Conservation

Genetic diversity of *Oenothera drummondii* (Onagraceae), a dune coastal herb: ecological and evolutionary implications

Diversidad genética de Oenothera drummondii *(Onagraceae), una herbácea de dunas costeras: implicaciones ecológicas y evolutivas*

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Abstract

We studied the diversity and genetic structure of *Oenothera drummondii* Hook. (Onagraceae), a dune plant with a mixed reproductive system, across 9 populations using 10 microsatellite markers. Plant genetic diversity is governed by intrinsic factors (i.e., reproductive system and dispersal) and extrinsic factors (i.e., population fluctuations and founder effects). We found moderate to low genetic diversity, with southern populations showing lower diversity, and northern populations higher. A separate study on self-compatibility revealed higher selfing in the south and lower in the north, suggesting a latitudinal gradient that may reduce genetic diversity in southern populations. Peripheral populations showed reduced diversity and greater differentiation, likely due to increased isolation and limited gene flow. Central populations near the Texas origin exhibited the highest diversity. Populations from Baja California (*O. drummondii* subsp. *thalassaphila*) formed a genetically distinct group, suggesting a separate species. Overall, genetic patterns in *O. drummondii* genetic diversity reflect historical and ecological influences, including mating system variation, floral traits, and pollinator dynamics. These findings support hypotheses such as center-periphery dynamics and climate-driven historical processes (e.g., post-glacial expansion), which may shape the species' genetic landscape and suggest possible local adaptations to environmental changes.

Keywords: Neutral genetic diversity; Coastal dunes; Microsatellites; Genetic bottleneck; Latitude; Selfing

Resumen

Evaluamos la diversidad y estructura genética de *Oenothera drummondii* Hook. (Onagraceae), planta de dunas costeras con sistema reproductivo mixto; se usaron 9 poblaciones y 10 marcadores microsatélites. La diversidad genética en plantas está influenciada por factores intrínsecos (dispersión y sistema reproductivo) y factores extrínsecos (fluctuaciones poblacionales y efecto fundador). Encontramos diversidad genética moderada a baja, con menor diversidad en poblaciones del sur (Ojoshal) y mayor en el norte (Bolívar). Un estudio complementario mostró mayor autocompatibilidad en Ojoshal y menor en Bolívar, sugiriendo un gradiente latitudinal con incremento de autocompatibilidad hacia el sur, con posible reducción de diversidad genética. Las poblaciones periféricas presentaron menor diversidad y mayor diferenciación, asociada a menor flujo génico y mayor aislamiento; las poblaciones centrales, cercanas al origen de la especie en Texas, mostraron mayor diversidad. Las poblaciones de Baja California (*O. drummondii* subsp. *thalassaphila*) formaron un grupo genético distinto, que podría representar a una especie separada. En conjunto, los patrones genéticos de *O. drummondii* reflejan procesos históricos y ecológicos relacionados con el sistema reproductivo, características florales y las dinámicas de polinización, lo cual podría significar adaptaciones locales a cambios ambientales, y patrones de distribución genética asociados con ellos, como la hipótesis de centro-periferia.

Palabras clave: Diversidad genética neutral; Dunas costeras; Microsatélites; Cuellos de botella; Latitud; Autocompatibilidad

Introduction

Genetic diversity may be considered a crucial factor in determining the ability of populations to adapt and evolve, thus increasing their evolutionary potential (He et al., 2024). Understanding the drivers of genetic variation among plant populations is essential in evolutionary biology as this diversity is the foundation for adaptive potential and long-term survival (Chung et al., 2023; Wright, 1969). In plants, the factors that shape genetic variation can be divided into: *a)* intrinsic biological properties (e.g., the genetic recombination system, including ploidy level, reproductive system, and meiotic behavior; the mode of dispersal and pollination; and the life form), and *b)* extrinsic dynamic processes (e.g., fluctuations in population size due to bottlenecks, founder effect, invasions, and changes caused by ecological succession) (Duminil et al., 2009). The reproductive system is the intrinsic biological property suggested to be the major driver of genetic diversity in plants (i.e., heterozygosity and population genetic differentiation) (Koelling et al., 2011; Wright, 1969), as it influences the mating patterns within a population by determining the extent to which selfing can occur (Charlesworth, 2006; Raduski et al., 2012). Self-incompatible populations, for instance, are composed of outcrossing plants with a predominantly outcrossing mating system. Conversely, in partially or completely self-compatible plants, the mating system can span from outcrossing to mixed mating to complete selfing (Holsinger, 1991).

Small, low-density populations experiencing habitat fragmentation and isolation, with limited mate or pollinator availability, exhibit increased propensity for self-fertilization (Devaux et al., 2014; Whitehead et al., 2018). In situations where self-fertilization is evolutionarily advantageous, selection will favor changes in traits that facilitate selfing (e.g., reductions in flower size, reduced nectar, pollen and reduced herkogamy) (Opedal, 2018; Shimizu & Tsuchimatsu, 2015; Sicard & Lenhard, 2011). These changes can have consequences at the genetic level, including elevated rates of inbreeding, a reduction in genetic diversity within populations, and increased genetic differentiation among populations (Barrett & Harder, 1996; Ingvarsson, 2002). Furthermore, a principal consequence of predominant self-fertilization is the reduction in heterozygosity and intra-population genetic diversity, coupled with an increase in inter-population differentiation, when compared to self-incompatible plants (Hamrick & Godt, 1996). In contrast, clonal species are expected to exhibit higher levels of heterozygosity than self-incompatible species, but lower levels of polymorphism within the population and higher levels of differentiation between populations (Levin, 2012).

