulting in a heterogeneous landscape. The Mediterranean climate that transitions into desert near the 30 °N parallel is the result of steep precipitation latitudinal gradient (González-Abraham et al., 2010; Vanderplank and Ezcurra, 2016). Average annual precipitation registered for our study area over the last 30 years is 276.4 mm ± 77.3 SD, and mean annual temperatures range from 10.3 to 23.5 °C (data from WorldClim 2; Fick & Hijmans, 2017). Eight populations occur in chaparral/Pinyon-juniper woodland ecotypes, the remaining five populations occur in desert scrub vegetation and are located in the southern edge of the species'

distribution range (Fig.1). Between 2013 and 2016, we visited these sites and collected 10 g of fresh leaf tissue from 15-20 individuals selected haphazardly at each site. To avoid including genetic clones in our analysis, we only sampled plants that were at least 10 m apart from each other. The final total sample size was 240 plants. We stored all samples at -80 °C prior to DNA extraction.

### 2.2.2 DNA extraction and microsatellite amplification

Because yucca leaves are fibrous and rich in polysaccharide compounds, we processed disrupted leaf tissue following Varela-Álvarez et al. (2006) before proceeding with the DNA extraction. Genomic DNA was obtained using the salt extraction protocol (Aljanabi and Martinez, 1997). Integrity of the genomic DNA was visualized in a 0.8 % agarose gel stained with GelRed™ (Biotium, Fremont, CA). We used fluorescent dye-tagged primers to genotype 240 individuals at 7 microsatellite loci. The oligonucleotides used in this study were developed by Sklaney et al. (2009) and Flatz et al. (2011) for *Y. filamentosa* and *Y. brevifolia*. Our PCR products were analyzed through capillary electrophoresis on an ABI Prism 3130 automated sequencer (Applied Biosystems, Foster City, CA) at SeqXcel (San Diego, CA).

### 2.2.3 Population genetics analysis

Allele sizes were visualized and scored manually using Peak Scanner v.2. We checked for genotyping errors using Micro-Checker (Van Oosterhout et al., 2004) and estimated null allele frequencies using the EM algorithm (Dempster et al., 1977) in FreeNA (Chapuis and Estoup, 2007). We tested for linkage disequilibrium (*LD*) using Fstat (Goudet, 1995), and deviation from HW equilibrium was tested via Markov chain permutation following Guo and Thompson (1992) in Arlequin 3.5 (Excoffier and Lischer, 2010).

To assess genetic diversity, for each population we estimated allelic richness ( $A_R$ ), expected and observed heterozygosity ( $H_{exp}$  and  $H_{obs}$ ), and the number of private alleles ( $A_P$ ) across loci using the 'adegenet' package (Jombart, 2008) in R 3.3.2 (R Development Core Team, 2016). To estimate genetic structure across populations, we computed an analysis of molecular variance (amova) in Arlequin (Excoffier et al., 1992) using standard allele frequencies. We performed exact tests of population differentiation on the

basis of pairwise  $F_{ST}$  values with Arlequin (Goudet et al., 1996; Raymond and Rousset, 1995) and we used 'ggplot2' to construct a heatmap of the  $F_{ST}$  pairwise comparisons to visualize genetic differentiation among populations. For significance, we considered an alpha of 0.05 in all statistical tests.

To assess the level of genetic admixture in our sample, we conducted a Bayesian assignment analysis with Structure (Pritchard et al., 2000). We tested  $K$  values from 1 to 15 without prior population assignment (LOCPRIOR), and for each value of  $K$ , we ran 25 independent iterations using a burn-in period of 250,000 and  $1 \times 10^6$  Monte Carlo Chains. To determine the number of clusters that best fit our data we screened the output runs for *i*) the highest likelihood value with least variance for  $K$ , and *ii*) the highest value of delta  $K$  (following Evanno et al., 2005) implemented in Structure Harvester (Earl and vonHoldt, 2012).

To analyze if genetic diversity in *Y. schidigera* declines with latitude, we used linear regression to fit two estimators of the species' genetic diversity (allelic richness and expected heterozygosity) with relation to the site's latitude. To test the influence of geographic distance and climatic variation on the genetic structure of populations, we used the package 'vegan' (Oksanen et al., 2016) to perform two partial Mantel tests: one controlling for the climatic variation, and a second one controlling for geographic distance. For these tests we used Rousset's (1997) standardized genetic distance ( $F_{ST} / 1 - F_{ST}$ ) and geographic distance was estimated using geoid-corrected linear distances among sites. For climatic distances we used the Euclidean multivariate distance calculated from the seven Worldclim2 variables used in the ENMs.

#### **2.2.4 Predictive models**

We used Environmental Niche Models (ENM) to predict climatically suitable areas for *Y. schidigera* under current and three different past times. This form of modeling uses occurrence and climatic data to predict suitable habitat for the species. The model can then be projected onto past climates to predict the extension of climatically suitable areas in the past. We used the R package 'Biomod2' (Thuiller et al., 2016) and used the following algorithms with their default parameters to model the potential distribution of *Y. schidigera*: generalized additive model (GAM), random forest (RF), and maximum entropy (MAXENT). To construct the model, we acquired *Y. schidigera* occurrence records from public databases that concentrate herbarium records:

- i) Consortium of California Herbaria (<http://ucjeps.berkeley.edu/consortium/>)
- ii) BajaFlora (<http://bajaflora.org>)
- iii) Global Biodiversity Information Facility (<http://www.gbif.org>)
- iv) SEINet (<http://swbiodiversity.org/seinet>)

We removed registries that were not consistent with the geographic distribution of the species. After including our own data from the field, the occurrence list summed up 909 records. To reduce model over-fitting from spatial autocorrelation, we used the R package ‘*raster*’ (Hijmans, 2016) to filter occurrence points down to a single registry per 0.1 degree of latitude. The final database consisted of 103 occurrence points, and represents a realized niche of *Y. schidigera*.

We used the present WorldClim 2 dataset (1970-2000; Fick and Hijmans, 2017) with a resolution of 30 arcsecs, and to reduce variable co-linearity we performed PCA using ‘FactoMineR’ (Lê et al., 2008). After removing highly correlated variables, seven bioclimatic variables that showed low correlation and were likely to be of high biological importance to *Y. schidigera* were used to construct the final models: *Bio1*-Annual Mean Temperature, *Bio5*-Max Temperature of the Warmest Month, *Bio6*-Min Temperature of the Coldest Month, *Bio8*-Mean Temperature of the Wettest Quarter, *Bio12*-Annual Precipitation, *Bio13*-Precipitation of the Wettest Month and *Bio18*-Precipitation of the Warmest Quarter. We used the climatic data from Braconnot et al. (2007) for the Mid-Holocene (MH; ~6 ka; 30 arc-sec) and Last Glacial Maximum (LGM; ~22 ka; available only at 2.5 arc-min). For the Last Interglacial (~120 ka), we used data from Otto-Bliesner et al. (2006) at 30 arc-sec resolution (at 32 ° of latitude, 30 arc-sec is equivalent to 925 m, and 2.5 arc-min is equivalent to 4625 m). Our set of records was randomly split into training (70 %) and testing sets (30 %). We evaluated model performance using the TSS and ROC metrics, and only kept model replicates with TSS > 0.75 and ROC > 0.90. We used ensemble forecasting to construct a consensus model for current and past period conditions. Finally, we converted predicted projections to a binary presence/absence value using the TSS threshold.

## 2.3 Results

### 2.3.1 Genetic diversity

We genotyped 240 individuals at 7 microsatellite loci. All seven microsatellite loci were highly polymorphic, allele size ranges were consistent with Flatz et al. (2011)) and we did not detect evidence of genotypic linkage disequilibrium. For the 13 sampled populations, we registered a total of 227 alleles across the seven loci (Mean=32.42  $\pm$  3.68 SE). The average number of effective alleles ( $A_E$ ) per population across all loci ranged from 4.725 to 7.605 (Table 1). Allelic richness ( $A_R$ ) averaged across all loci ranged from 7.71 to 11.57 among populations, and the overall mean was 10.11  $\pm$  0.38 SE (Table 1). We found an excess of homozygotes across all sampled populations, with 53.84 % of the 91 exact tests resulting in significant departure from HW equilibrium. Accordingly, across all populations, mean observed heterozygosity ( $H_{obs}$  = 0.584  $\pm$  0.021 SE) was lower than mean expected heterozygosity ( $H_{exp}$  = 0.791  $\pm$  0.011 SE). Mean population  $H_{obs}$  ranged from 0.513 to 0.73 and  $H_{exp}$  ranged from 0.727 to 0.843 (Table 1). We detected higher genetic variation in populations located in the Juárez (population A) and San Pedro Mártir foothills (populations F and G). These chaparral and woodland populations had the highest values of  $H_{exp}$  across our samples (A=0.843; F=0.83 and G=0.841). Three of the desert scrub populations, located in the southern region of our study area, showed lower genetic diversity (J= 0.727, L=0.765 and M=0.727). We found significant associations between both estimators of genetic variation and latitude (Fig. 2, for allelic richness  $R^2$ =0.38,  $P$  = 0.023; for expected heterozygosity  $R^2$  = 0.32;  $P$  = 0.042), supporting our hypothesis of a colonization front that expanded north to south across the species' distribution in the BC Peninsula.

**Table 1.** Estimates of genetic variability in *Yucca schidigera* across the 13 populations sampled in this study. Column headers:  $A_R$  = Allelic richness (mean number of alleles across loci); SE= Standard error;  $A_E$ =Number of effective alleles;  $H_{obs}$ = Observed heterozygosity;  $H_{exp}$ = Expected heterozygosity;  $f$  = Average fixation index;  $N$ =sample size.

Site	$A_R \pm 1 SE$	$A_E$	$H_{obs} \pm 1 SE$	$H_{exp} \pm 1 SE$	$f$	$N$
A	11.57 $\pm$ 1.36	7.61 $\pm$ 1.16	0.536 $\pm$ 0.11	0.843 $\pm$ 0.03	0.365	20
B	10.71 $\pm$ 1.47	6.36 $\pm$ 1.1	0.588 $\pm$ 0.07	0.807 $\pm$ 0.04	0.259	20
C	11 $\pm$ 1.74	6.53 $\pm$ 1.49	0.537 $\pm$ 0.07	0.781 $\pm$ 0.05	0.299	20
D	8.71 $\pm$ 1.51	4.98 $\pm$ 0.94	0.520 $\pm$ 0.06	0.756 $\pm$ 0.04	0.303	20
E	10.85 $\pm$ 1.91	6.63 $\pm$ 1.38	0.544 $\pm$ 0.08	0.808 $\pm$ 0.04	0.338	20
F	11.57 $\pm$ 1.36	7.04 $\pm$ 1.21	0.69 $\pm$ 0.06	0.830 $\pm$ 0.03	0.169	20
G	10.57 $\pm$ 0.95	6.7 $\pm$ 0.65	0.73 $\pm$ 0.08	0.841 $\pm$ 0.02	0.134	15
H	9.43 $\pm$ 0.95	5.88 $\pm$ 0.66	0.527 $\pm$ 0.04	0.813 $\pm$ 0.03	0.339	15
I	10.86 $\pm$ 1.87	6.48 $\pm$ 1.36	0.635 $\pm$ 0.07	0.799 $\pm$ 0.04	0.213	20
J	8.14 $\pm$ 1.18	4.73 $\pm$ 0.87	0.559 $\pm$ 0.07	0.727 $\pm$ 0.06	0.207	20
K	10.14 $\pm$ 1.12	5.6 $\pm$ 0.82	0.570 $\pm$ 0.04	0.788 $\pm$ 0.04	0.257	20
L	8 $\pm$ 0.96	4.73 $\pm$ 0.64	0.513 $\pm$ 0.10	0.765 $\pm$ 0.03	0.337	15
M	7.71 $\pm$ 1.04	4.79 $\pm$ 0.87	0.637 $\pm$ 0.09	0.727 $\pm$ 0.07	0.105	15
Mean	9.94 $\pm$ 0.38	6 $\pm$ 0.28	0.584 $\pm$ 0.02	0.791 $\pm$ 0.01	0.256	18.46

### 2.3.2 Genetic structure

The AMOVA detected low, but significant genetic differentiation among populations ( $F_{ST} = 0.067$ ;  $P < 0.0001$ ), and most of the genetic variance (92.3 %) was found within the *Y. schidigera* populations (Table 2). Population pairwise genetic differentiation was low but statistically significant for all paired comparisons, except for A-H, C-D, and G-H (Fig. 3). Pairwise differentiation values ranged from  $F_{ST}=0.02$  to  $F_{ST}=0.14$ , and populations located across the southern edge of the species' distribution showed slightly higher genetic differentiation in relation to central populations (Fig. 3).

