Botanical Sciences 100 (3): 579-599. 2022
DOI: 10.17129/botsci.3001

Received: July 26, 2021, Accepted: October 27, 2021
On line first: March 22, 2022

Ecology / Ecología

Variability in leaf morphological traits of an endemic Mexican oak (*Quercus mexicana* Bonpl.) along an environmental gradient

Variabilidad en caracteres morfológicos foliares de un encino endémico mexicano (*Quercus mexicana* Bonpl.) a lo largo de un gradiente ambiental

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Abstract
Background: Phenotypic and functional traits of plant populations vary with environmental conditions at local and regional scales. The analysis of these traits along environmental gradients provides information on the differential response of populations to climate changes.
Objective: We analyzed the leaf morphological variation of an endemic oak to identify the degree of population differentiation along an environmental gradient.
Study species: *Quercus mexicana* Bonpl. (Fagaceae).
Study site and dates: Samples were collected from 39 populations in the Sierra Madre Oriental and east of the Trans-Mexican Volcanic Belt from 2014 to 2016.
Methods: We measured eight macromorphological traits in 5,507 leaves and three micromorphological traits in 228 leaves. We performed univariate and multivariate statistical analyses to assess the morphological differentiation among populations, and the relationship between variation in leaf traits and environmental variables related to temperature and water availability.
Results: Populations of *Q. mexicana* showed leaf morphological differentiation along its distribution. Significant linear correlations were found between leaf traits and environmental variables. Smaller and thicker leaves with lower density of trichomes and smaller stomata were found in populations located in more arid regions. In contrast, larger and thinner leaves with higher trichome density and larger stomata occurred in more humid places.
Conclusions: Populations of *Q. mexicana* are adapted to a wide range of climatic conditions. Considering the predictive future climatic changes for the region (*i.e.*, warmer and drier conditions), *Q. mexicana* populations with traits better adapted to a more humid and cooler environments could be negatively affected.
Keywords: climate change, drought tolerance, endemic oaks, leaf variation, morfo-functional traits, Sierra Madre Oriental.

Resumen
Antecedentes: Los caracteres fenotípicos y funcionales de las poblaciones de plantas varían a escalas local y regional. El análisis de estos caracteres a lo largo de gradientes ambientales proporciona información sobre la respuesta diferencial de las poblaciones a cambios climáticos.
Objetivo: Analizamos la variación foliar morfológica de un encino endémico para identificar el grado de diferenciación poblacional a un gradiente ambiental.
Especie de estudio: *Quercus mexicana* Bonpl. (Fagaceae).
Sitio de estudio y fechas: Se colectaron 39 poblaciones en la Sierra Madre Oriental y del este de la Faja Volcánica Transmexicana de 2014 al 2016.
Métodos: Medimos ocho caracteres macromorfológicos en 5,507 hojas y tres micromorfológicos en 228 hojas. Se realizaron análisis estadísticos univariados y multivariados para evaluar la diferenciación morfológica entre poblaciones, y las relaciones entre la variación foliar y variables relacionadas con la temperatura y la disponibilidad de agua.
Resultados: Las poblaciones de *Q. mexicana* están diferenciadas a lo largo de su distribución. Se encontraron correlaciones significativas entre caracteres foliares y variables ambientales. Hojas pequeñas y gruesas con baja densidad de tricomas y estomas pequeños se encontraron en sitios más áridos. En contraste, hojas más grandes y delgadas con mayor densidad de tricomas y estomas más grandes ocurrieron en sitios más húmedos.
Conclusiones: Las poblaciones de *Q. mexicana* están adaptadas a un amplio intervalo de condiciones climáticas. Considerando los futuros cambios climáticos para la región (*i.e.*, condiciones más secas y cálidas), las poblaciones de *Q. mexicana* con caracteres adaptados a ambientes más húmedos y fríos podrían afectarse negativamente.
Palabras clave: cambio climático, caracteres morfo-funcionales, encinos endémicos, tolerancia a la sequía, variación foliar, Sierra Madre Oriental.