In addition to the intrinsic biological properties of the species, spatial distribution and demography strongly influence processes such as genetic drift, gene flow, and natural selection, which in turn shape the genetic characteristics of populations (Eckert et al., 2008). For example, species range size tends to influence gene flow and genetic differentiation between populations.

Large distances between populations with large ranges would be barriers to gene flow (Lawrence & Fraser, 2020). Peripheral populations are predicted to exhibit reduced gene flow, greater isolation, and higher genetic differentiation. In contrast, populations in the center of the distribution would have higher gene flow and be less differentiated (Lawrence & Fraser, 2020). This may be exacerbated if the peripheral populations experience rapid cycles of colonization and extinction, and have associated bottleneck events or founder effects (Eckert et al., 2008). Dispersal ability also influences the amount of gene flow between core and peripheral populations. This has implications for the maintenance of genetic diversity. For example, species with large ranges but limited mobility have reduced gene flow between populations, resulting in greater genetic differentiation (Pelletier & Carstens, 2018).

The genus *Oenothera* (Onagraceae) is widespread with the majority of species concentrated in western North America (Overson et al., 2023), with some taxa extending to Central Mexico and South America (Wagner et al., 2007). Like other Onagraceae members, *Oenothera* species originated in the Nearctic region. Their diversification began 20 million years ago during the Miocene, an epoch characterized by colder and drier than the present. These conditions triggered both altitudinal and latitudinal forest retreats, creating ecological opportunities for herbaceous species that prefer open environments and can resist low temperatures and low humidity (Dietrich & Wagner, 1988).

The species of *Oenothera* contributed significantly to the early development of plant genetics, cytogenetics, and evolutionary biology. Since the work of De Vries in 1900 (Cleland, 1972), a great deal of information has been collected on the ecology, morphology, cytology, and genetics of the genus, giving it a great advantage as a model for study (Greiner & Köhl, 2014), especially on reproductive systems (Johnson et al., 2009; Raven, 1979; Wagner et al., 2007). The intricate evolutionary history of the genus reports several transitions in reproductive systems, which are diverse (Cleland, 1972; Johnson et al., 2011; Rauwolf et al., 2008). In addition, *Oenothera* has a unique genetic recombination system (Cleland, 1972). In some species, meiosis is accompanied by a rearrangement of chromosome arms that form structures known as rings. These rings segregate into the next generation and have significant implications for inheritance (Golczyk et al., 2014). They act as barriers to homologous recombination and alterations to the linkage balance (Overson et al., 2023; Rauwolf et al., 2008).

Our study focuses on *Oenothera drummondii* Hook. (Onagraceae), an herbaceous, short-lived perennial species belonging to the subsection *Raimmania* within *Oenothera* (Overson et al., 2023). The species presents different chromosomal configurations during meiosis, including the formation of bivalents and rings, but is not a permanent translocation heterozygote; so its reproduction is not expected to be clonal (Dietrich & Wagner, 1988). In contrast, the species displays a mixed mating system, encompassing both self- and cross-pollination (Dietrich & Wagner, 1988; Gallego-Fernández & García-Franco, 2021b). Despite exhibiting self-compatibility, its herkogamous large yellow flowers, and elevated stigma suggest predominantly outcrossing, particularly in North American populations (Gregory, 1964). Nevertheless, the species is capable of self-pollination in the absence of pollinators (i.e., sphingid moths and some hymenopterans) or under specific environmental conditions, such as wind, sand burial, and wave action during tropical storms, which are common in dunes and have an impact on the survival of individuals (Dietrich & Wagner, 1988; Gallego-Fernández & García-Franco, 2021; Gregory, 1964). Recent studies in North America showed that levels of self-compatibility vary from population to population, decreasing with increasing latitude (Gallego-Fernández & García-Franco, 2021b). Also, flower size decreased significantly with increasing latitude (Gallego-Fernández & García-Franco, 2021a). Given that all these characteristics could be reflected in the genetic diversity and genetic structure of the populations, we combined existing reports on life history traits, germination and self-compatibility levels, floral traits, dispersal modes, and genetic recombination system with our results of the genetic diversity and structure of 9 populations covering the North American distribution of *O. drummondii* (Gulf of Mexico and Baja California). In this study, we addressed the following questions: *1*, Does the genetic diversity vary between populations? Because different environmental conditions allow different evolutionary pressures to act in each population, we expect variation in genetic diversity and related genetic parameters such as heterozygosity and inbreeding. *2*, Does the predicted variation in genetic diversity relate to intrinsic biological properties of the populations, such as selfing or changes in floral traits? If self-fertilization is the predominant mating system in the population, we expect populations to show smaller flower size, higher inbreeding, lower heterozygosity, and reduced genetic diversity (Ingvarsson, 2002). *3*, Do populations closer to the center of the distribution have greater genetic diversity and less genetic differentiation than peripheral populations? Large distances between populations of species with large ranges would be barriers to gene flow (Lawrence & Fraser, 2020). Also, species living in coastal dunes commonly experience rapid cycles of colonization
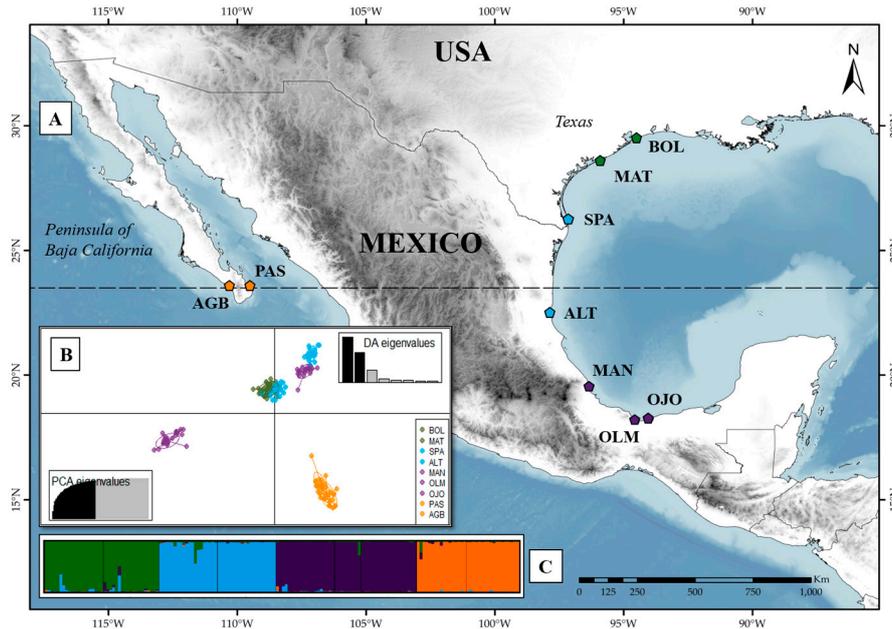
Figure 1. A, Distribution of *Oenothera drummondii* in North America. BOL: Bolívar, Texas-USA. MAT: Matagorda, Texas-USA. SPA: South Padre Island, Texas-USA. ALT: Altamira, Tamaulipas-México. MAN: La Mancha, Veracruz-México. OLM: Olmeca, Veracruz-México. OJO: Ojoshal, Tabasco-México. AGB: Agua Blanca, Baja California-México. PAS: Punta Arena del Sur, Baja California-México. B, Discriminant analysis of principal components DAPC. C. STRUCTURE bar plot of individual assignments of genetic groups (K = 4). The probability that an individual belongs to a particular group is represented by the colored bars. The order of the groups is the same as in A.