The Structure analysis suggested three genetic clusters. Both methods of estimating  $K$  converged in the same clustering solution ( $K=3$ ). Individual assignment probability plots show moderate geographic structuring across *Y. schidigera* populations (Fig. 1C). Most individuals in the northern populations (A and B) were assigned to a genetic cluster (blue, Fig. 1C). For those populations located between 31° N and



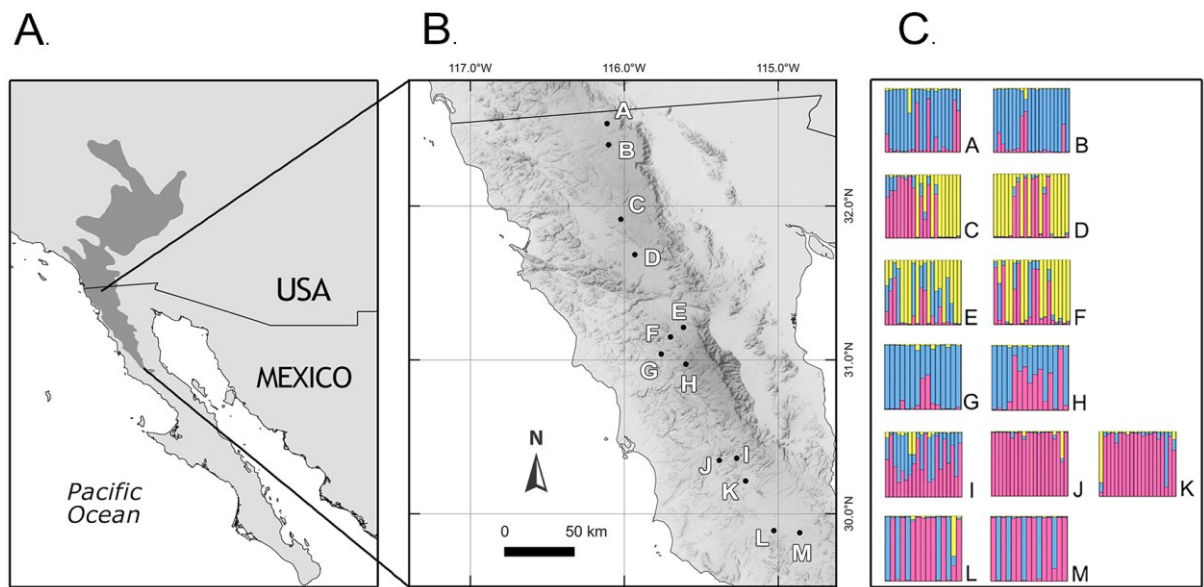
32° N (C, D, E and F), the Structure analysis assigned most individuals to a distinct cluster (yellow, Fig. 1C), with some individuals showing admixture with a third cluster (pink, Fig. 1C). South of these populations, sites G and H were assigned mainly to the blue cluster, similarly to populations A and B (Fig. 1C). These sites (G and H) are montane populations located within the foothills of the San Pedro Mártir sierra. Finally, individuals from populations I, J, K, L and M, consistently shared a high probability of belonging to a third genetic cluster (pink, Fig. 1C). These last five sites are all located within desert scrub, south of the sierra foothills. The partial Mantel test detected a strong and significant isolation by distance correlation between geographic distance and genetic differentiation across populations ( $P = 0.004$ ;  $r = 0.38$ ; Fig.4). In contrast, the isolation by environment correlation between climatic and genetic distance was not significant when controlling for the geographic distance ( $P = 0.78$ ;  $r = -0.11$ ).

**Table 2.** Analysis of molecular variance (amova). Column headers: df= degrees of freedom; SS=Sum of squares; and % of Var.= percentage of the variance explained.

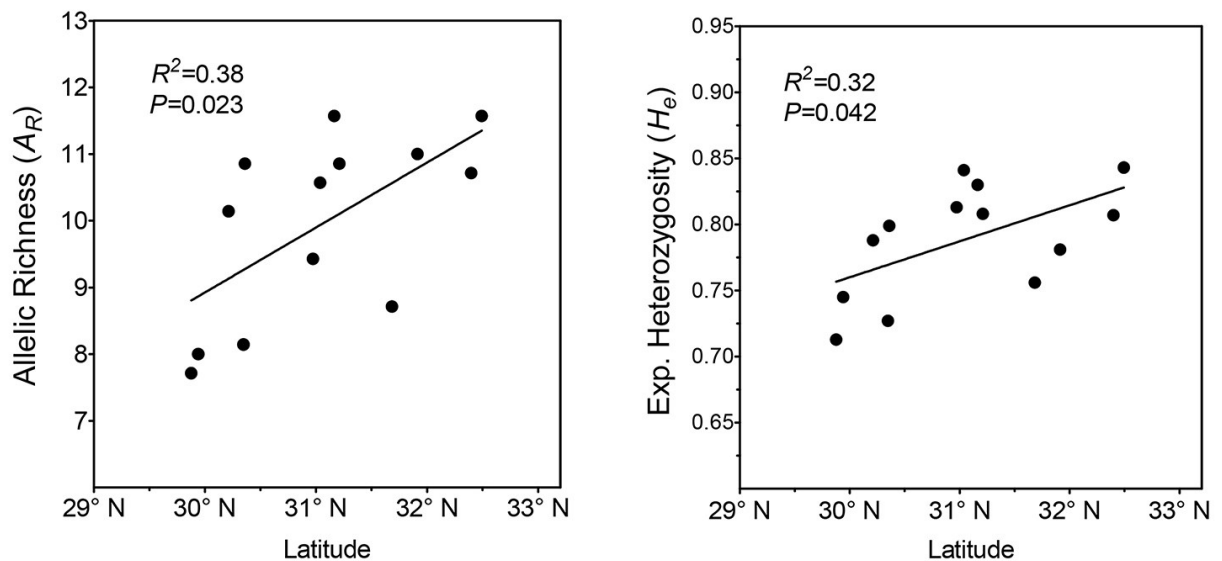
Source of variation	df	SS	% of Var.	P-value
Among populations	12	127.98	6.78 %	<b>&lt;0.001</b>
Within populations	467	1352.51	93.22 %	
<b>Total</b>			100 %	
F-statistic	Value	P-value		
F <sub>ST</sub>	0.0678	<0.001		
F <sub>IS</sub>	0.211	<0.001		
F <sub>IT</sub>	0.281	<0.001		

### 2.3.3 Predictive models

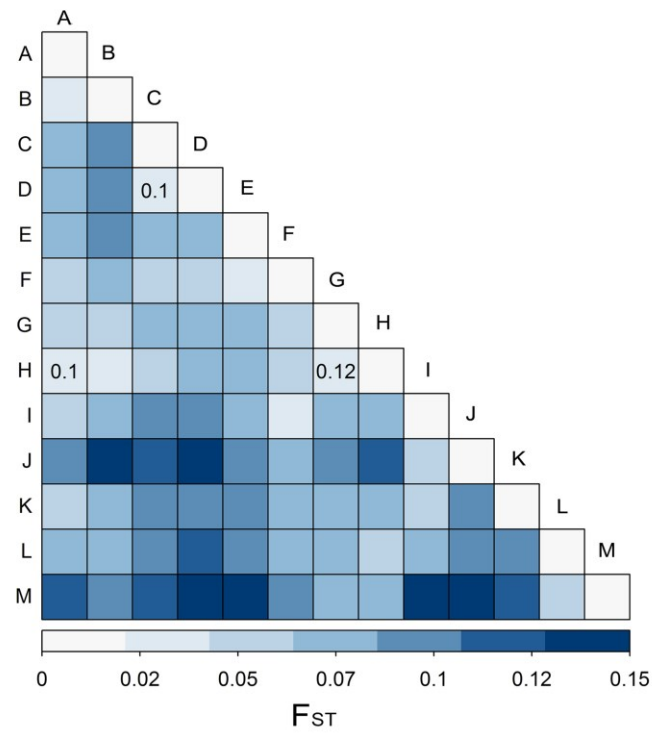
The suitable range predicted by our environmental niche model is consistent with the species' distribution described by Turner et al. (2005). The model performed well, with AUC values averaged for 5 replicates = 0.94. The climatic variables that contributed the most were the mean temperature of the wettest quarter, contributing 40.3 %, the minimum temperature of the coldest month (21.9 %), and precipitation of the warmest quarter (16.3 %). Together, these variables contributed with 78.5% of the explanatory power of the model. Finally, the ENM projections for paleoclimatic conditions indicate a very ample increase in suitable climatic conditions from the LIG to the LGM (Fig.5), with a large potential distribution area that remained through the LGM until the Mid-Holocene, although it decreases until its current distribution.



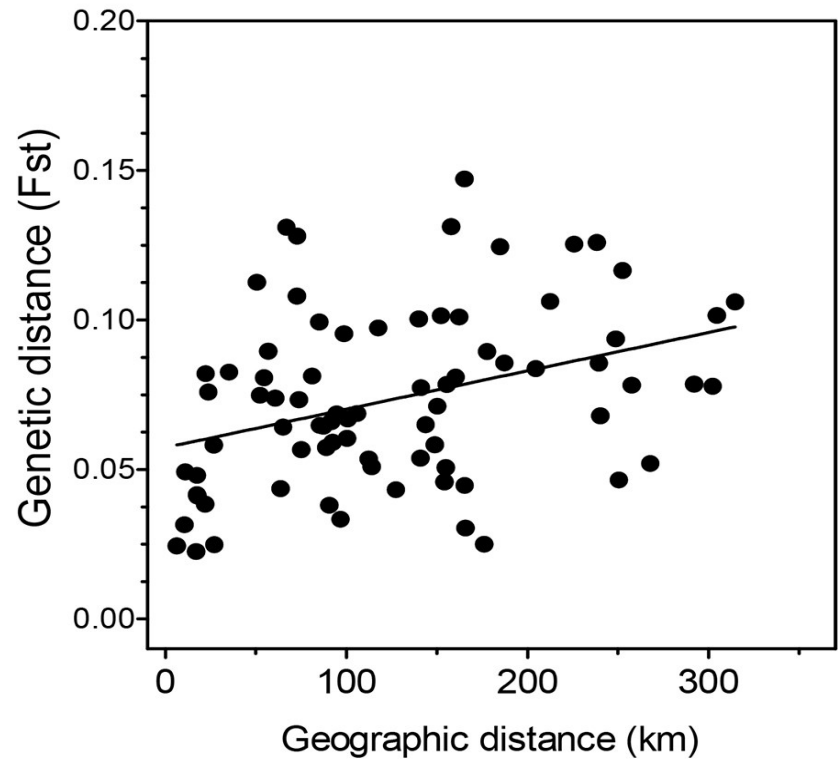
**Figure 1.** Sampling area and genetic admixture in 13 populations of *Yucca schidigera* located in the Baja California Peninsula. Panel A shows current distribution range of *Y. schidigera* (shaded in dark grey; modified from Turner et al., 2005). Panel B shows the geographic distribution of the 13 sampling sites. The estimation of genetic clustering in *Y. schidigera* populations of Baja California inferred by the Structure algorithm is shown in panel C. Individual assignment probability plots show levels of genetic admixture for each population when  $K=3$ . Each vertical bar represents an individual, and its partition indicates the probability of membership to each of the genetic clusters detected.



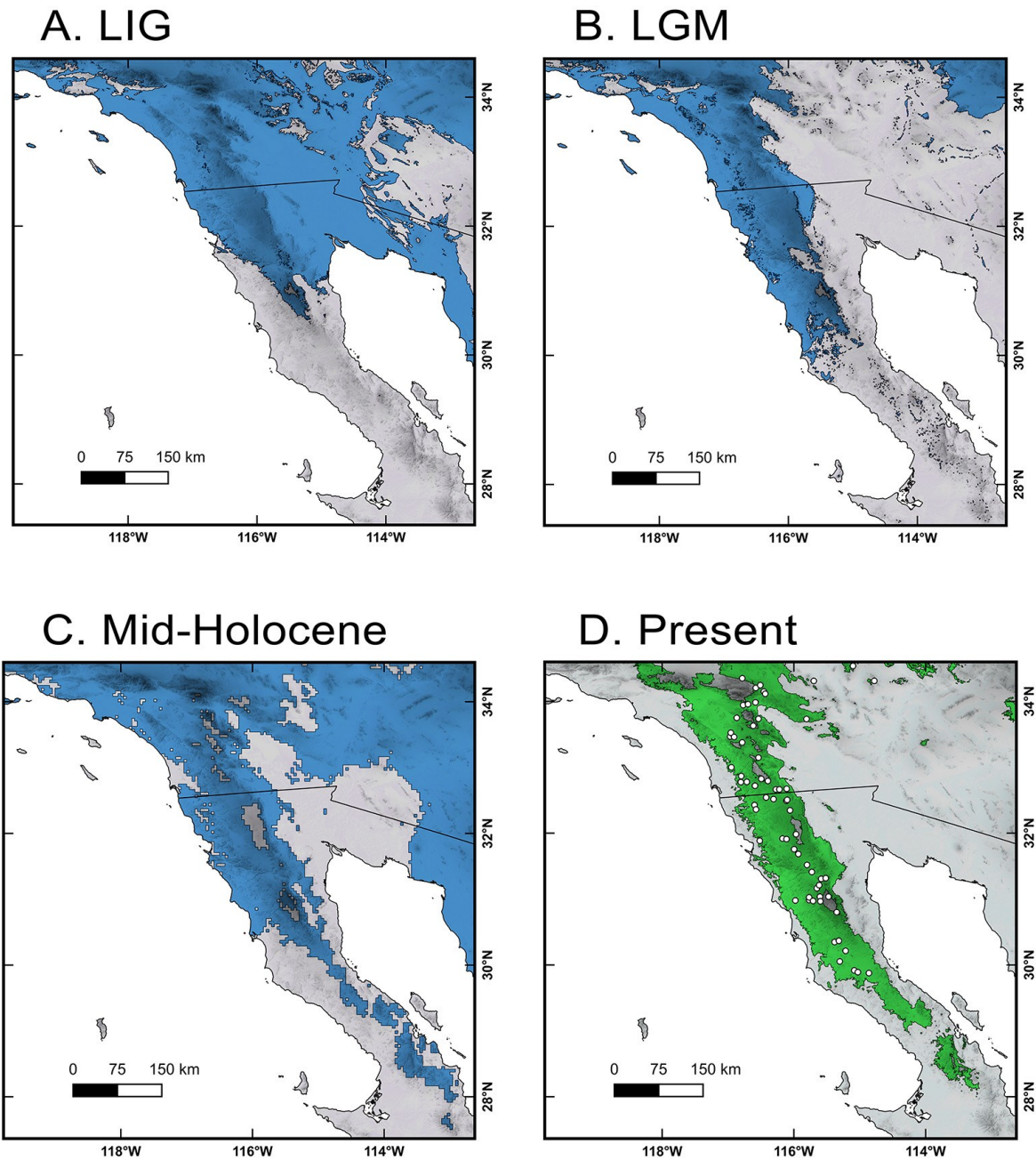
**Figure 2.** Relationship between site latitude and genetic diversity across *Y. schidigera* populations in the Baja California Peninsula. Each point represents one population and the significant regression lines are shown in each plot. Left panel shows the linear regression model between site latitude and allelic richness. Right panel shows the linear regression model between site latitude and expected heterozygosity.



**Figure 3.** Heat map representation of the pairwise  $F_{ST}$  values among *Y. schidigera* populations. All comparisons presented are statistically significant at a level of significance of 0.01 unless the p-value is reported in the overlay. Significance is based on 10100 permutations.



**Figure 4.** Relationship between genetic distance ( $F_{ST}$ ) and geoid-corrected Euclidean geographic distance in *Y. schidigera* populations across Baja California ( $P = 0.004$ ;  $r = 0.38$ ). Mantel statistic based on Pearson's product-moment correlation based on 1010 permutations.



**Figure 5.** Geographic projections of the Environmental Niche Models (ENMs) for *Y. schidigera*. Colored surfaces represent highly suitable habitat area. Each projection represents different climatic periods: A-Last Interglacial (LIG, 120 ka). B-Last Glacial Maximum (LGM, 22 ka). C-Mid-Holocene (MH, 6 ka). D-Present Time. White circles in panel D represent occurrence records used to construct the predictive models.