Variation in climatic conditions at local and regional scales determines the phenotypic and physiological expression of plants and, at larger scales, the geographical distribution patterns of plant species (Pollock *et al*. 2012, Stahl *et al*. 2014, Riordan *et al*. 2016). Phenotypic variability in functional traits determines the limits of species distributions (Pollock *et al.* 2012, Stahl *et al.* 2014, Körner *et al.* 2016) through the intraspecific responses of plants to specific environmental factors (Albert *et al.* 2010, Laforest-Lapointe *et al.* 2014, Henn *et al.* 2018), in turn determining the ecological differentiation among populations (Albarrán-Lara *et al.* 2019, Martínez-Blancas & Martorell 2020). Identifying the relationships between morphological traits and environmental changes increases our understanding of the differential responses of populations, the potential shifts in species distributions, and the potential responses of plant species to climate change (Valladares *et al.* 2014, Henn *et al.* 2018).

Temperature and precipitation are two of the main environmental drivers of leaf variation at a global scale (Moles *et al.* 2014, Valladares *et al.* 2014, Wright *et al.* 2017). Patterns of foliar variation associated with gradients of temperature and precipitation have been identified for some leaf traits. For instance, plants with smaller leaves occur at sites with lower mean annual precipitation (Wright *et al.* 2017) along latitudinal (Uribe-Salas *et al.* 2008) or elevational (Tang & Ohsawa 1999) gradients. Similarly, leaf dry mass per area and leaf thickness have shown positive relationships with high solar radiation and temperature and negative relationships with rainfall at the multispecies level at a global scale (Niinemets 2001). However, these patterns vary at the intraspecific level at a regional scale. Variation among populations has been observed for some traits; for instance, a larger leaf area has been associated with high rainfall (Gouveia & Freitas 2009), and greater leaf vein density has been observed in drier environments (Zhu *et al.* 2012).

The density and type of trichomes also provide evidence of the functional responses of plants exposed to different environmental conditions. Under dry conditions, trichomes reduce the absorbance of solar radiation (Benz & Martin 2006), providing resistance to low temperatures (Agrawal *et al.* 2004) and UV ray protection (Schilmiller *et al.* 2008). Similarly, the importance of stomata in sensing and driving environmental change has been observed (Hetherington & Woodward 2003). Reduced stomatal density has been observed at higher CO_2 concentrations (Casson & Gray 2008), while stomatal density tends to increase in plants located at sites with elevated temperature and drought stress (Yan *et al.* 2017).

In this study, we analyzed the intraspecific variation in leaf morphological and functional traits in response to environmental gradients for an endemic red oak from northeastern Mexico: *Quercus mexicana* Bonpl. (Section *Lobatae*). The populations of *Q. mexicana* are distributed over a wide area, including subhumid regions, temperate forests, and xeric areas (Pérez-Mojica & Valencia-Á. 2017), in which conditions of temperature, precipitation and humidity display a high degree of variation. The objectives of this study were to determine the variation and degree of differentiation in leaf morphological traits among the populations of *Q. mexicana* and assess the relative influences of climatic and geographic factors on this phenotypic variation throughout the entire distribution of this species.

Materials and methods

Study species. Quercus mexicana is a tree species that grows between 6 to 12 m tall. This species presents coriaceous leaves that are elliptic, or oblong to oblong-obovate, from 2.8 to 8 cm in length and 1.5 to 2.5 cm in width and bear 7 to 12 secondary veins. Leaves present stellate trichomes at the base of the adaxial side and fasciculate trichomes on the abaxial side (Pérez-Mojica & Valencia-Á. 2017). *Quercus mexicana* is distributed mainly in the Sierra Madre Oriental (eastern Mexico) and at the eastern boundaries of the Mexican Plateau and the Trans-Mexican Volcanic Belt (Figure 1). It has been recorded in pine forests, oak forests, and xeric shrublands at elevations ranging from 1,600 to 2,700 m in clay soils with rocky outcrops (Pérez-Mojica & Valencia-Á. 2017).

Study area. The Sierra Madre Oriental is a mountain chain with marked climatic and environmental variation and a noticeable difference in the composition of the biotas. Hernández & Carrasco (2004) reported arid to warm humid climates (temperature ranges between 12 and 25 °C, and annual precipitation ranges between 300 mm and 4,000 mm).

The northern and southern regions of the Sierra Madre Oriental are separated by the Pánuco Depression; a warm and humid climate dominates on the Gulf of Mexico slope, while it becomes arid and warm towards the western slope and the south, and colder towards the north, particularly in the highlands (Hernández & Carrasco 2004, Figure 1). The climatic differences of the Sierra Madre Oriental with respect to the Trans-Mexican Volcanic Belt and Mexican Plateau are also striking, with colder temperatures and higher humidity or aridity in the last two regions (Figure 1).