and extinction, and have associated bottleneck events or founder effects (Eckert et al., 2008). This effect would be exacerbated in peripheral populations (Lawrence & Fraser, 2020). We expect that populations at the edge of the distribution would have lower genetic diversity and higher differentiation than central populations.

Materials and methods

Study system

Oenothera drummondii is the sixth member of the series *Raimannia* within the subsection *Raimannia* in the genus *Oenothera* (Dietrich & Wagner, 1988). Its center of origin is in North America (Texas, USA) and consists of 2 subspecies with a disjunct distribution: *O. drummondii* subsp. *drummondii*, which is distributed in the coastal dunes of the Gulf of Mexico (from North Carolina in the USA to Campeche in Mexico), and *O. drummondii* subsp. *thalasaphilla* along the Pacific coast at the Southern tip of Baja California in Mexico (Dietrich & Wagner, 1988; Hernández-Espinosa et al., 2020). The 2 subspecies can be distinguished morphologically: *O. drummondii* subsp. *drummondii* have large flowers and long pubescent leaves, while *O. drummondii* subsp. *thalasaphilla* is characterized by having small flowers and small succulent leaves (Dietrich & Wagner, 1988). *O. drummondii* subsp. *drummondii* has been accidentally introduced into coastal dune systems around the world, and is considered invasive in several of them (Castillo-Infante et al., 2021; Dietrich & Wagner, 1988). The species is restricted to coastal dunes, inhabiting the dunes from the back beaches to the first dune ridges and embryo-dunes of the Gulf of Mexico and Southeastern USA (from Campeche to North Carolina) (Dietrich & Wagner, 1988; Gallego-Fernández & García-Franco, 2021b; Moreno-Casasola, 1988). From Louisiana to North Carolina in USA, *O. drummondii* shares its distribution with *O. humifusa,* another species within subsection *Raimmania. Oenothera humifusa* is an autogamous, permanent structural heterozygote (PTH), very similar to *O. drummondii* in growth form and habitat requirements. In the overlapped distribution, 2 basic types can be distinguished, although they are linked by intermediate forms that result from crosses between *O. drummondii* and *O. humifusa* (Dietrich & Wagner, 1988; Wagner et al., 2007). To ensure that the collection represented only *O.*

drummondii and excluded the possibility of hybrids, the sampling was made systematically, beginning in Texas, USA, and continuing southward to Tabasco. Some of the populations collected were small and restricted to limited areas, in which all individuals were collected. The most illustrative example was the population of Ojoshal, composed of 9 individuals. We studied 169 individuals from 9 North American populations, and the 2 subspecies of *O. drummondii* (Fig. 1; Supplementary material: Table S1). Of these, 2 populations are from *O. drummondii* subsp. *thalassaphila* (Agua Blanca and Punta Arena del Sur), and the remaining 7 are from subsp. *drummondii* (Bolívar, South Padre Island, Matagorda, Altamira, La Mancha, Olmeca, and Ojoshal). We had information on self-compatibility and floral traits for 4 of the 9 populations evaluated (Bolívar, South Padre Island, La Mancha, and Ojoshal), since Gallego-Fernández and García-Franco (2021a, b) used the same populations in their studies (Supplementary material: Table S2).

Vegetal material

Leaf material was collected from reproductive adults of each population, for which 5 leaves per plant were sampled in paper bags with silica gel, and the samples were subsequently stored at -20 °C. When population density exceeded 30 individuals, 20 were randomly selected, and if within a population less than 20 individuals were found, as in Ojoshal, the entire population was sampled (Supplementary material: Table S1).