## 2.4 Discussion

The large body of investigation on the geology, climate and biology of the BC Peninsula suggests that peninsular biogeographical patterns have followed a timescale governed by processes occurring at

three different periods: i) geological events involving plate tectonics which occurred during the Tertiary, ii) midpeninsular marine incursions that interrupted connectivity among northern and southern populations during the Plio-Pleistocene, and iii) glacial-interglacial climatic cycles that resulted in significant temperature and precipitation changes across the BC region (Dolby et al., 2015). Climatic oscillations linked to the glacial-interglacial cycles of the Quaternary resulted in changes in the distribution of plant species across the BC Peninsula (e.g., Garrick et al., 2009; Klimova et al., 2017, 2018). An example of the large distribution shifts that happened in Baja California during the LGM are the isolated strands of chaparral (e.g. California sclerophyll vegetation) that occur over sky islands in the Central Desert of the peninsula. The fact that these areas currently host relict chaparral vegetation as far south as 27.5° N (Bullock et al., 2008) supports the paleoecological evidence gathered from fossil packrat middens in the BC Peninsula (Rhode, 2002; Wells, 2000). This fact also indicates that during the LGM, climatic conditions in the Central Desert of the peninsula allowed temperate plant species to migrate southward across it.

Genetic studies of native Baja California plant species support the idea that post-glacial range expansions have played an important role in shaping genetic diversity of several long-lived perennial species. Among these, *Lophocereus schotti* (Nason et al., 2002), *Stenocereus gummosus* (Clark-Tapia and Molina-Freaner, 2003); *Encelia farinosa* (Fehlberg and Ranker, 2009) and *Euphorbia lomelii* (Garrick et al., 2009) experienced range expansion after the LGM, with the latter following a southward directionality during colonization. More recently, approaches that incorporate geographic patterns of historic distributions with genetic information have shown how close native peninsular species respond different to past climatic changes (Klimova et al., 2017, 2018).

In this sense, we followed an integrative approach by combining genetic data analysis with ENM to investigate possible post-glacial range expansion of *Y. schidigera*, a long-lived desert perennial of the BC Peninsula. This is also the first detailed population genetic study of this species in the southern range of its distribution. We found high genetic variation that decreased with latitude towards populations located near the southern edge of the species distribution. This reduction of genetic diversity is located within an area where available suitable habitat increased since the Last Glacial Maximum (LGM; 21.5 ka), according our ENMs. Both evidences, genetic and climatic, suggests a recent range expansion of *Y. schidigera* across the southern edge of its distribution.

We found high genetic variation across the whole study area ( $H_{exp}=0.79 \pm 0.01$  SE), similar in magnitude to previous reports of other species of the genus *Yucca*: *Y. brevifolia* ( $H_{exp}=0.77 \pm 0.01$  SE; (Flatz et al.,

2011), *Y. capensis* ( $H_{\text{exp}}=0.83 \pm 0.03$  SE; Luna, 2018). Long-lived perennial species with large distributions have been documented to show high genetic variation (e.g., Massey & Hamrick, 1998). Breeding systems also have been known to influence levels of genetic variation across plant species, with outcrossing species showing high genetic variation and low inter-population differentiation (Hamrick and Godt, 1996). *Yucca* species are predominantly outcrossed. For example, allozyme analysis in *Y. filamentosa* suggested very high rates of outcrossing (Pellmyr et al., 1997), and self-pollinated *Yucca* flowers had a higher probability of being aborted compared to outcrossed flowers (Huth and Pellmyr, 2000). Thus, wide geographical ranges and mechanisms reducing the success of self-pollination can help explain the maintenance of high levels of genetic diversity in this plant.

We found that *Y. schidigera* populations in the BC Peninsula show weak genetic structure. Our amova results demonstrate low genetic differentiation among *Y. schidigera* populations ( $F_{ST} = 0.067$ ). The magnitude of this differentiation is very similar to what has been reported in populations of *Y. brevifolia* ( $F_{ST}=0.061$ ; Starr et al., 2013), *Y. capensis* ( $F_{ST}=0.022$ ; Luna, 2018) and other Asparagaceae distributed across the deserts of North America, particularly, species belonging to Agavoidea. For example, two subspecies of *Agave cerulata* that share their distribution with *Y. schidigera* in the north of the peninsula, showed genetic differentiation ( $F_{ST}=0.098$ ; Navarro-Quezada et al., 2003) as well as populations of *A. lechuguilla* ( $F_{ST}=0.083$ ; Silva-Montellano & Eguiarte, 2003), and *A. potatorum* ( $F_{ST}=0.099$ ; Aguirre-Dugua & Eguiarte, 2013).

Genetic differentiation patterns among *Yucca* populations are partially influenced by seed dispersal and pollen movement. Seed dispersal in contemporary populations is carried out by vertebrates such as corvids and rodents, and was probably mediated by megaherbivores during the Pleistocene (Lenz, 2001). On the other hand, pollen movement is restricted to the yucca moth's dispersal ability. Massey and Hamrick (1999) found that yucca moths are able to transfer pollen  $118 \text{ m} \pm 73 \text{ SD}$  on average within populations of *Y. filamentosa*. Under a stepping stone scenario and considering that *Yucca* plants are long-lived and have the potential to reproduce annually, pollen-mediated gene flow could reach populations located far away from its source. The yucca moth *T. mojavella* is the species pollinating the *Y. schidigera* populations sampled in this study (De la Rosa-Conroy, Arteaga, et al., 2019). Even if we do not have information on the distance and frequency that this yucca moth is capable of moving pollen among populations, we should not discard the possibility that low genetic differentiation can result from long-distance gene flow (via pollen). Studies involving species-specific systems such as the mutualism between fig trees (*Ficus*, Moraceae) and fig wasps (*Agaonidae*, Hymenoptera), support that mutualistic

pollination systems can result in strong pollen flow, limiting local genetic differentiation (Tian et al., 2015).

The genetic admixture pattern detected in *Y. schidigera* populations by the STRUCTURE algorithm shows that northern populations (Fig.1C; populations A and B) in the peninsula share a genetic component with some of the central populations (G and H). The fact that geographically separated populations have high proportion of the same component can indicate an historical contact, probably mediated by megaherbivores through long distance seed dispersal. Other possibility is that landscape features affect yucca moths that transfer pollen across neighboring *Yucca* plants, favoring higher pollen dispersal among some populations. In general, the observed admixture pattern across this side of the *Y. schidigera* geographic distribution indicates a complex history of this species that needs to be explored with a higher genomic resolution.

Finally, the genetic differentiation pattern among *Y. schidigera* populations show signs of a probable colonization history. We found that genetic differentiation between populations located at the southern edge of the distribution range was slightly higher than in the northern. Models suggest that populations associated with colonization fronts of long-lived perennials tend to increase their genetic differentiation over time (Austerlitz et al., 2000).

Our ENMs showed an expansion, through lower latitudes, in the range of potential available conditions for *Y. schidigera*, following the Last Interglacial and continuing until present time. This type of range expansion (e.g., following a southward direction during the LGM) has been documented previously for other plant species in the BC peninsula (Garrick et al., 2009) and is consistent with the paleoecological evidence that during the LGM the distribution range of chaparral and Pinyon-juniper woodland vegetation reached the 30 °N parallel across the peninsula (Rhode, 2002; Wells, 2000). Indeed, the fact that highly suitable habitat conditions for *Y. schidigera* have been available along the north of the BC Peninsula since the LGM supports the idea that multiple generations with large effective population sizes could have accumulated such large magnitudes of genetic variation (e.g., Ortego et al., 2015).

Overall, we showed that genetic variation of *Y. schidigera* in the BC Peninsula declines with latitude, possibly because of historic range expansions associated to the climate of the LGM. The weak genetic differentiation and high genetic variation suggests that *Yucca* populations maintained large populations in this region, and it is possible that populations have not yet achieved equilibrium between drift and

genetic flow. Further studies using other molecular markers and that include the complete distribution of *Y. schidigera* will help to better understand the evolutionary history of this species.



## Chapter 3. Patterns of variation of vegetative and floral traits across *Yucca schidigera* (Asparagaceae) populations in Baja California

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### 3.1 Introduction

Morphological divergence across populations reflects relevant aspects about the evolutionary history of a species, potentially explaining adaptation to local conditions. In plants, morphological variation is fundamental because phenotypic traits have a significant effect on fitness (Farris and Lechowicz, 1990; Milla et al., 2009). Variation in phenotype results from evolutionary processes operating over genes, individuals and populations across the geographic range of a species (Darwin, 1859; D. J. Futuyma and Moreno, 2003; Rieseberg et al., 2002). For plants, spatial heterogeneous conditions of soil, climate and biological interactions can promote local adaptation, which can be detected in the phenotypic features of populations (Godsoe et al., 2008; Scheepens et al., 2011). In this sense, if selection outweighs the effects of gene flow, populations become differentiated and traits that promote performance under particular environments will be selected (Aguirre-Liguori et al., 2019, 2017; Joshi et al., 2001; Kawecki and Ebert, 2004).

Because terrestrial plants are sessile, local environmental conditions usually impose strong selection over phenotypes along the different stages of individual development. Local environmental conditions are composed by a suite of physical (e.g., climatic), geochemical (e.g., edaphic) and biotic factors (e.g., pollinators, pathogens, herbivores) which can affect plants differently across their multiple life stages. Different conditions result in selective pressures that may promote differentiation and local adaptation among plant populations (Colautti et al., 2010; Colautti and Lau, 2015; Thompson, 2005; White and Walker, 1997). For species showing a broad geographic distribution, environmental heterogeneity may result in contrasting selection pressures which can promote phenotypic differentiation among local populations (Domínguez et al., 1998). This may even be reinforced if selection acts over migrant genotypes, or if local phenotypes show poor plasticity when exposed to novel selective pressures.

Vegetative and reproductive traits have been used to investigate phenotypic differentiation among plant populations. Among vegetative traits, total mass and dry weight are two features commonly used when comparing plant growth, however, for long-lived species, non-destructive measurements are preferred. Because no standardized measures of body size exist in plants (Chazdon, 1991), total plant height, stem length and stem width are good proxies of carbon allocation and can be used to compare among individuals and between populations. Leaf morphology (i.e., morphometric traits) reflects a number of key adaptive features which are of fundamental importance for the photosynthetic capacities of plants.

For example, the analysis of variation in leaf length and leaf width have been used to investigate adaptive variation among plant species and plant populations (Tomás et al., 2013; Wright et al., 2004).

Likewise, different reproductive traits of plants such as flowers, fruits and seeds undergo selection by both mutualistic and antagonistic species. Therefore, the phenotype of plant traits may show signals of adaptive variation, limiting the variability to adaptive values. In the case of animal pollinated plants, phenotypic selection on floral traits can reflect the strength of the interactions between plants and their pollinators (Kato and Kawakita, 2017). Plants with specialized pollination systems are expected to show a lower level of variability in flower size and architecture than plants with a generalized system of pollination. Field and experimental studies have demonstrated that natural selection favors the evolution of well integrated phenotypes that result in efficient pollination, which increases fitness in plants (Fenster et al., 2004). Thus, measurements of phenotype are likely to reveal the adaptive variation in floral traits in relation to specific pollinators.

Plants in the genus *Yucca* are large, long-lived perennial monocots distributed across the deserts of North America. Because yucca moths (*Tegeticula*: Prodoxidae), are the sole pollinators of *Yucca* plants (Pellmyr, 2003), matching floral and moth phenotypes have been used to study how co-evolution has shaped variation of phenotypic attributes across the plant's distribution range (Godsoe et al., 2008). In the *Yucca* – yucca moth mutualism system, pollinators exert selection on floral traits such as the stigma or the style, with remarkable functional constraints acting on reproductive traits of *Yucca* plants (Smith et al., 2009) leading to a series of evolutionary consequences, such as reproductive isolation and ecological co-speciation among both groups (Pellmyr, 2000; Pellmyr et al., 1996, 1997; Starr et al., 2013). However, because *Yucca* species usually distribute across contrasting climatic environments (Turner et al., 2005) it is possible that the abiotic environment forces selection on *Yucca* plants. This has been investigated in *Y. brevifolia*, which occurs in both desert and montane forest habitats (Godsoe et al., 2009). It has also been studied in *Y. capensis*, a subtropical species with its distribution restricted to the Baja California cape region (Arteaga et al., 2015).

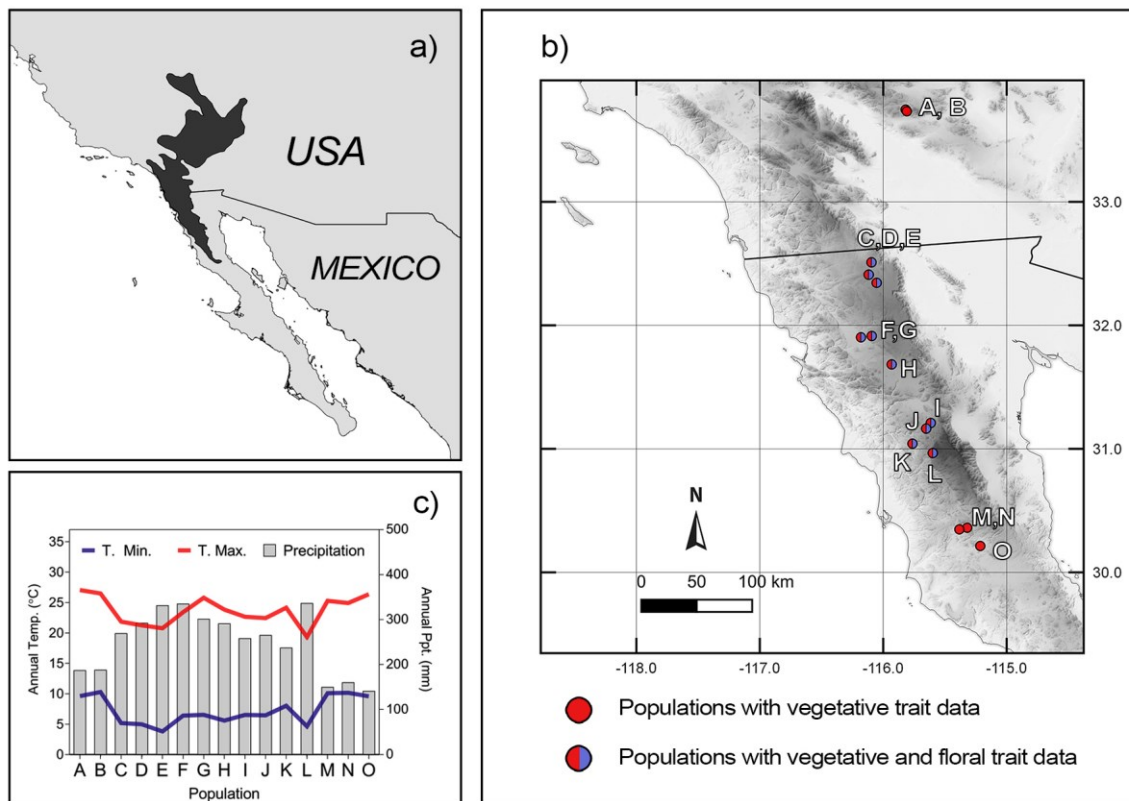
In this study, we examined phenotypic variation across a set of *Y. schidigera* populations located in northwestern Baja California. *Yucca schidigera* is a large, long-lived perennial monocot with a distribution that extends from 36 °N to 29 °N across the Mojave, Sonoran and Baja California deserts in North America. We assessed whether vegetative and floral traits showed a different level of variability. In doing so, we aim to understand the ability of this plant to respond to heterogeneous environmental conditions across its geographic range of distribution. We expect that *Y. schidigera* populations show high phenotypic variation in vegetative traits as a consequence of the wide range of environmental

conditions experienced by this species across this section of its distribution. In contrast, considering that *Yucca* species have developed a tight interaction with its pollinators, and due to the fact that *Tegeticula mojavella* is the sole pollinator of *Y. schidigera* along our study area, we expect to find low phenotypic variation in the set of floral traits that we analyzed.