DNA extraction and microsatellite loci amplification

Ten microsatellite loci transferred to *O. drummondii* from other species of the *Oenothera* (Hernández-Espinosa et al., 2020) were used in this study: OenhaB105, OenhaD102, OenbidiA_C10, Oenbi2triA_A1, Oenbi2triA_D3, Oenbi39tri10, Oenbi2triA_H1, Oenbi39tri4, Oenbi2triA_E4 and Oenbi39di2. The extraction of genomic DNA was carried out according to 2 protocols: *a)* CTAB extraction, using 50 mg of dry leaf tissue (González & Vovides, 2002). The extracted DNA was subsequently purified using the PCR Clean-up & Gel Extraction Purification Kit (QIAGEN). *b)* DNeasy Plant Mini Kit using 20 mg dried leaf tissue following the manufacturer's instructions (QIAGEN). The quantity and quality of the extracted DNA were verified on 1% agarose gels stained with Red Gel.

Microsatellite amplification was performed using 2 protocols. An initial PCR in a final volume of 10 µL containing total DNA, 2X reaction buffer, 0.2 mM of each dNTP, 1.6 mM MgCl2, 0.5 ng/µL BSA, 0.025 U/µL Taq polymerase, 0.5 µM reverse and forward primers, and 0.5 µM universal primer M13. For sample visualization, each forward primer was modified by the addition of an M13 sequence at the 5' end (5'-TGT AAA ACG ACG GCC AGT-3') that is complementary to an M13 primer labeled with either NED (yellow), HEX (green), or 6-FAM (blue) fluorophores. The amplification program consisted of an initial cycle of 3 min at 94 °C; followed by 25 cycles of 94 °C for 40 s, 50 °C for 40 s, and 72 °C for 60 s; 8 cycles of 94 °C for 60 s, 53 °C for 60 s, and 72 °C for 60 s, where hybridization of the M13 primer occurs; and a final extension at 72 °C for 10 min. A second PCR was performed using the Type-it microsatellite kit (QIAGEN), 1 µL of total DNA, and 0.2 µM of the forward, reverse, and M13 primers in cases where no amplification products were obtained from the first PCR after verification in 1% agarose. The amplification program consisted of a 15-minute cycle at 95 °C, followed by 20 cycles at 94 °C for 40 s, 50 °C for 90 s, and 72 °C for 60 s; 15 cycles at 94 °C for 60 s, 53 °C for 60 s, and 72 °C for 60 s. The final extension was performed at 60 °C for 30 min. A standard 400 bp marker and an ABI 3730 Gene Analysis System (Macrogen) were used to analyze the PCR products resulting from amplification. GeneMarker (Softgenetics, State College, PA, USA) was used to identify allele sizes manually. *Oenothera drummondii* is a diploid species, with amplifications consisting of 1 or 2 alleles per individual.

Statistical analysis

Presence of null alleles was evaluated using the FreeNA software with the EM algorithm (Chapuis & Estoup, 2007). The frequency of null alleles was estimated for each locus and population. F_{ST} values were calculated with FreeNA with the ENA algorithm for the correction of null alleles. The presence of clones in the populations was assessed with GenAIEx 6.503 software (Peakall & Smouse, 2012). Deviations from Hardy-Weinberg equilibrium for each locus in each population were assessed by the X^2 test using the Benjamin-Hochberg procedure in GenAIEx 6.503 software (Peakall & Smouse, 2012). Linkage disequilibrium (LD) between loci was evaluated in Arlequin 3.5.2 (Excoffier & Lischer, 2010).

The percentage of polymorphic alleles (P), average number of alleles per locus (A), average number of effective alleles (Ae), observed heterozygosity (H_O), expected heterozygosity (H_E), and fixation index (F) were calculated in GenAIEx 6.503 (Peakall & Smouse, 2012). To estimate the patterns of genetic variation between and within the populations, we calculated the F-statistics (i.e., F_{IS}, F_{IT} and F_{ST}) (Wright, 1978). Additionally, F_{IS} per population, considering null allele frequency, was estimated using INEST 2.2 (Chybicki, 2017), applying Bayesian method with 300,000 steps, sampling every

1,000 steps and burn-in of 30,000 steps. For the bottleneck analysis, the Wilcoxon signed rank test was performed for 3 mutation models: Infinite Allele Model (IAM), Stepwise Mutation Model (SMM), and Two-Phase Model (TPM). INEST 2.2 was used to run 100,000 simulations for each mutation. The relationship between latitude and parameters of genetic diversity (A, P, H_O and H_E) was evaluated using regression models (Sokal & Rohlf, 1995) in the R 4.2.0 program (R Core Team, 2020).

To estimate the molecular variation within and between populations, an analysis of molecular variance (AMOVA) was performed using 1,000 permutations in poppr package in R 4.2.0 software (R Core Team, 2020). To determine isolation by distance (IBD), a Mantel test (Mantel, 1967) was performed between the matrix of genetic differentiation (F_{ST}) with INA null allele correction, and population geographic distances (Euclidean) using 999 permutations in the vegan R package (R Core Team, 2020). We measured current dispersal rates (*m*) using BAYESASS, in which *m* is interpreted as the fraction of migrants per generation in one population that is derived from another population (Wilson & Rannala, 2003). The Bayesian clustering algorithm implemented in the Structure 2.3.4 software (Pritchard et al., 2010) was used to clarify the genetic structure of the samples by assigning individuals to genetic groups. The simulation was carried out with correlated allele frequencies and with mixture models according to ancestry. All analyses used 100,000 replicates, a Markov chain Monte Carlo (MCMC) burn-in period of 10,000 steps, and 10 replicates per K. The results obtained from Structure were processed with Structure Harvester (Earl & VonHoldt, 2012) to select the optimal K (highest value of ΔK) using the Evanno method (Evanno et al., 2005). Clumpak (Kopelman et al., 2015) and Distruct (Rosenberg, 2004) were used to visualize the bar graphs obtained in Structure. Discriminant analysis of principal components (DAPC) using the R package adegenet with 1,000 permutations for validation was also used to analyze the genetic structure of *O. drummondii*. It was based on the genetic distances (Cavalli-Sforza & Edwards, 1967) for each pair of populations, using the INA correction (Chapuis & Estoup, 2007) for the presence of null alleles.