## 3.2 Methods

### 3.2.1 Sampling effort

During March and April 2015, and June 2016, we measured vegetative and floral traits from 423 *Y. schidigera* individuals belonging to 15 populations located two in California (US) and 13 in Baja California (Mexico). This sampling area is located across a southern section of the complete geographic distribution of *Y. schidigera* (Fig.6). This region includes various climatic conditions, which are mainly: arid hot desert climate, temperate climate with hot and dry summers and arid-hot steppe (Peel et al., 2007)



**Figure 6.** Location of the southern California and Baja California populations of *Yucca schidigera* populations that were sampled in this study (b). Inset map (a) shows the geographic distribution range of *Y. schidigera* based on Turner et al. (2005). Inlay Bar plot (c) shows the annual average climatic conditions for each site (data from Fick & Hijmans, 2017).

**Table 3.** Sample sizes (number of individuals) and distribution of the *Yucca schidigera* populations sampled in southern California (US) and northern Baja California (Mexico) included in this study.

CODE	Site	Vegetation Type	Latitude	Longitude	Elevation (m)	Sample Size	
						Vegetative Traits	Floral Traits
A	Joshua Tree 1	Desert scrub	33.7467	-115.8177	945	30	0
B	Joshua Tree 2	Desert scrub	33.7330	-115.8081	940	30	0
C	Japá 1	Chaparral	32.4899	-116.1150	1288	34	20
D	Japá 2	Chaparral	32.4099	-116.1169	1310	31	21
E	Japá 3	Woodland	32.3442	-116.0525	1430	31	16
F	Ojos Negros 1	Chaparral	31.9135	-116.0925	1201	30	16
G	Ojos Negros 2	Chaparral	31.9030	-116.1797	777	27	12
H	Agua Blanca	Chaparral	31.6833	-115.9307	1167	30	4
I	San Matías 1	Chaparral	31.2089	-115.6140	1206	30	14
J	San Matías 2	Chaparral	31.1637	-115.6510	1252	30	16
K	El Coyote	Chaparral	31.0412	-115.7624	889	15	16
L	San Pedro Mártir	Woodland	30.9655	-115.5981	1917	15	14
M	Los Mártires 1	Desert scrub	30.3605	-115.3188	627	30	0
N	Los Mártires 2	Desert scrub	30.3475	-115.3823	639	30	0
O	Los Mártires 3	Desert scrub	30.2128	-115.2128	591	30	0
Total						423	149

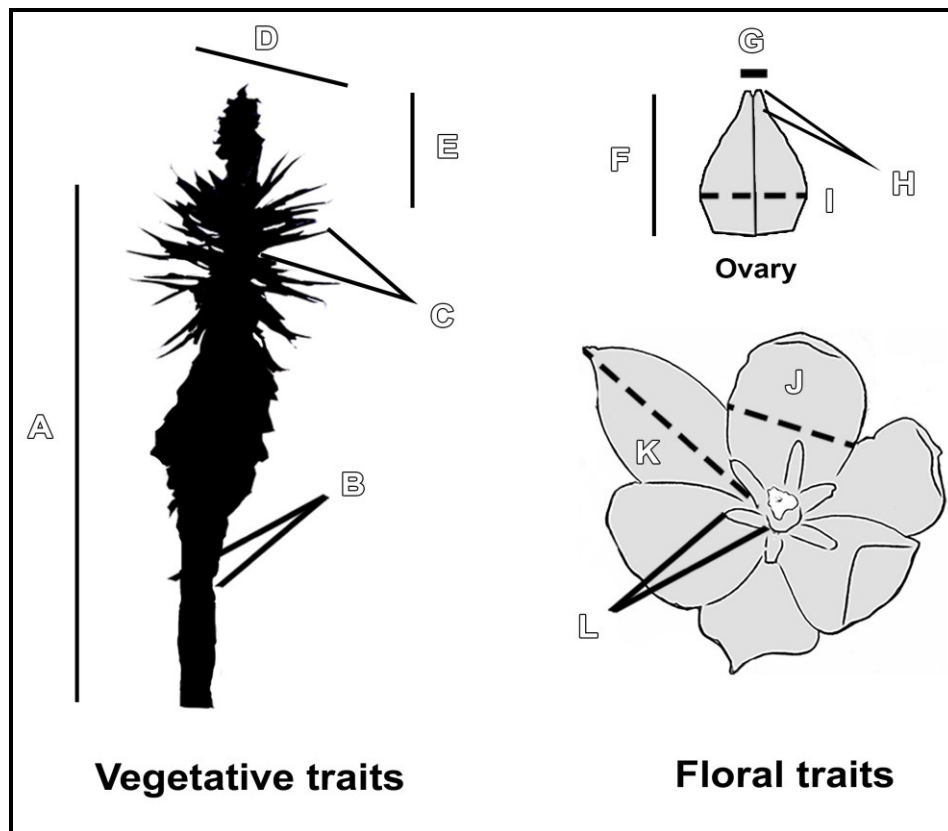
### 3.2.2 Vegetative morphometry

In each population, we measured between 10 and 30 *Y. schidigera* plants haphazardly selected (Mean=28.2  $\pm$  5.53 SD). In each individual we measured 7 vegetative traits (see Fig. 7 for a description of a subset of these traits). In total, we recorded measurements from 423 plants belonging to 15 populations. To avoid sampling several stems from the same individual due to clonal growth, we only included plants that were at least 5 m apart from each other. Measurements of vegetative morphometry were conducted following Arteaga et al. (2015). Briefly, we counted the number of stems and rosettes

produced by each individual, a total height measurement was taken from the base of the stem to the tip of the rosette of the largest trunk. Stem width was measured at 0.5 m above ground level, and for the same stem, rosette diameter, leaf length and maximum leaf width were registered.

### 3.2.3 Floral morphometry

During the flowering season in 2015, on the months of February and March, we collected a sample of three flowers from up to 15 individuals in each locality. From each plant, we three flowers from one inflorescence taking care that they were fresh and at full anthesis. We measured ten floral traits to the nearest 0.1 mm using digital calipers. We obtained the average of the three independent measurements per individual, and used these values in our analyses. For floral measurements we followed Godsoe et al. (2008) and registered maximum length and width of one interior and one external tepal, length and width of the ovary, maximum length and width of the stigma, length of the filament and length of the anther base (Fig. 7).



**Figure 7.** Morphometric traits of vegetative and floral structures measured in *Y. schidigera*: A- Plant Height, B- Stem Width, C-Leaf Length, D-Rosette Width, E-Inflorescence Length, F-Pistil Length, G-Stigma Width, H-Stigma Length, I- Pistil Width, J-Tepal Width, K-Tepal Length, L-Anther Base Length.

### 3.2.4 Statistical analyses

We obtained the coefficient of variation (% CV) across all populations for the phenotypic traits, and then proceeded to rank them in descending order. This allowed us to compare the degree of variation among the variables. To evaluate for differences in phenotypic traits among populations we used ANOVA models and pairwise Tukey HSD comparisons. We considered population as a fixed effect and individual variation as random effects with individual variation nested within the population. We used the percentage of variance explained among populations and among individuals within populations to compare variation among vegetative and reproductive traits. All analyses were executed using R (R Development Core Team, 2016) and JMP v.10.0.0 (SAS institute, 2012).

## 3.3 Results

### 3.3.1 Phenotypic variation of the vegetative structure

The average number of *Yucca* plants per population was 28.2 (mean)  $\pm$  1.37 SE. Vegetative traits showed substantial phenotypic variation across *Y. schidigera* populations (Tables 4 and 5), with most variation distributed within the populations (Fig. 8).

**Table 4.** Population variation (mean  $\pm 1$  SE) of vegetative traits in *Y. schidigera*.

Site	Plant Height	Stem Width	Leaf Length	Leaf Width	Rosette Width	No. of Stems	No. of Rosettes
A	207.60 $\pm 6.76$	25.57 $\pm 0.62$	61.28 $\pm 2.88$	3.29 $\pm 0.09$	114.57 $\pm 5.61$	4.17 $\pm 0.37$	7 $\pm 0.65$
B	186.77 $\pm 8.74$	23.37 $\pm 0.66$	62.50 $\pm 3.98$	3.01 $\pm 0.10$	127.40 $\pm 5.47$	4.10 $\pm 0.65$	6.67 $\pm 1.01$
C	173.71 $\pm 7.47$	15.94 $\pm 0.74$	64.51 $\pm 2.81$	2.85 $\pm 0.12$	120.21 $\pm 5.34$	4.85 $\pm 0.51$	8.29 $\pm 0.62$
D	175.68 $\pm 8.44$	15.59 $\pm 0.43$	65.57 $\pm 2.82$	2.70 $\pm 0.06$	130.19 $\pm 5.05$	3.29 $\pm 0.42$	5.42 $\pm 0.52$
E	152.79 $\pm 8.43$	14.11 $\pm 0.57$	55.66 $\pm 2.09$	2.38 $\pm 0.06$	105.93 $\pm 5.28$	3.94 $\pm 0.43$	7.16 $\pm 0.68$
F	145.85 $\pm 9.27$	13.57 $\pm 0.63$	64.93 $\pm 1.72$	2.77 $\pm 0.08$	118.47 $\pm 4.76$	2.96 $\pm 0.45$	7.23 $\pm 0.81$
G	161.13 $\pm 13.02$	15.50 $\pm 0.62$	71.23 $\pm 2.46$	2.77 $\pm 0.10$	127.70 $\pm 5.31$	2.75 $\pm 0.51$	9.56 $\pm 1.33$
H	174.40 $\pm 7.03$	14.24 $\pm 0.33$	69.22 $\pm 2.97$	2.91 $\pm 0.09$	121.77 $\pm 5.60$	4.80 $\pm 0.80$	8.30 $\pm 0.88$
I	258.85 $\pm 9.02$	20.55 $\pm 0.63$	71.70 $\pm 2.79$	3.40 $\pm 0.11$	160.77 $\pm 8.14$	3.62 $\pm 0.85$	6.93 $\pm 1.33$
J	287.08 $\pm 11.06$	19.97 $\pm 0.67$	59.50 $\pm 2.56$	2.92 $\pm 0.07$	127.80 $\pm 5.46$	3.37 $\pm 0.33$	7.83 $\pm 0.72$
K	219.33 $\pm 16.35$	16.15 $\pm 0.88$	57.87 $\pm 2.99$	2.83 $\pm 0.12$	120 $\pm 8.13$	4.21 $\pm 0.79$	12.13 $\pm 1.57$
L	215.99 $\pm 11.27$	24.13 $\pm 1.79$	63.39 $\pm 3.82$	3.07 $\pm 0.12$	122.77 $\pm 11.37$	3.27 $\pm 0.38$	6.40 $\pm 0.70$
M	132.48 $\pm 5.59$	15.23 $\pm 0.48$	47.72 $\pm 1.79$	2.93 $\pm 0.08$	91.50 $\pm 3.65$	6.00 $\pm 0.86$	8.63 $\pm 1.18$
N	173.72 $\pm 4.85$	15.20 $\pm 0.49$	52.17 $\pm 1.62$	2.84 $\pm 0.08$	107.03 $\pm 4.56$	5.90 $\pm 0.72$	9.20 $\pm 1.29$
O	260.93 $\pm 10.02$	19.55 $\pm 0.43$	54.02 $\pm 1.99$	3.05 $\pm 0.09$	108.87 $\pm 5.35$	5.30 $\pm 0.55$	7.90 $\pm 0.77$
Mean	<b>192.83</b> <b><math>\pm 3.13</math></b>	<b>17.78</b> <b><math>\pm 0.24</math></b>	<b>61.32</b> <b><math>\pm 0.72</math></b>	<b>2.91</b> <b><math>\pm 0.02</math></b>	<b>119.95</b> <b><math>\pm 1.59</math></b>	<b>4.21</b> <b><math>\pm 0.16</math></b>	<b>7.74</b> <b><math>\pm 0.25</math></b>

**Table 5.** Overall mean  $\pm 1$  SE, coefficients of variation (% CV) and sample size of seven vegetative traits registered in *Y. schidigera* populations of southern California (US) and Baja California (Mexico). Vegetative traits are sorted following a descending order in the magnitude of the overall variation.