Results

Frequency of null alleles
The 10 microsatellite loci amplified successfully in 161 individuals from 9 populations. Statistical tests of Hardy-Weinberg equilibrium for each locus in each population showed that most cases (50 of 78) did not deviate from equilibrium. For each population, the linkage disequilibrium analysis showed significant deviations in at least one locus. Mean null allele frequencies for all populations were low (0.06 ± 0.04). The mean null allele frequencies per population were moderate to low (0.01 ± 0.02-0.13 ± 0.14). Five loci exhibited high allele frequencies (> 0.2) in at least one population when analyzed across all population-locus combinations (Supplementary material: Tables S3, S4).

Genetic diversity
We detected 99 alleles for the 10 microsatellite loci. The total number of alleles detected had an average of 9.9 ± 5.6 alleles per locus, ranging from 4 for OenhaD102 to 23 for OenbidiA_C10. The level of polymorphism was high in all populations (mean 86.67 ± 14.14). The lowest percentage of polymorphism was found in Ojoshal (OJO), where 4 loci were monomorphic. The number of alleles per locus ranged from 1.80 in Ojoshal to 4.60 in Bolívar, with an overall mean number of alleles per locus of 2.28 ± 0.48. We found a total of 35 private alleles, ranging from 1 in La Mancha to 8 in Bolívar and Agua Blanca. The average effective number of alleles was 2.28 ± 0.48. It ranged from 1.58 in Olmeca to 3.06 in Bolívar (Table 1).

Expected heterozygosity ranged from 0.283 in Olmeca to 0.599 in Matagorda. The overall mean was 0.442 ± 0.104. The observed heterozygosity was also lowest in Olmeca (0.221), and highest in Matagorda (0.547). The mean total observed heterozygosity was 0.409 ± 0.097. The average observed heterozygosity was lower ($H_O < H_E$) than expected, indicating an overall homozygote excess. This is consistent with the results of the Hardy-Weinberg equilibrium test, which showed heterozygote deficiency for at least 1 locus in all populations. However, the Hardy-Weinberg equilibrium test also showed heterozygote excess in 4 populations: South Padre Island, Olmeca, Ojoshal, and Punta Arena del Sur. Of these, Ojoshal showed heterozygote excess at 5 of the 6 polymorphic loci (Table 1).

The coefficients of inbreeding obtained using the INEST ranged from 0.0051 in Altamira to 0.2446 in Olmeca (Table 2). Three models were evaluated to detect the presence of recent genetic bottlenecks. The infinite allele model (IAM), the two-phase model (TPM), and the step mutation model (SSM). The SMM model has been suggested as a statistically conservative approach to detect microsatellite bottlenecks (Luikart et al., 1998). However, given the instability and wide range of mutation rates inherent to these markers, the TPM model may be the one that best explains the predominant mutation process at most loci (Di Rienzo et al., 1994). According to the TPM model, 4 populations in *O. drummondii* show possible recent bottlenecks: Altamira, Ojoshal, Matagorda

and La Mancha (Table 2). Regression analyses revealed significant latitudinal clines in genetic diversity, with both alleles per locus (r = 0.69, $p < 0.005$) and expected heterozygosity (r = 0.65, $p < 0.005$) showing strong negative relationships with latitude. Latitude significantly predicted the percentage of polymorphic loci (r = 0.4, $p < 0.05$), whereas no significant relationship was observed for heterozygosity (Supplementary material: Table S5).

Genetic structure

When evaluating the distribution of genetic variation within and among populations, mean values of global inbreeding ($F_{IT} = 0.416 \pm 0.163$) and local inbreeding ($F_{IS} = 0.059 \pm 0.195$) indicated significant heterozygosity deficits. The mean value of genetic differentiation (F_{ST}) was 0.376 ± 0.132, indicating that 38% of the genetic variation in *O. drummondii* was due to differences

Table 1

Parameters of neutral genetic diversity of *O. drummondii* at 10 microsatellite loci. N = Sample size; P = percentage of polymorphic loci; A = mean allele number by locus; Ae = mean effective allele number; Ap = private alleles; H_O and H_E = mean observed heterozygosity, and mean expected heterozygosity; F = fixation index. Hardy-Weinberg equilibrium deviations ($p < 0.05$), test is the number of polymorphic loci evaluated, and (-) is the number of loci with deficiency and excess of heterozygotes (+).