Trait	Mean (cm)	% CV	n
Number of Stems	4.22 $\pm$ 0.16	78.36	415
Number of Rosettes	7.75 $\pm$ 0.25	66.96	429
Plant Height	192.83 $\pm$ 3.13	33.65	429
Stem Width	17.79 $\pm$ 0.24	28.06	417
Rosette Width	119.96 $\pm$ 1.59	27.54	429
Leaf Length	61.33 $\pm$ 0.72	24.48	429
Leaf Width	2.91 $\pm$ 0.03	18.38	429

Six out of the eight vegetative traits showed coefficients of variation larger than 25 %, supporting that substantial variation in vegetative traits occurs across *Y. schidigera* populations. The most variable traits showed CV's of 78.36 % and 66.96 % and these were the number of stems and the number of rosettes, respectively. The number of stems per individual ranged from 1 to 23 across our sample, and the total number of rosettes ranged from 1 to 30. However, because *Y. schidigera* does not branch as often as other *Yucca* species, these traits are highly correlated for this species.

Stem length and total plant height —which are also highly correlated in this species—were also highly variable across our sample (stem length showed CV = 54.41 %; and plant height showed CV= 33.65 %). This variation resulted plant size ranging from 68 cm to 442 cm across the global sample. Distribution of variance components from the ANOVA models indicated that the two vegetative traits to be more differentiated among populations were plant height and stem width (Fig.8). However, according to the Tukey HSD comparisons, populations with different plant height did not match populations with differences in stem width. While some populations had taller individuals, other populations were represented by plants with stems that were more massive.

Foliar traits were less variable than stem length and total plant height. Leaf length —which is strongly correlated with rosette width— ranged from 14.9 cm to 120 cm (%CV=24.48). Leaf width was the less variable trait, ranging from 1.5 cm to 6 cm across all populations (%CV=18.38).



### 3.3.2 Phenotypic variation of the reproductive structure

We sampled a total of 468 across 10 populations (Table 3). Mean number of plants sampled per site was  $14.9 \pm 1.47$  SE. We were not able to collect a sample of the floral structure from all 15 populations because some *Y. schidigera* individuals did not produce an inflorescence during the sampling year. Populations A and B were not visited during flowering season because these two localities were added to the sampling effort on the last year of the study.

**Table 6.** Population-level variation (mean  $\pm 1$  SE) of floral traits of *Y. schidigera* across 10 populations in Baja California (Mexico). Unless noted, results are reported in mm.

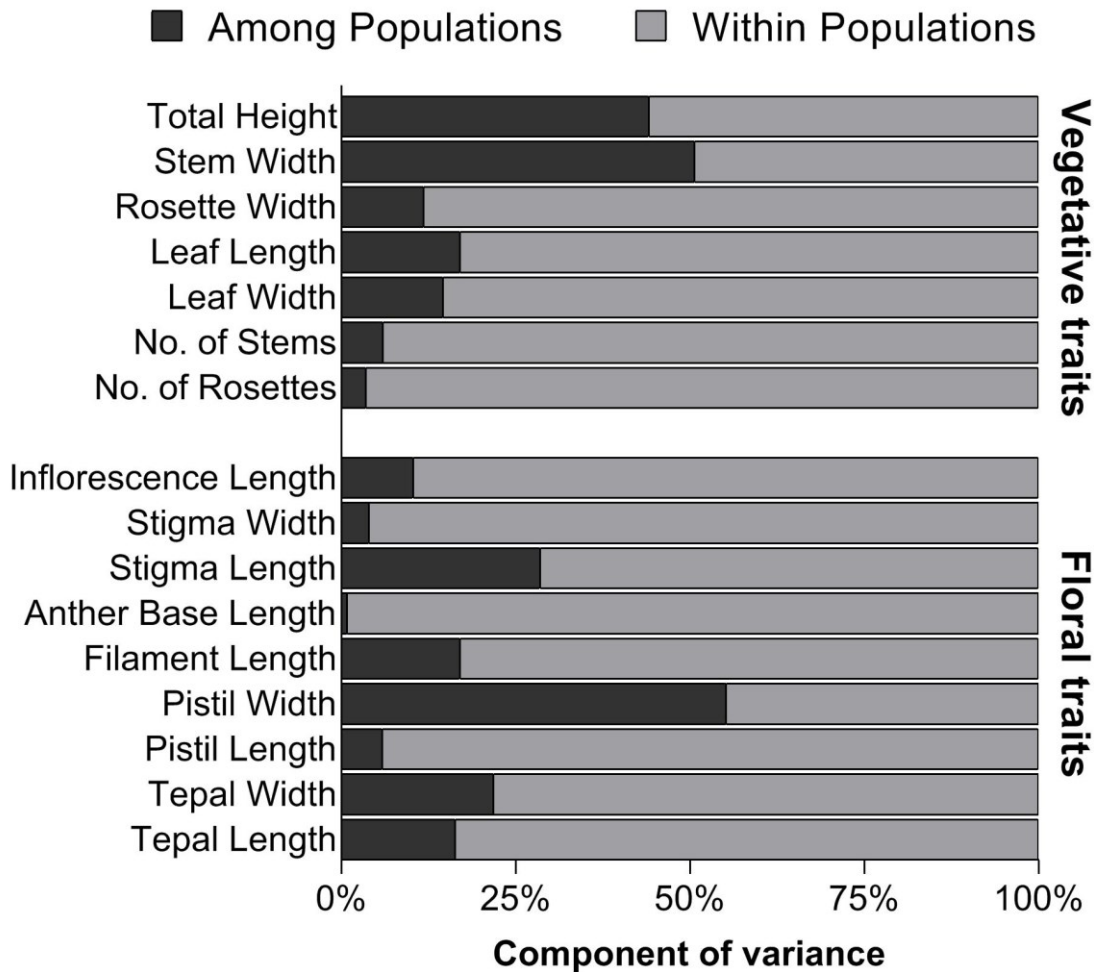
Site	Tepal Length	Tepal Width	Pistil Length	Pistil Width	Anther Filament Length	Anther Base Length	Stigma Length	Stigma Width	Inflorescence Length (cm)
C	44.89 $\pm 0.74$	18.04 $\pm 0.24$	27.08 $\pm 0.36$	9.09 $\pm 0.16$	15.43 $\pm 0.26$	5.05 $\pm 0.10$	3.79 $\pm 0.06$	2.77 $\pm 0.05$	70.53 $\pm 2.09$
D	48.34 $\pm 0.98$	19.48 $\pm 0.30$	27.42 $\pm 0.37$	8.70 $\pm 0.11$	16.53 $\pm 0.33$	4.81 $\pm 0.13$	3.81 $\pm 0.07$	2.75 $\pm 0.05$	79.39 $\pm 2.89$
E	50.67 $\pm 1.09$	18.01 $\pm 0.41$	27.21 $\pm 0.50$	7.91 $\pm 0.13$	16.71 $\pm 0.26$	4.84 $\pm 0.13$	3.79 $\pm 0.07$	2.71 $\pm 0.05$	59.69 $\pm 2.95$
F	49.58 $\pm 1.28$	17.62 $\pm 0.25$	29.73 $\pm 0.38$	8.23 $\pm 0.12$	19.18 $\pm 0.34$	4.67 $\pm 0.12$	3.14 $\pm 0.07$	2.60 $\pm 0.05$	73.16 $\pm 3.41$
G	46.04 $\pm 0.92$	18.17 $\pm 0.36$	28.62 $\pm 0.68$	7.69 $\pm 0.19$	18.35 $\pm$ 0.32	4.97 $\pm 0.15$	3.41 $\pm 0.07$	2.66 $\pm 0.05$	69.14 $\pm 5.63$
H	48.04 $\pm 0.82$	18.63 $\pm 0.24$	29.44 $\pm 0.28$	8.69 $\pm 0.13$	20.14 $\pm 0.36$	4.69 $\pm 0.26$	4.37 $\pm 0.13$	2.75 $\pm 0.09$	68 $\pm 5.21$
I	40.48 $\pm 1.65$	14.76 $\pm 0.39$	27.12 $\pm 0.58$	6.44 $\pm 0.15$	16.14 $\pm 0.39$	5.08 $\pm 0.11$	3.66 $\pm 0.10$	2.48 $\pm 0.06$	76.01 $\pm 2.93$
J	42.53 $\pm 1.22$	17.07 $\pm 0.39$	28.31 $\pm 0.59$	7.05 $\pm 0.12$	17.32 $\pm 0.41$	4.96 $\pm 0.12$	3.53 $\pm 0.10$	2.61 $\pm 0.08$	58.89 $\pm 2.03$
K	38.66 $\pm 1.05$	15.67 $\pm 0.30$	26.32 $\pm 0.51$	7.34 $\pm 0.17$	16.38 $\pm 0.39$	5.20 $\pm 0.17$	3.06 $\pm 0.06$	2.46 $\pm 0.05$	71.33 $\pm 4.02$
L	44.03 $\pm 1.12$	16.80 $\pm 0.33$	29.68 $\pm 0.51$	8.0 $\pm 0.13$	17.59 $\pm 0.39$	5.04 $\pm 0.15$	3.31 $\pm 0.08$	2.59 $\pm 0.05$	64.98 $\pm 3.74$

**Table 7.** Overall mean ( $\pm 1$  SE), coefficient of variation and sample size of eight floral traits registered in 10 *Y. schidigera* populations located across Baja California (Mexico). Floral traits are sorted in descending order of the magnitude of the overall variation.

Trait	Mean (mm)	% CV	N <sub>plants</sub>	N <sub>flowers</sub>
Inflorescence Length	690.2 $\pm$ 11.4	20.71	156	NA
Tepal Length	45.35 $\pm$ 0.39	18.71	149	465
Anther Base Length	4.95 $\pm$ 0.04	18.52	149	465
Stigma Length	3.56 $\pm$ 0.03	17.29	149	468
Pistil Width	7.99 $\pm$ 0.06	16.24	149	468
Stigma Width	2.64 $\pm$ 0.02	15.29	149	468
Tepal Width	17.5 $\pm$ 0.12	14.95	149	465
Pistil Length	27.88 $\pm$ 0.16	12.74	149	468
Filament Length	17.37 $\pm$ 0.46	11.85	149	456

Coefficients of variation of floral traits in *Y. schidigera* showed a lower range than the vegetative traits. The length of the inflorescence stalk showed the highest range of variation for reproductive traits with a % CV= 20.71, and a range of 31.5 cm to 106 cm; this trait was weakly differentiated among populations. Among floral traits, the tepal length was the most variable trait (% CV=18.71) ranging from 22.70 mm to 76.16 mm. The tepal width was less variable than the tepal length, and ranged from 8.8 to 26.55 mm (% CV=14.95). These two traits were moderately differentiated among populations (Fig.8). The stigma length (CV=17.29 %) ranged from 2.14 mm to 5.27 mm in its length, and the stigma width (CV=15.29 %) ranged from 1.4 mm to 4.39 mm across our sample. Over 25 % of the variance documented for the stigma length was distributed among populations (Fig.8). The pistil's length was the least variable trait of the floral structure (CV= 12.74 %; Table 4), but the pistil's width (%CV =16.24) was more variable and over 50% of its variance was distributed among populations. The nested ANOVA models were statistically

significant for all eight floral traits, suggesting differences among populations. However, Tukey HSD post-hoc did not show differences among populations for anther base length and pistil length.



**Figure 8.** Phenotypic variation across southern populations of the distribution range of *Y. schidigera*. Barplots depict the components of variance for the different traits expressed in percentages. Variance proportions were extracted from the ANOVA models, and all models resulted in high statistical significance ( $P < 0.001$ ).

### 3.4 Discussion

Our study found substantial variation in vegetative and reproductive traits of *Y. schidigera*. There was weak but significant population differentiation in both trait groups, which is consistent with the pattern of low genetic differentiation and moderate genetic admixture found in these *Y. schidigera* populations (De la Rosa-Conroy et al., 2019). One explanation for the low genetic and phenotypic differentiation registered is the lack of sufficient time to differentiate, which is a result of a relatively

recent colonization history. Low population differentiation is also the result of homogenization carried out by moderate gene flow among populations. There is evidence that gene flow through pollen and seed dispersal is sufficient to maintain similarities in genotypes (and phenotypes) across populations. Despite the fact that this *Yucca* species shows a discontinuous distribution across its geographic range in Baja California, and restricted gene flow would be expected, vertebrate species with a large home ranges have been reported to carry out long-distance seed dispersal in *Yucca* (Lenz, 2001), meaning that distances between populations may not limit establishment of new migrants from neighboring populations.

Vegetative and reproductive morphometric traits of this study vary accordingly to other studies in *Y. schidigera* (Baldwin et al., 2012; Webber, 1953). However, we noticed differences in leaf length and inflorescence length ranges. The range for the leaf length in this study was 14.9 – 120 cm, while Baldwin et al. (2012) reported larger leaf lengths (30– 150 cm). The length of the inflorescence in our study ranged from 31.5 – 106 cm, which is also a wider range than what is reported in the Jepson Flora (60 – 120 cm). Differences in size ranges between this study could be explained by two reasons: i) differences in sample size, and ii) geographic location of the sampled populations. Sample size in previous studies is lower in relation to this study, possibly resulting in a larger variance than what is reported in this study. Our study included 423 plants sampled from 15 populations, neither of the two studies published that report *Y. schidigera* morphometry specify their sample size. In Baldwin et al. (2012) there is reference to *Y. schidigera* populations located in southwestern San Diego County and the Mojave Desert (both in California), in contrast, our sample was mostly collected from populations in the Baja California peninsula.

Even if different age classes within populations could account for a fraction of the variance registered on vegetative features, we only included mature plants for this study, thus, variation associated to age should be controlled to a certain degree. Of course, the age of the individual gradually increases traits such as plant height and stem diameter, but traits related to leaf size and floral architecture should be less affected by the plant's age.

Our findings indicate that vegetative traits are more variable than floral traits in this species, this suggests that vegetative and floral phenotypes respond differently to environmental conditions. Phenotypic variation has been documented for other species in *Yucca*, for example, Arteaga et al. (2015) conducted a study in *Y. capensis*, which is a subtropical species restricted to the cape region of the Baja California Peninsula. In their study, Arteaga et al. (2015) showed that stem length, and rosette diameter

had large variation, whereas foliar traits were the least variable characters for that particular species. Our results in populations of *Y. schidigera* partially resemble the patterns of phenotypic variation observed in *Y. capensis*, suggesting that low variation in foliar traits is conserved through peninsular *Yucca* species. An explanation for this is that variation in stem traits are the result of an interaction between different abilities of plant growth (e.g., different genotypes) and environmental conditions that promote growth (e.g., water availability, temperature). Lower levels of variation observed on leaf traits could reflect selection for features that increase efficiency in biomass investment by the plant, which can be related to a physiological response to hydric stress, which is frequent for plant species distributed in Mediterranean climate (Verdú et al., 2003) such as chaparral species present in the Baja California peninsula (Axelrod, 1978).

Variation of the floral phenotype of *Yucca schidigera* was substantially lower in relation to most vegetative traits. Flowers are commonly under the selective pressure of pollinators, thus, floral phenotypes that match features which promote fitness on its pollinators, will be positively selected, reducing the amount of variation in flower traits. Consequently, selective pressures exerted on floral traits by the pollinator species *Tegeticula mojavelle* and *T. californica* should represent a strong component limiting floral morphometry on *Y. schidigera* flowers. For example, Godsoe et al. (2008) showed that floral trait specialization evolved dramatically far more rapidly than vegetative features within two subspecies of *Y. brevifolia* that occur sympatrically in the Mojave Desert. Evidence from Godsoe et al (2008) and Smith et al. (2009) show that phenotypic matching in highly specialized pollination systems, such as the one between yucca plants and yucca moths, can reach great extents and become highly specialized, supporting the idea that coevolution, rather than environmental factors, may drive the evolution of traits involved in an interaction. The high specificity in the mechanism of pollination of the moth explains the highly conserved level of variation in flower traits of *Yucca* plants (Darwell and Althoff, 2017), reflecting the way strong selection imposed on the size and architecture of the flower reduces the frequency of plant phenotypes with large and small flowers.

The action of natural selection over populations largely depends on the heritable variation which populations carry to respond to pressures abiotic and biotic environment. The phenotype of organisms develops in a concerted manner in order to function optimally so that organisms are able to adaptively grow in their environment. In this sense, groups of traits which exert different functions would not be expected to share similar degrees of morphological variation. A broad range of functions involve phenotypic traits, while some roles are expected to show a certain degree of relationship (e.g., reproduction and growth), other group of traits are definitely not linked, for the reason that they can

independently respond to different pressures acting on their environments (Rundle et al., 2000). As genetic variation expresses on the phenotype, natural selection will assign different evolutionary trajectories to different populations.

## **Chapter 4. Population variation in the intensity of fruit infestation and pre-dispersal seed predation in *Yucca schidigera* (Asparagaceae) by its obligate pollinator.**

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### **4.1 Introduction**

Variation in the occurrence and the intensity of insect-plant interactions is common in natural ecosystems. Understanding the nature of factors that influence the spatial structure of such interactions is central to the study of ecology and evolution simply because insects have been an important driver of the genetic and phenotypic diversity in plant populations and species (Farrell et al., 1992; Weber and Agrawal, 2014)). Interactions like pollination and indirect defense have beneficial effects for plants, whereas antagonistic interactions such as herbivory and pre-dispersal seed predation are accompanied by negative impacts on plant fitness (Katz, 2016; Kolb et al., 2007). These antagonistic and mutualistic interactions have shown ample variation in their intensity across populations (Castillo et al., 2014; Thompson and Cunningham, 2002; Toju and Sota, 2006), generating geographic mosaics of fitness costs and benefits for the species involved (Thompson, 2005). For example, the detrimental cost of pre-dispersal seed predation on plant populations has been found to be temporally and spatially heterogeneous (Borchert and DeFalco, 2016; Dimitri et al., 2018; Leimu et al., 2002). In part, local conditions such as the abundance of plants, pollinators, and seed predators likely influence the intensity of species interactions.

Many plant species are involved in interactions with pollinators that act as pre-dispersal seed predators (Kato and Kawakita, 2017). However, pollination and pre-dispersal seed predation exert opposite effects on plant fitness. While pollination positively influences plant fitness by increasing the number of fertilized seeds, pre-dispersal seed predation reduces seed production, and can even alter germination and seedling survival during early stages of growth (Han et al., 2018; Koptur, 1998). In this sense, seed predation reduces plant fitness by affecting population growth (Katz, 2016). The simultaneous effects of pollination and seed predation on plant fitness can be difficult to quantify and the complexity of the effects of are not equally predictable across populations. Documenting variation in the strength of a highly host-specific interaction by measuring seed predation and analyzing the influence on fitness of both participants in several populations has been suggested as an advantageous approach to understand how fitness costs can shape insect-plant interactions (Bronstein, 2001).

In particular, studies of pre-dispersal seed predation have found that the intensity of infestation and seed predation are accompanied by different costs across populations (Leimu and Lehtilä, 2006).

Variation in the magnitude of the cost of pre-dispersal seed predation has been found to depend on the efficacy of pollination service. For example, seed production can be limited if pollinator abundance is low and/or the amount and type of pollen reduce the probability of ovule fertilization (Ashman et al., 2004; Baer and Maron, 2018). In a scenario where pollen limitation results in plants with reduced seed production, the negative impacts of seed predation would increase in relation to plants with larger seed production. In terms of fitness, the cost for a plant that produces a low number of seeds would be higher in relation to a plant that can produce a larger number of seeds (Boege and Domínguez, 2008). Moreover, this condition would occur even when plants show similar infestation rates by seed predators (Bello-bedoy et al., 2011). Thus, differential production of fertile and infertile seeds can influence the potential cost of pre-dispersal seed predation.

As all other *Yucca* species, *Y. schidigera*, is engaged in an obligate mutualism two species of yucca moths of the genus *Tegeticula* (Prodoxidae). In this system, adult female moths pollinate yucca flowers by actively pushing pollen into the stigma after ovipositing into the ovary (Riley, 1982). During pollination, the female moth lays its eggs, and latter hatching larvae feed on a fraction of the developing seeds. To prevent overexploitation by the pollinating moth, plants selectively abscise flowers with a relatively large number of eggs (Marr and Pellmyr, 2003; Shapiro and Addicott, 2004; Wilson and Addicott, 1998) and flowers that receive low amounts of pollen (Pollination quality; Pellmyr & Huth, 1994). The number of fertile seeds is expected to increase with the number of pollinator visits, which would confer fitness benefits for the plant. However, with each visit the moth will oviposit in the flower's ovary, increasing the potential number of seed-consuming larvae, and reducing the probability of flower retention (Csotonyi and Addicott, 2001; Pellmyr and Huth, 1994). Thus, hatching larvae feed on a fraction of the seeds that develop within the fruit, reducing the benefits of pollination (Pellmyr and Huth, 1994).

Despite the strong co-dependence between *Yucca* and its pollinator, studies quantifying the cost of mutualisms throughout pre-dispersal seed predation in *Yucca* have found differences in the intensity of fruit infestation and pre-dispersal seed predation when they compare between and within species (J. F. Addicott, 1986; Keeley et al., 1984; Pellmyr and Huth, 1994; Ziv and Bronstein, 1996). Only a few studies have examined infestation and pre-dispersal seed predation in multiple plant populations of the same *Yucca* species (Dodd and Linhart, 1994; Harrower and Gilbert, 2018), limiting our understanding of the ecological and evolutionary dynamic processes driven by the cost seed consumption. Increasing the number of populations across heterogeneous landscape allows to evaluate the degree of variation in the cost of pre-dispersal seed predation for different *Yucca* populations and predict whether these differences are relevant for demographic or evolutionary processes of populations.



In this study, we examined the variation in the intensity of fruit infestation and pre-dispersal seed predation in multiple populations of *Y. schidigera* and *Tegeticula mojavella* to examine the magnitude of the cost for the plant. Seed predation and its cost were quantified by obtaining the number of unfertile and fertile damaged seeds. Likewise, we examined variation of *T. mojavella* infestation over *Y. schidigera* fruits by counting the number of moth larvae within each yucca fruit. Finally, we discuss the evolutionary consequences of variation in seed predation for the maintenance of mutualism.

To examine the variation in the cost of pre-dispersal seed predation on fertile seed production, we asked the following specific questions:

- i. Does production of fertile and infertile seeds vary between populations?
- ii. Do fruit infestation rates and pre-dispersal seed predation vary among plant populations?
- iii. Are seed mass and germination rates correlated with the strength of the interaction?

## 4.2 Methods

### 4.2.1 Study species

Mojave yucca, *Yucca schidigera* Roezel (Asparagaceae), is a long-lived caulescent, woody monocot that is native to the Mojave desert, fringes of the Sonoran desert in Arizona, California and Baja California, and chaparral throughout the coastal foothills and mountains of southern California and Baja California. Mojave yucca populations occur at altitudes of 100 m to ~2000 m, from coastal bluffs to interior deserts, and within a latitudinal range that extends from 30° to 36° N (Turner et al., 2005; Fig.10).

In *Y. schidigera*, flowering occurs between February and May. During this period, plants produce large panicle inflorescences with as many as 500 flowers. Fruits are indehiscent capsules that complete maturation three to four weeks after being fertilized (Baldwin et al., 2012). This plant is pollinated by two moth species, *Tegeticula mojavella* and *T. californica* (Lepidoptera: Prodoxidae; Pellmyr, 1999). Reports of *T. californica* are mostly restricted to low altitudes sites within a 40 km coastal stretch in southern California (Althoff et al., 2006; Pellmyr et al., 2008). By means of molecular genotyping (850 bp, mtDNA, n=50), we detected exclusively *T. mojavella* moths in our sample. *Yucca* plants are self-compatible, and although pollen transfer within a same inflorescence has been registered to be frequent (55 % of total

observations,  $N=270$ ; Marr et al., 2000), self-fertilized flowers are more likely to be aborted than flowers that were outcrossed (Huth & Pellmyr, 2000).

#### 4.2.2 Sampling effort

We studied nine populations of *Y. schidigera* distributed in northwestern Baja California (Fig.10). These locations have a semiarid, Mediterranean climate, and are located at altitudes of 850 - 1,950 m (Table 8), with an annual regional precipitation mean of  $234.7 \text{ mm} \pm 78.2 \text{ SD}$  (data from the WorldClim2 dataset; Fick & Hijmans, 2017). Precipitation annual averages during the winters of 2013, 2014 and 2015 were lower (58.5, 88.3 and 32.9 mm, respectively) than the 30 year average for this region (rain data taken from the CONAGUA weather station in Ojos Negros, Baja California).

In March 2015, we marked 15 flowering plants at each site during the flowering season. We registered latitude and longitude for each population using a GPS (Garmin eTrex 10). We registered plant density at each site by counting the number of *Y. schidigera* individuals within two adjacent 30 m x 50 m quadrats and then estimating the mean. Densities ranged from 28 to 129.5 ind./ha across all nine populations (Table 8). In late August, when fruits were fully ripe, we visited each site and counted the number of fruits produced by the marked plants. From each plant, we collected a sample of 10 fruits, whenever possible. To avoid losing any larvae during transportation, we placed the fruits in individual plastic cups that we covered with nylon mesh. The plastic cups were labeled to maintain population and plant identity. A total of 650 fruits belonging to 73 plants were collected from the nine populations. In the laboratory, we dissected the fruits and registered the number of *Tegeticula* larvae within each fruit. By examining the exocarp, we also registered the number of perforations bored by the larvae when escaping mature fruits; each exit hole was scored as a surrogate of a larva that developed within the fruit.

**Table 8.** Latitude, elevation (masl—meters above sea level), climatic variables (Ann. Ppt.—Mean annual precipitation; Ann. Temp.—Mean annual temperature), vegetation type (after González-Abraham et al., 2010), number of sampled plants (n), and plant density of nine *Yucca schidigera* populations in Baja California.

Site	Latitude (°N)	Altitude (masl)	Ann. Ppt. (mm)	Ann. Temp. (°C)	Vegetation Type	n	Plant density (ind./ha)
A	32.5129	1,330	268	30.6	Chaparral	8	66.5
B	32.4094	1,285	292	29.5	Pinyon-juniper Woodland	11	49
C	32.3440	1,421	331	29.0	Pinyon-juniper Woodland	8	28
D	31.9135	1,206	335	30.0	Chaparral	7	45.5
E	31.9030	786	301	33.3	Chaparral	3	52.5
F	31.2088	1,199	258	29.8	Chaparral	14	129.5
G	31.1636	1,254	265	29.5	Chaparral	10	105
H	31.0412	883	237	31.1	Coastal Sage Scrub	9	28
I	30.9655	1,916	336	26.1	Chaparral	3	42

#### 4.2.3 Seed number

We registered the number of fertile and infertile seeds per fruit. Fertile seeds have a black seed coat, whereas infertile seeds are white and lack endosperm. We quantified the number of intact and damaged seeds per fruit. Pre-dispersal seed predation was analyzed using fertile seeds only, and to have an indicator of the cost imposed on the plant by the yucca moth larvae, we calculated the proportion of damage on fertile seeds ( $d$ ) as follows:

$$d = \frac{\text{damaged fertile seeds}}{\text{total fertile seeds}}$$

#### 4.2.4 Seed mass and germination rate

We registered seed mass and germination rate in a subset of 55 fruits belonging to eight populations (3 - 6 plants per population), with 20 seeds per fruit ( $N_{\text{seeds}} = 940$ ). Each seed was weighed to the nearest 0.1 mg using an analytical balance (Mettler-Toledo, Columbus, OH, USA). To assess germination rate, we sowed all seeds ~3 cm deep in plastic trays filled with BM2 soil (Berger, Quebec, Canada) maintaining the identity of each seed (e.g. fruit, plant and population number). Trays were kept inside a greenhouse at environmental conditions and the plantings were soaked daily. We conducted observations every 24 h and scored germination when the radicle was visible at the soil surface.

#### 4.2.5 Statistical analyses

To assess differences in yucca moth infestation, seed production and pre-dispersal seed predation across populations, we carried out an independent nested ANOVA for each variable. These models included the fixed effects of the site and the plant nested within its population to control individual variation in each variable. To examine the effect of larvae on seed production, we included the number of larvae per fruit as a covariate of the population. To test whether infestation by *T. mojavella* larvae affects seed mass and germination, we conducted independent ANCOVA models, including the fixed effect of the site, the plant nested within its population and each plant's mean number of larvae per fruit as a covariate. We fitted mean seed mass per fruit and the proportion of seeds that germinated after a 60-day trail as separate response variables. Seed damage ratio and germination rate were log transformed before conducting further statistical analysis (Warton et al., 2011). In all cases, a post-hoc HSD Tukey test was used to compare means among populations, and we used an alpha level of 0.05 for statistical significance. Finally, we conducted independent linear regression of mean infestation and mean seed predation as response variable on latitude and altitude. Statistical analyses were performed with JMP 10 (SAS Institute, Cary, NC, USA).