Population	N	P	A	A_e	A_p	Ho	H_E	F	H-W deviations		
									Test	(+)	(-)
Bolívar	20	100	4.60	3.06	8	0.410	0.557	0.264	10	-	4
Matagorda	19	100	4.20	2.75	3	0.547	0.599	0.086	10	-	1
South Padre Island	20	80	3.60	2.54	2	0.385	0.433	0.110	8	1	1
Altamira	20	100	3.10	2.27	1	0.485	0.487	0.003	10	-	2
La Mancha	20	90	3.10	2.28	3	0.420	0.464	0.094	9	-	3
Olmeca	19	70	3.00	1.58	6	0.221	0.283	0.219	7	1	2
Ojoshal	9	60	1.80	1.61	0	0.489	0.301	-0.623	6	5	1
Agua Blanca	17	90	4.10	2.31	8	0.324	0.435	0.256	9	-	4
Punta Arena del Sur	18	90	3.30	2.11	4	0.400	0.420	0.047	9	1	2
Mean ± SD	18 ± 3.54	86.7 ± 14.1	3.42 ± 0.83	2.28 ± 0.48		0.41 ± 0.097	0.44 ± 0.10	0.05 ± 0.27			

Table 2

Bottleneck estimation for 9 populations of *O. drummondii*. Significant values are shown in bold. SSM: Step mutation model, TPM: two-phase model.

| Population	FIS (INest)	FIS (INest) 95% HDPI	Bottleneck test (*p*-value)	
			SSM	TPM
Bolívar (BOL)	0.2061	0.2061-0.3218	0.385	0.3473
Matagorda (MAT)	0.1221	0.0023-0.2274	0.0656	**0.0421**
South Padre Island (SPA)	0.0356	0.0011-0.0715	0.1913	0.1907
Altamira (ALT)	0.0051	0.0000-0.0049	**0.0322**	**0.0245**
La Mancha (MAN)	0.0214	0.0044-0.0568	0.0820	**0.0486**
Olmeca (OLM)	0.2446	0.2277-0.3479	0.9845	0.9845
Ojoshal (OJO)	0.0133	0.0000-0.0217	**0.0264**	**0.0268**
Agua Blanca (AGB)	0.0977	0.0586-0.1955	0.973	0.9625
Punta Arena del Sur (PAS)	0.0196	0.0000-0.471	0.5443	0.4556
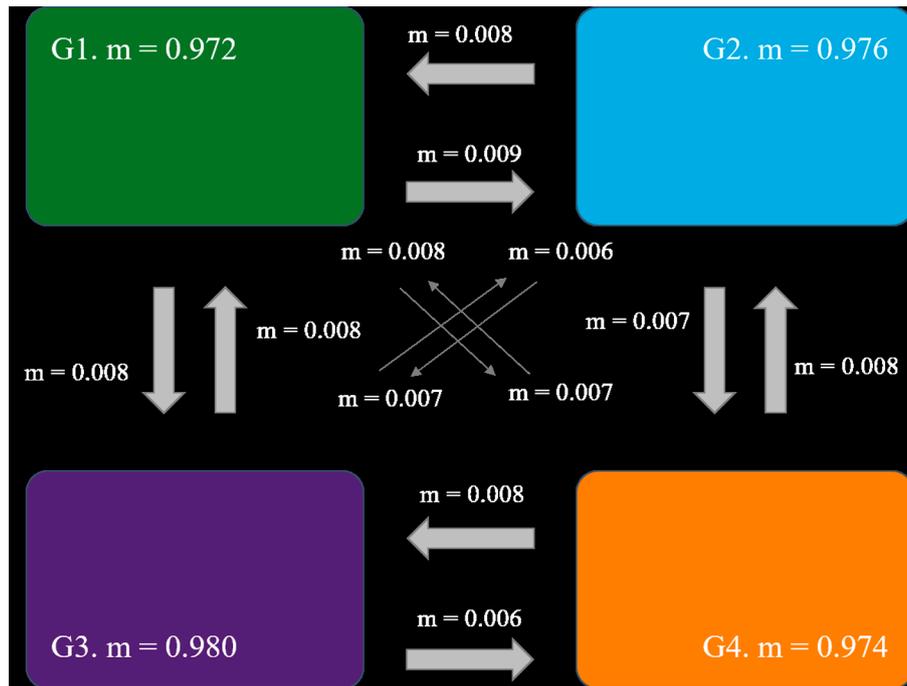
Figure 2. Migration rates *m* in the groups found by STRUCTURE. Group colors match those found in Bayesian clustering: G1, Bolívar (BOL) and Matagorda (MAT). G2, South Padre Island (SPA) and Altamira (ALT). G3, La Mancha (MAN), Ojoshal (OJO), and Olmeca (OLM). G4, Agua Blanca (AGB) and Punta Arena del Sur (PAS).

between populations. Furthermore, genetic differentiation between pairs of populations was mostly high (> 0.15). Matagorda and Bolívar had the lowest differentiation (0.06), followed by Punta Arena del Sur and Agua Blanca (0.17), and Bolívar and South Padre Island (0.18). The analysis of molecular variance (AMOVA) showed that most genetic variation was found among individuals (54%), followed by that found among populations (38%) (Table 3).

The Mantel test detected significant isolation by distance (r = 0.64, *p* = 0.002). The BAYESASS analysis revealed high diagonal *m*-values (G1 = 0.972 – G3 = 0.980), suggesting high gene flow within the individual groups. Higher gene flow was observed from G1 to G2 ($m_{1 \text{ to } 2}$ = 0.009) (Fig. 2). The results of the Bayesian clustering are shown in Supplementary material: Fig. S1. The most probable K with the highest value of ΔK = 479.023 was K = 3, followed by K = 4 with ΔK = 101.129. For K = 3, 3 clearly defined genetic groups were observed, with a very low proportion of admixture among them. In this case, the first group consisted of the Texas populations: Bolívar, Matagorda, and South Padre Island, and the northernmost Mexican population, Altamira. The second group included the 3 remaining Mexican populations in

the Gulf of Mexico: La Mancha, Olmeca and Ojoshal, while the third group comprised the 2 populations on the Baja California Peninsula. For K = 4, the clustering is similar to K = 3. The main difference in this case was that the fourth cluster split the previous cluster containing the Texas and Altamira populations, indicating greater genetic correspondence between Bolívar and Matagorda (populations further north in the Gulf) and between South Padre Island and Altamira. Admixture was low, indicating that *Oenothera* populations are highly structured.