## 4.3 Results

### 4.3.1 Variation in seed production

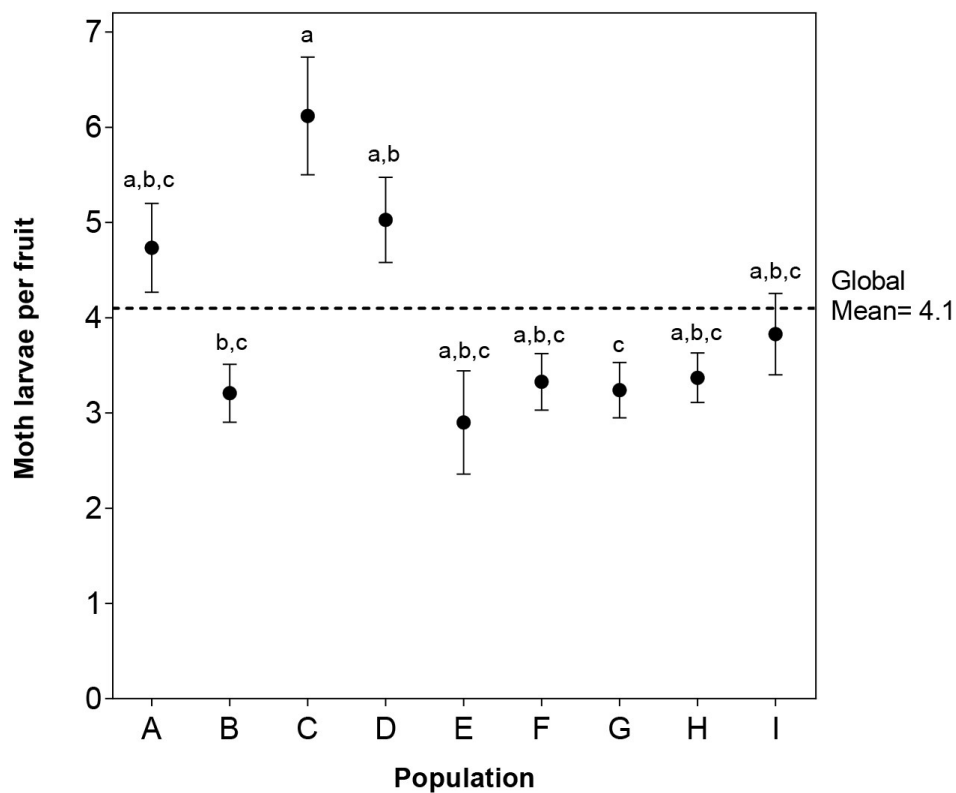
Population means for total seed production per fruit ranged from  $53.10 \pm 2.55$  SE to  $72.48 \pm 3.19$  SE across populations. The total number of seeds per fruit differed among populations ( $F_8 = 8.60$ ;  $P < 0.0001$ ,  $N = 650$ ; Table 9) and within populations ( $F_{64} = 11.19$ ,  $P < 0.0001$ ). Likewise, the number of fertile seeds and infertile seeds showed significant differences among plant populations (fertile:  $F_8 = 21.7$ ,  $P < 0.0001$ ; infertile:  $F_8 = 51.95$ ,  $P < 0.0001$ ; Table 9) and among individuals within populations (fertile:  $F_{64} = 11.53$ ,  $P < 0.0001$ ; infertile:  $F_{64} = 9.81$ ,  $P < 0.0001$ ). The mean number of fertile seeds in non-infested fruits was  $34.98 \pm 2.48$  SE ( $N=73$ ), and ranged from  $15.25 \pm 3.5$  SE to  $59.66 \pm 12.73$  SE across populations. In turn, the mean number of fertile seeds across infested fruits was  $46.86 \pm 1.05$  SE ( $N=577$ ), and ranged from  $33.95 \pm 2.47$  SE to  $60.55 \pm 3.13$  SE across populations.

**Table 9.** Population-level variation in seed production and seed predation in nine *Y. schidigera* populations in Baja California. Columns 3—8 report the number of seeds per fruit (population averages  $\pm 1$  SE). Column 8 shows the proportion of fertile seeds within each fruit damaged by yucca moth larvae.

Site	Fruits (n)	Total	Infertile	Fertile	Fertile Intact	Fertile Damaged	Cost (d)
A	89	71.84 $\pm$ 3.26	22.88 $\pm$ 1.56	48.88 $\pm$ 2.45	38.70 $\pm$ 2.59	10.19 $\pm$ 0.96	0.26 $\pm$ 0.02
B	87	64.63 $\pm$ 3.18	13.28 $\pm$ 0.73	51.34 $\pm$ 2.76	46.40 $\pm$ 2.58	4.95 $\pm$ 0.42	0.12 $\pm$ 0.01
C	67	67.72 $\pm$ 2.59	19.00 $\pm$ 1.25	48.71 $\pm$ 2.59	41.47 $\pm$ 2.40	7.23 $\pm$ 0.78	0.15 $\pm$ 0.01
D	74	72.49 $\pm$ 3.20	31.55 $\pm$ 1.89	40.49 $\pm$ 2.35	33.35 $\pm$ 2.17	7.79 $\pm$ 0.81	0.21 $\pm$ 0.02
E	20	65.35 $\pm$ 3.86	22.6 $\pm$ 2.78	42.75 $\pm$ 4.39	39.10 $\pm$ 4.58	3.84 $\pm$ 0.61	0.11 $\pm$ 0.02
F	105	66.75 $\pm$ 3.19	28.85 $\pm$ 1.91	37.89 $\pm$ 2.13	33.42 $\pm$ 2.14	4.98 $\pm$ 0.65	0.18 $\pm$ 0.03
G	96	53.10 $\pm$ 2.55	22.27 $\pm$ 1.72	30.83 $\pm$ 2.25	25.96 $\pm$ 2.06	5.13 $\pm$ 0.54	0.18 $\pm$ 0.02
H	81	64.83 $\pm$ 3.22	4.40 $\pm$ 0.64	60.41 $\pm$ 3.12	55.08 $\pm$ 3.07	6.35 $\pm$ 0.68	0.12 $\pm$ 0.01
I	31	62.00 $\pm$ 4.70	4.32 $\pm$ 0.88	58 $\pm$ 5.08	49.16 $\pm$ 4.00	9.8 $\pm$ 1.81	0.15 $\pm$ 0.02
Mean	72.22	65.39 $\pm$ 1.21	19.86 $\pm$ 0.64	45.53 $\pm$ 0.98	39.22 $\pm$ 0.94	6.66 $\pm$ 0.27	0.16 $\pm$ 0.01

### 4.3.2 Fruit infestation

We registered *Tegeticula mojavella* larvae in 100% of plants and in 85.5% of the total fruit sample (N=650). The number of larvae found within the fruits ranged from 0 to 26 and the grand mean was  $4.06 \pm 0.14$  SE. Across populations, mean number of larvae per fruit ranged from  $2.9 \pm 0.541$  SE to  $6.11 \pm 0.61$  SE (Fig.9) and larvae infestation differed between populations after controlling individual variation within populations ( $F_{8, 64} = 5.81$ ,  $P < 0.0001$ ). Plant density did not correlate with the level of infestation ( $r=-0.36$ ,  $P=0.33$ ).

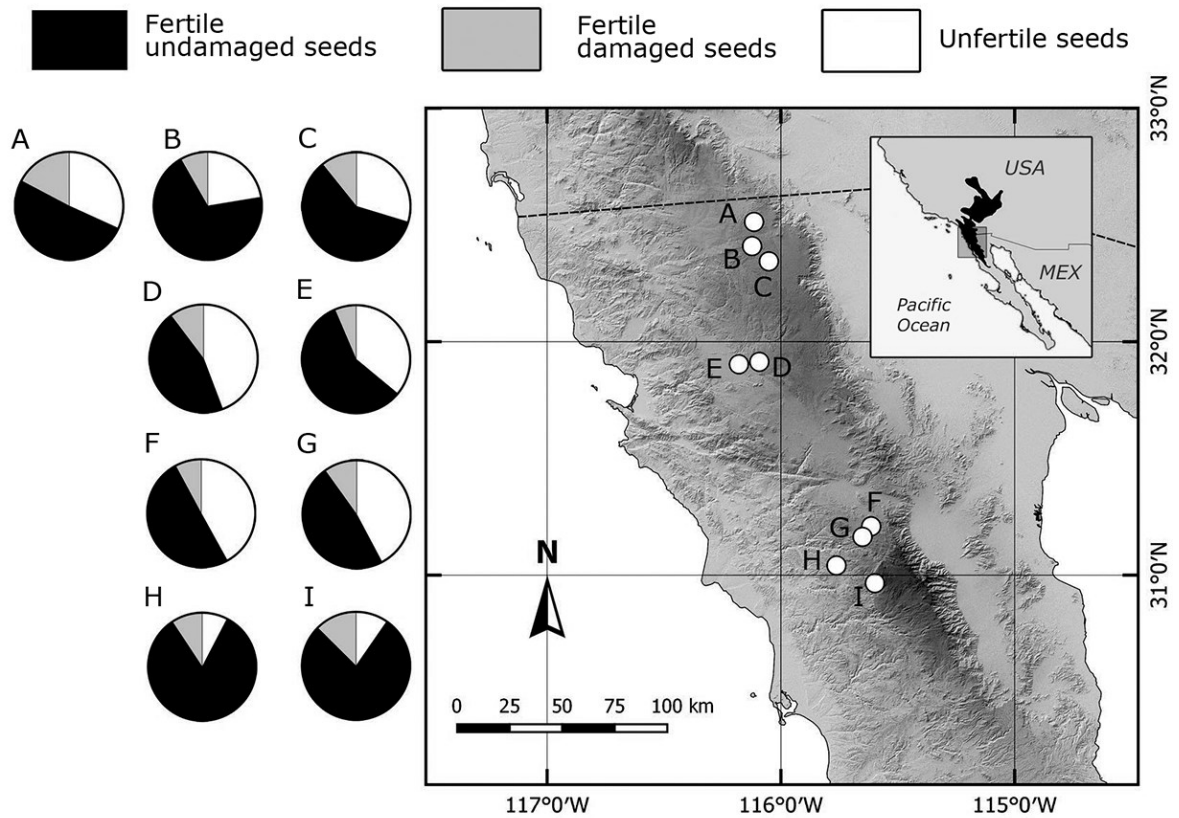


**Figure 9.** Variation in yucca moth larvae infestation in nine populations of *Y. schidigera* (Mean  $\pm$  1 SE). Letters (smallcaps) show TUKEY HSD test groups of the nested Plant(Population) ANOVA model.

### 4.3.3 Pre-dispersal seed predation

The nested ANCOVA detected variation across populations in the number of fertile seeds damaged by *T. mojavella* larvae. Mean number of fertile damaged seeds per fruit differed between populations ( $F_{8, 64} = 6.23$ ,  $P < 0.0001$ ,  $N = 650$ ; Table 10) and within populations ( $F_{8, 64} = 2.51$ ,  $P < 0.0001$ ; Table 10). Plant density did not correlate with seed predation ( $r=-0.12$ ,  $P=0.73$ ). Moreover, the interaction between

population and the number of larvae was statistically significant ( $F_8 = 8$ ;  $P < 0.024$ ; Table 10), indicating that the effect of seed damage differed between populations, even at similar levels of fruit infestation. The proportion of damaged fertile seeds ( $d$ ) ranged from 10% to 26% across populations (Table 10; Fig.10), indicating that the cost of seed predation is variable among populations.



**Figure 10.** Geographic variation of pre-dispersal seed predation in nine *Y. schidigera* populations in Baja California. Pie charts depict population averages of the proportion of seeds per fruit for: fertile seeds that escaped predation (black), damaged fertile seeds (grey) and the sum of infertile seeds (white). Inset map shows the distribution range of *Y. schidigera* (modified from Turner et al., 2005).

**Table 10.** Nested ANOVA and nested ANCOVA models for seed production and damage on fertile seeds by the *Tegeticula* larvae. Nested ANCOVA model for damaged seeds included larvae number and the Population x Larvae number interaction.

Trait	Source of variation	DF	MS	F	P
Mean total seed production	Population	8	27045.39	8.60	<0.0001
	Plant <sub>[Population]</sub>	64	280579.28	11.15	<0.0001
Mean fertile seed production	Population	8	46804.61	21.70	<0.0001
	Plant <sub>[Population]</sub>	64	198919.14	11.53	<0.0001
Mean infertile seed production	Population	8	42421.22	51.95	<0.0001
	Plant <sub>[Population]</sub>	64	64083.18	9.81	<0.0001
Mean damage on fertile seeds	Population	8	1154.64	6.23	<0.0001
	Plant <sub>[Population]</sub>	64	3715.34	2.51	<0.0001
	Larvae number	1	1704.96	73.59	<0.0001
	Population × Larvae number	8	413.31	2.23	0.024

#### 4.3.4 Seed mass and germination

Seed mass ranged from 22.3 mg to 276.1 mg and averaged 110.95 mg  $\pm$  1.08 SE across all populations. Overall germination rate was 32.68% and population germination rates ranged from 0.71% for site D, up to 90% for site I. Seeds began germination after 14 d, and out of the 353 seeds that germinated, 80% did so during the first 28 d. Mean seed mass and germination rate of fertile undamaged seeds were significantly different between populations (seed mass:  $F_7 = 6.18$ ,  $P = 0.01$ ; germination rate:  $F_7 = 10.28$ ,  $P < 0.0001$ ). However, mean number of larvae per fruit did not affect mean seed mass or germination rate (mean seed mass:  $F_1=2.73$ ,  $P=0.13$ ; germination rate:  $F_1=0.12$ ,  $P=0.72$ ).



## 4.4 Discussion

In this study, we quantified seed production by *Yucca schidigera* and seed predation by consumption of its obligate pollinator, *Tegeticula mojavella*, across their distribution in Baja California. Our data found significant variation in the proportions of fertile and infertile seeds produced in fruits across populations suggesting that plants experienced variation in pollination environments in each site. Also, we found that the number of moth larvae developing in the fruits and the proportion of seeds consumed by yucca moth larvae differed among populations (Fig.9; Fig.10). Damage on fertile seeds caused by the larvae varied between populations, even at similar infestation levels (i.e., significant population x larvae interaction). Although variation in infestation intensity was relatively small, the number of damaged seeds across *Yucca* populations resulted in differences in the cost of seed predation (Fig.10). This heterogeneous fitness cost for the plant, suggests that larvae might exert natural selection of different magnitude among populations. We did not find differences in seed mass and germination rate in seeds derived from fruits with different level of infestation by larvae of the yucca moth. Altogether, our data suggest that interplay between the beneficial effects of pollination and the antagonistic effects of seed predation can account for the mutualism costs at the population level.

Our study found large differences in the production of fertile and infertile seeds across populations. Variation in infertile seed production could be explained by differences in the abundance of pollinators and/or in the quality of pollen available for pollination (Ashman et al., 2004). Studies in *Yucca filamentosa* have shown that manually pollinated flowers with selfed and low amounts of pollen resulted in lower fruit retention in relation to flowers pollinated with abundant and outcrossed pollen (Huth and Pellmyr, 2000). These results suggest that environments with low pollinator efficacy can reduce an individuals' seed production. Furthermore, our sampled populations occur in different environmental conditions, and this environmental heterogeneity could reflect in differences in pollinator abundance across populations.

We found significant differences in the rate of yucca moth larvae infestation across *Y. schidigera* populations. The level of infestation found in this study was considerably larger in relation to reported values for other *Y. schidigera* populations. For instance, Keeley et al. (1984) reported a mean of 0.6 larvae per fruit. In contrast, we found 4.06 larvae per fruit. Such difference in the level of infestation can be explained by differences in the moth's population density, as well as differences in oviposition rates. As is the case in several insect-plant systems, host plant density can influence for population-level differences in infestation intensity (Harrower and Gilbert, 2018; Skoracka et al., 2017). However, plant

densities in our populations were not associated to infestation or to fertile seed damage, ruling out the role of plant density.

Another explanation for variation in the number of larvae is that regulatory mechanisms that operate to limit yucca moth densities vary among populations. For instance, flower abscission is a common mechanism employed by *Yucca* plants to prevent overexploitation. Plants avoid opportunistic behaviour of pollinators by abscising flowers which ovaries have been damaged by an excessive number of ovipositor insertions (Marr and Pellmyr, 2003), and by selectively aborting developing fruits with heavy egg loads (Pellmyr and Huth, 1994; Wilson and Addicott, 1998). However, a question is whether plants from all populations are equally sensitive to ovary damage and oviposition. In our study, three populations had large larvae numbers in relation to the rest (Fig.2). It is possible that differences in moth infestation result from different abilities across plants to tolerate ovipositor damage. These three populations are candidates to test hypotheses of tolerance to oviposition in field experimental manipulation.

Pre-dispersal seed predation in *Y. schidigera* differed within and between populations. Overall, seed predation on fertile seeds was higher in relation to previous values previously reported for this species (Keeley et al., 1984). Two factors can explain variable rates of seed predation: first, large seed predation rates observed in this study are associated to a high number of larvae per fruit; and second, larvae could consume different amounts of seeds in each population, as indicated by a significant population  $\times$  larvae interaction. Thus, when comparing among fruits with similar number of larvae, *Yucca* populations experienced seed predation differently. Studies in other *Yucca* species have reported similar results, suggesting that variation in consumption rate by seed predators is not a rare event (Dodd and Linhart, 1994; Harrower and Gilbert, 2018). Some populations would experience higher costs even with similar levels of infestation, partially explaining variability in the cost of pre-dispersal seed predation.

In regard of the evolutionary implication of variation in pre-dispersal seed predation, our data demonstrated that individuals within populations and between populations suffered different costs of seed predation, suggesting that natural selection might operate on these fitness-related traits if they are genetically based. Studies have demonstrated that natural levels of damage by pre-dispersal seed predators can select for structural traits, such as plant size (Kolb et al., 2007) or features related to the fruit (Toju and Sota, 2006). *Yucca* plants interact with yucca moths through flowers, the developing fruit and seeds, so selection exerted by *T. mojavella* should favor the evolution of reproductive structures that make the plant a better host for the moth's larvae. Studies in *Yucca* species have reported that different

moth species have preferences to oviposit eggs in different parts of the ovary (Pellmyr and Leebens-Mack 2000), and this behavior has evolved more than once during the evolution of the interaction (i.e., in the evolutionary transition from seed predator to a complete mutualism, and to parasitism in some cases).

Differences between individuals and populations in fruit infestation and seed predation intensities in our study suggest that selection could promote phenotypic changes in traits that maximize the beneficial effect of pollination, while efficiently regulating larvae density (even plant tolerance) to minimize seed loss (i.e., plant fitness). Thus, a study of the selective role of damage caused by pollinators and seed predators in the phenotypic evolution *Y. schidigera* flowers, fruits and seeds could reveal if selection varies across populations.

Our results demonstrate that the intensity of infestation and pre-dispersal seed predation by *T. mojavella* can differ between *Y. schidigera* populations. Even if most populations showed similar levels of infestation, the fruits collected from three populations bore more larvae than the rest of the sites. This suggests that overexploitation is controlled in most populations, but plants from other populations can be more tolerant to ovipositor damage and host more larvae per fruit. In addition, our data demonstrated that individuals within populations and between populations suffered different costs of seed predation. It is possible that natural selection might operate on fitness-related traits. Further studies in several populations in the *Y. schidigera*—*T. mojavella* system should examine whether variability in the cost of seed predation exerts selective pressure in reproductive traits, generating a complex geographic mosaic of coevolution.

## Chapter 5. General discussion

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Genetic variation, a basic unit of biodiversity and the evaluation of its geographic distribution of is a fundamental aspect of species conservation (Frankham et al., 2002). Documenting population genetic variation is of special importance in species with populations that have been subject to human harvesting because extraction of individuals can perturb genetic subdivision across populations (Allendorf et al., 2008). Conservation Genetics has emerged as a multidisciplinary research focused on studying the genetic makeup of species to understand and preserve their evolutionary potential. In addition to concern for effective population sizes, conservation strategies have started to implement actions based on the amount of genetic variation in wild populations. In this sense, neutral genetic variation is used to evaluate in broad terms the “*genetic health*” of populations because its estimates reveal aspects of the microevolutionary dynamics experienced by a species (Fox et al., 2001; J. Futuyma, 2009). Also, in many organisms, genetic variation is correlated with population fitness (Reed and Frankham, 2003) and can represent a significant component of the evolutionary potential of the species of interest. The analysis of variation across a species’ phenotype adds an additional dimension to the matrix of factors endured by a species along its evolutionary history. This is because the phenotype determines the ability of individuals to successfully interact with their environment. Ecological interactions, in turn, can affect how populations establish, grow and adapt to their environment (Thompson, 2005).

In my thesis, we analyzed three aspects of biological variation across Baja California populations of *Yucca schidigera*. These three lines of biological diversity are genetic variation, phenotypic variation and a number of reproductive and ecological traits associated with the pollination mutualism of *Y. schidigera*. These three components of biological variation can determine a large amount of a species evolutionary potential. By quantifying variation along these three axes, I conducted an integrative analysis of how biological variation is partitioned among *Y. schidigera* populations. Influenced by different processes, at different temporal scales, these three aspects stem from the most fundamental feature of a species: genetic traits. Because these traits are expressed through the phenotype, natural selection will act differentially over genotypes, which then will interact at the ecological realm, in particular during species interactions. Because *Y. schidigera* is involved in a highly specific mutualism with its pollinators, a great fraction of *successful* traits (e.g., plant features that will be selected) respond to benefits related to the interaction with the *Tegeticula* moths. We were able to assess variation in reproductive traits and we interpreted our results in the context of the pollination/seed predation mutualism with the yucca moths.

Some literature studies suggest that obligate pollination/seed predation mutualisms are among the tightest, most finely specialized interactions on Earth (Kato and Kawakita, 2017). Although the theory of the factors spatially shaping this type of interactions is relatively recent (e.g. geographic mosaic of co-evolution; Thompson, 2005), there are detailed eco-evolutionary studies of the *Yucca-yucca* moth system (for instance, Addicott, 1998; Althoff et al., 2006; Pellmyr & Huth, 1994; Pellmyr & Leebens-Mack, 1999, among others) and key aspects of the regulation of its mutualism have now become clear. This gives us solid context to interpret and understand *Y. schidigera* populations.

Throughout the three main chapters of this work, we found our results consistent with the existing *Yucca-yucca* moth literature, and the aspects of biological diversity that we studied revealed a similar pattern among *Y. schidigera* populations: low genetic and phenotypic differentiation among populations together with high genetic variability within populations, suggesting high connectivity across *Y. schidigera* distribution.

The ecological data offered a detailed picture about the structure of the mutualism across neighboring populations. Key features of the interaction were differentiated among populations but held no relationship with geography. We argue that this reflects the aspects of the mutualism that occur locally within populations. For example, regulation of excessive seed predation by intrinsic mechanisms such as flower abscission as has been shown to be common in *Yucca* (J. F. Addicott and Bao, 1999; Pellmyr and Huth, 1994), density-dependent effects of the pollinator or ecological factors related to the arthropod community that uses *Yucca* inflorescences during flowering season. However, these are all factors that need to be investigated over extensive periods.

Analysis of the molecular data in conjunction with historical projections of the climate in the peninsula led understanding the biographical history of *Y. schidigera*. By using ecological niche models projected to three different times during the Quaternary, we were able to support the hypothesis of that *Yucca schidigera* populations colonized the northern extent of the Baja California peninsula during the Pleistocene. Although we lacked paleoecological records to support this claim, the genetic data suggested a pattern that our climatic models supported. The fact that a broad extent of suitable habitat could have been available for this species during the LGM is consistent with the findings of Rhode (2002) and Wells (2000) showing the presence of temperate chaparral and woodland vegetation between the 28<sup>th</sup> and 30<sup>th</sup> parallels in what is currently Baja California's mid-peninsular desert. Our results are also supported by genetic evidence that shows other long-lived plant species from the Baja California peninsula expanding their distribution during the Pleistocene (Clark-Tapia and Molina-Freaner, 2003;

Garrick et al., 2009; Gutiérrez-Flores et al., 2016; Nason et al., 2002). We attempted preliminary genetic analyses using slower-evolving markers during early stages of this project but the chloroplast coding regions we used were only slightly polymorphic in *Y. schidigera*. Future exploration of genomic variation would allow a robust phylogeographic analysis, which would shed more light on the colonization process of *Y. schidigera* populations.

A species' geographic distribution reflects evolutionary responses to spatial and ecological habitat variation even in the absence of geographic barriers. Features like dispersal biology or the length of life-history stages affect the strength of population differentiation across the distribution range of a species. Another factor that influences the way biological traits become structured across populations is demographic history, which is strongly influenced by environmental factors such as climate. Global fluctuations of the Earth's climate have certainly influenced the natural histories of species by shifting their distributions (Hewitt, 2000). The history of environmental conditions can promote or reduce demographic growth while modifying the extent of a species' distribution, changing the connectivity among populations. As a result, climate change influences the amount of gene flow that populations experience. On the other hand, environmental heterogeneity, which is common in species with large distribution ranges, can promote population differentiation and eventually isolate populations from each other. The environmental factors that shape biological variation among populations has proven to be complex and dynamic across time. However, by using quantitative methods in ecology, it is possible to detect the influence of past conditions and recurring patterns which are inherent of each species natural history or their environment.

The heterogeneous landscape of the Baja California peninsula promotes local environmental differentiation of populations resulting in a high proportion of endemics (Tang et al., 2014). This heterogeneity is the result of local climatic conditions that are influenced by the cold California current in the Pacific Ocean, the steep mountain ranges that run along the middle of the peninsula and a steep precipitation gradient that runs north to south shifting the Mediterranean climate region into desert in a few hundred kilometers. Our sampling sites were located within these environmental gradients and *Y. schidigera* populations extend from the Mojave desert through the Mediterranean climate, into the Baja California scrubland desert. The fact that phenotypic variation was not geographically structured suggests that *Y. schidigera* can express a wide range of plasticity in its phenotype. Further studies, including genetic expression could determine the extent to which these plants vary in response to strong climatic gradients in their distribution. When interpreted in the context of the natural history of the Baja California peninsula, patterns of variation that we evidenced for *Y. schidigera* by studying its genetic,

phenotypic and ecological attributes, all show favorable conditions for a wide distribution and probable large effective population sizes for the species.

Because mutualistic interactions in many cases originate as antagonistic relationships that evolve into an obligate interaction where benefits outweigh the costs for both species (Bronstein, 2001), opportunistic behavior is easily selected back as it may increase fitness of one of the species involved in the (former) mutualism. Exploitation of the counterpart's resources to increase benefits can quickly result in diversification of species, but obviously creates a conflict of interests for the interaction. *Yucca* plants and their yucca moths, however, have extended their history of co-evolving relationship for over 20 Myr (Flores-Abreu et al., 2019; McKain et al., 2016).

Despite the number of yucca moth lineages that have diversified into cheater species (e.g., non-mutualistic species; Addicott, 1996; Darwell et al., 2016; Pellmyr et al., 1996), the number of pollinating (e.g., mutualistic) moth species outnumbers the cheater species. This may be because *Yucca* plants have evolved a series of mechanisms that regulate overexploitation of its resources by the pollinator. Our study contributes with the study of variation in this dynamic interaction, showing that even under similar climatic environments, the output of the mutualism will be variable. Thus, more focus on studying the mechanisms that regulate these dynamics is encouraged.

The Yucca-Yucca moth model has become an ideal model to study ecological and evolutionary dynamics of insect-plant interactions. Studies such as this one have great potential to document a great number of co-evolutionary processes that have been predicted theoretically. Because the number of species in the *Yucca* phylogeny is sufficient, a wide number of studies could be completed to test such theoretical predictions. Among these, we suggest comparing the phenology of the mutualism among *Yucca* species that experience contrasting climate regimes. Also, investigating the differences in the reproductive output of *Yucca* species that are pollinated by yucca moth species occurring sympatrically with opportunistic moths should result in very useful input on the interaction ecology of this system. Whether this type of mutualism actually promotes diversification of both counterparts has been addressed using molecular data (Smith et al., 2008). However, differences in diversification rates between species subject to moderate isolation, as on the peninsula, and groups of *Yucca* species that have a history of sympatric distribution could be very informative about hybridization and speciation processes in the mutualism.

The Yucca- yucca moth mutualism offers the possibility to address many questions about the ecology and evolution of insect-plant interactions. Because a large fraction of Earth's biodiversity is supported by

the diversification of Angiosperms, investigating topics such as ecological divergence, species diversification and phylogeography within the Yucca-yucca moth model will certainly contribute substantially to the study of ecology and evolution.



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