The Discriminant Analysis of Principal Components (DAPC), like the Bayesian method, also showed 4 groups (Fig. 1C; Supplementary material: Fig. S2). Consistent with the results of Structure, one group was formed by the populations of Baja California (Punta Arena del Sur and Agua Blanca). However, in this case, La Mancha was no longer grouped together with Ojoshal and Olmeca, which formed the second group. Altamira and La Mancha formed a third group that is closer to the fourth formed by the Texan populations (Bolívar, Matagorda, and South Padre Island). The DAPC results indicated the possibility that the Texas group was the ancestral group of the central Gulf of Mexico populations (Altamira and La Mancha). The other 2 groups were markedly more differentiated.

Table 3

Molecular variance analysis (AMOVA) of *Oenothera drummondii* populations.

Source of variation	df	Sum sq	Mean sq	Variance component	Total variance (%)	*p* values
Between populations	8	841.68	105.21	2.78	37.39	0.001
Among populations	153	807.84	5.28	0.61	8.21	0.001
Within individuals	162	657	4.05	4.05	54.39	0.001
Total	323	2,306.53	7.14	7.45	100	

Discussion

In this research, we evaluated the genetic variation between and within populations of *Oenothera drummondii* throughout its range in North America, for which we used microsatellite markers. These markers demonstrated sufficient variability to resolve population genetic structure at high resolution, consistent with a previous study (Hernández-Espinosa et al., 2020). We also found that all populations were in linkage disequilibrium for at least one locus. Deviations may result from limited homologous recombination during meiosis when chromosomal rings are formed (Rauwolf et al., 2008). In *O. drummondii*, as in many *Oenothera* species, this limited recombination is reported to cause changes in the linkage equilibrium (Cleland, 1972; Rauwolf et al., 2008; Raven, 1979), as described in other ring-forming species of the genus: *O. biennis* (Larson et al., 2008; Levin, 1975; Levy & Levin, 1975), *O. harringtonii* (Skogen et al., 2012), *O. hartwegii* and *O. gayleana* (Lewis et al., 2016). The unique recombination system in the *Oenothera* is an attribute that has allowed them to colonize new environments from their origin in the Miocene (ca. 20 Ma), until recent times, successfully establishing themselves first in the Neotropics and then in Europe (Dietrich et al., 1997; Wagner et al., 2007).

Compared to other plants with similar characteristics in lifeform, reproductive system, geographic range, and dispersal mechanism, we found that *Oenothera drummondii* showed moderate to low genetic diversity. This is a short-lived perennial, self-compatible species with a mixed reproductive system and wide distribution (Dietrich & Wagner, 1988), and a variety of dispersal mechanisms (Gallego-Fernández et al., 2021). Mean levels of genetic diversity reported for other plants with mixed reproduction (N = 15, mean H_E = 0.60), short-lived perennial life form (N = 29, mean H_E = 0.55), wide distribution (N = 31, mean H_E = 0.62), and water or wind dispersal (N = 28, mean H_E = 0.61) (Nybom & Bartish,

2000), were higher than the level found in *O. drummondii* (mean H_E = 0.442).

Historically, genetic diversity in *Oenothera* has been attributed to structural genomic rearrangements, particularly hybridization and reciprocal translocations that alter linkage relationships translocations (Cleland, 1972; Rauwolf et al., 2011), as the genomes in *Oenothera* were considered to be essentially "non-recombining" due to the formation of chromosomal rings during meiosis (Cleland, 1972; Golczyk et al., 2014; Rauwolf et al., 2008). In contrast, the reported genetic diversity in *Oenothera* species appears strongly influenced by their predominant reproductive system. *O. biennis*, a permanent translocation heterozygote (PTH), maintains higher genetic variation than *O. drummondii*, which exhibits a mixed mating system (mean H_E = 0.69 vs. 0.44) (Larson et al., 2008). This agreed with the expectation of the "functional" asexual reproductive system of *O. biennis*, in which autogamy is almost complete and offspring are mostly clonal (Mather, 1943; Stebbins, 1957). Consistent with expectations for obligate sexual reproduction, self-incompatible, bivalent-forming *Oenothera* species (*O. harringtonii* with mean H_E = 0.77, *O. gayleana* with mean H_E = 0.53 and *O. hartwegii* with mean H_E = 0.49 vs *O. drummondii* with H_E = 0.44) maintained significantly higher genetic diversity than the mixed-mating *O. drummondii* (H_E = 0.44) (Levin, 1975; Rhodes et al., 2014; Skogen et al., 2019).

In terms of its reproductive system, the self-compatible *O. drummondii* generally has large flowers and an elevated stigma, suggesting a high propensity for sexual reproduction, although it can self-pollinate in the absence of pollinators or in situations where self-fertilization is evolutionarily advantageous (Gregory, 1964; Sicard & Lenhard, 2011). Gallego-Fernández and García-Franco (2021b) assessed the level of self-compatibility of *O. drummondii* in the Gulf of Mexico, through germination essays. They evaluated 4 of the 9 populations in this study (Bolívar, South Padre Island, La Mancha and Ojoshal), and found that self-compatibility increased in

low-latitude populations (La Mancha and Ojoshal), while lower for higher-latitude populations (Bolívar and South Padre Island). These results suggest a latitudinal shift towards greater selfing to the south of the distribution. It has been suggested that greater selfing in populations leads to a decrease in genetic diversity (Ingvarsson, 2002), which is consistent with the pattern of genetic variation found in this study. In agreement with the results of Gallego-Fernández and García-Franco (2021a), we found that populations with higher reported self-compatibility also had lower genetic diversity. On the other hand, in partial selfing species, reductions in genetic diversity are associated with changes in traits that facilitate selfing (i.e., reduced flower size, reduced nectar, pollen and reduced herkogamy) (Barrett & Harder, 1996; Shimizu & Tsuchimatsu, 2015; Tedder et al., 2015). According to the theory, it would be expected that the populations of *O. drummondii* with a higher self-compatibility, and lower genetic diversity (i.e., Ojoshal) would also show a tendency to reduce flower size, and populations with lower self-compatibility, and higher genetic diversity (i.e., Bolívar) would show an increase in flower size. However, Gallego-Fernández and García-Franco (2021a) found the opposite: populations showed larger flower sizes following a latitudinal gradient from subtropical (i.e., Bolívar and South Padre Island) to tropical (i.e., La Mancha and Ojoshal) climates. This result suggests that in *O. drummondii* there is no latitudinal correlation between a reduction in flower size and an increase in selfing, and thus to a reduction in genetic diversity. Differences in floral traits among *O. drummondii* populations appear to respond to both biotic (i.e., pollinators) and abiotic factors (i.e., temperature, precipitation), suggesting local adaptations to environmental changes (Gallego-Fernández & García-Franco, 2021a).

In *O. drummondii* the genetic diversity showed a significant increase with latitude, with its lowest values in Ojoshal and Olmeca, while the higher values were found in Bolívar and Matagorda, populations in the highest latitudes. The genus *Oenothera* appears to have originated in northern Mexico and the adjacent USA, and subsection *Raimmania*, to which *O. drummondii* belongs, is centered in this area (Dietrich & Wagner, 1988). Since older clades tend to have more genetic diversity (Willi et al., 2018), it would be expected that the greatest genetic diversity would be found in populations at latitudes closest to the center of origin (i.e., Bolívar and Matagorda) and that it would lower as populations moved away to the periphery of the distribution, as was found here. In addition to reducing population genetic variation through genetic drift, habitat suitability often decreases from the core to the edge of a species' geographic range, leaving edge

populations small and isolated (Sagarin & Gaines, 2002), as in Ojoshal. However, despite the small population size of Ojoshal, inbreeding was low, presumably because of its high selfing index (Gallego-Fernández & García-Franco, 2021b). Inbreeding depression is predicted to be lower in selfing species because recessive deleterious alleles are expected to be efficiently "purged" from populations (Winn et al., 2011), and selfing favors the selection of new recessive beneficial mutations (Burgarella & Glémin, 2017; Charlesworth et al., 1993).

The pattern of distribution of the genetic diversity found in *Oenothera drummondii* seems to be shaped by demographic and evolutionary processes (Durka, 1999; Hewitt, 1996). Historical climate-driven changes are known to still affect the present-day genetic diversity (Alvarez et al., 2009; Hewitt, 2000). As a result of sequential founder events during post-glacial recolonization, contemporary populations show reduced genetic diversity and increased genetic differentiation, especially at range margins (Eckert et al., 2008; Sagarin & Gaines, 2002). The above pattern was found in 512 North American herbs, showing that the shifting and fragmentation of species' geographical ranges in the past 20,000 years has played an important role in shaping the genetic variation of contemporary populations (Benavides et al., 2019).

Regarding the populations in Baja California (i.e., Agua Blanca and Punta Arena del Sur), the results of both genetic diversity and genetic structure showed that they formed an independent genetic group, as expected given that the populations belong to a different subsp. of *O. drummondii*. However, based on the genetic differences detected and the evidence of differences in life history, vegetative and floral characteristics observed in the field at the time of collection, it is suggested that *O. drummondii* subsp. *thalassaphila* is a distinct species from *O. drummondii* subsp. *drummondii* (Table 4). A detailed taxonomic study and additional genetic data from new populations *of O. drummondii* subsp. *thalassaphila* will be needed to support this.

We conclude that the genetic diversity and genetic structure across the range of *O. drummondii* in North America suggest a latitudinal shift towards increased selfing and a decline in genetic diversity in the low-latitude populations. However, there is no positive relation between reductions in floral traits and increased selfing in the populations. Differences in floral traits appear to respond to biotic and abiotic factors, suggesting local adaptations to environmental changes. In *O. drummondii*, genetic parameters may reflect historical processes like climate-driven changes (i.e., the last glaciation), and patterns of genetic distribution associated with them,

Table 4

Distinctive morphological attributes between *O.drummondii* subsp. *drummondii* and O. *drummondii* subsp. *thalassaphila*. Data taken from Benavides et al. (2019).

	O.drummondii subsp. *drummondii*	O. *drummondii* subsp. *thalassaphila*
Habit	Annual herb	Perennial sudshrub
Stem pubescence	Strigillose to villous	Strigillose
Glandular puberulent hairs	Present	Absent
Cauline leaves	1-8cm, oblanceolate to obovate, densely villous, margins entire to remotely sinuate dentate	1-4.5cm, oblong lanceolate to oblanceolate, strigillose, marginis entire to coarsely dentate
Floral tube	2.5-5cm, strigillose to densely villous	2-3.5cm, strigillose
Sepals	2-3cm, strigillose to villous	1.3-2.5cm, strigillose, occasionally red doted
Petal length	2.5-4.5cm	2-3.5cm
Capsule	2.5-5.5cm, strigillose to villous	2-4cm, strigillose
Seeds	1.1-1.7mm	1.5-2mm, smooth

such as the center-peripheral hypotheses. The genetic attributes evaluated here suggest that populations of *O. drummondii* subsp. *thalassaphila* may represent a distinct species, but further evaluation will be required to confirm this hypothesis.

Acknowledgments

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