Sampling strategy. Leaf samples were collected from 39 populations of *Q. mexicana* throughout its geographic distribution (Figure 1, Appendix 1) (Valencia-Á. 2004). In each population, 10 individuals separated by at least 20 m apart were randomly selected. From each tree, 15 mature leaves were collected from the lower branches. The collected leaves were pressed and dried in an oven at 70 °C.

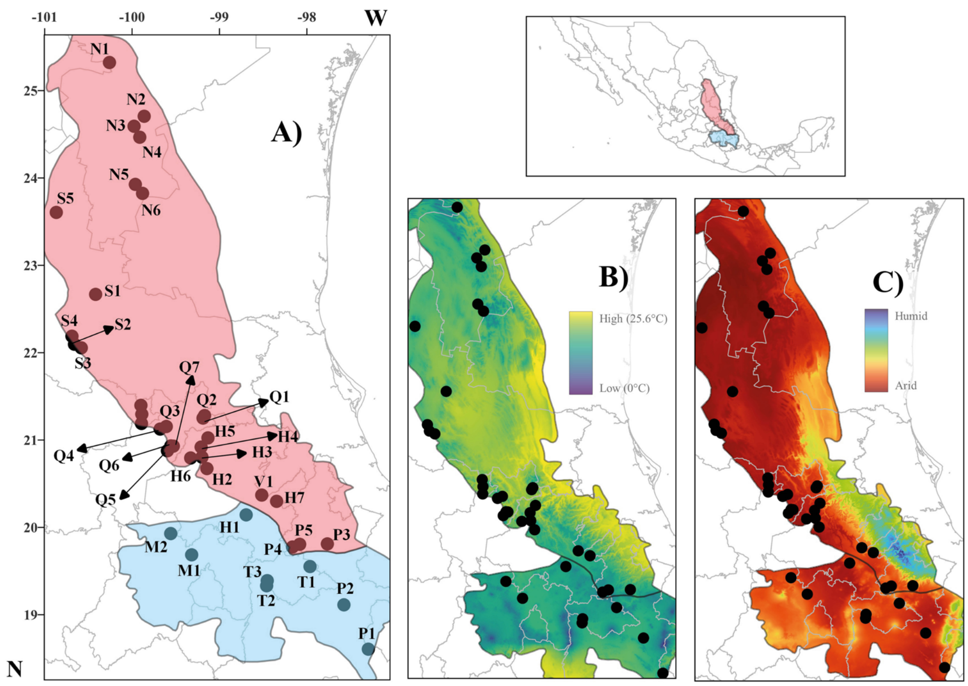

Figure 1. Study area. (A) Geographic locations of the 39 populations of *Quercus mexicana* across the Sierra Madre Oriental (SMO in red) and adjacent regions (in blue). Maps of (B) annual mean temperature and (C) aridity index in the study area. Population abbreviations are described in Appendix 1.

Leaf macromorphological traits. For each of the 5,507 sampled leaves, leaf length and thickness were measured with a digital Vernier caliper. Measurements of dry weight were performed with an analytical balance (precision = 0.001 g). Leaf area (LA, cm^2) was estimated through a quantitative evaluation of the area and leaf shape contours using elliptical Fourier descriptors with SHAPE ver. 3.1 software (Iwata & Ukai 2002). With this procedure, a matrix of eight macromorphological traits (M_{MAC}, Table 1) was constructed: leaf length, leaf width, leaf length/leaf width ratio, number of secondary veins, leaf thickness, specific leaf area, petiole length and petiole width.

Leaf micromorphological traits. We randomly selected four to six leaves per population of *Q. mexicana* for a total of 228 leaves to measure leaf trichomes and stomata using scanning electron microscopy (SEM). Three images corresponding to the X100, X200 and X500 fields for each leaf were taken; in total, 684 images were analyzed. For each population, trichome density (trichome number/area) was quantified using X100 field images and stomatal density (stomatal number/area) with X200 field images with the Digimizer software (www.digimizer.com). The X500 images were used to measure stomatal aperture length in µm. The three variables were integrated into a micromorphological matrix (M_{MIC}, Table 1) for further analysis.

Table 1. List of macro- and micromorphological leaf traits and environmental variables used to identify the differentiation among populations of *Quercus mexicana*.

Leaf morphological traits (M_{MOR})			
Macromorphological (M_{MAC}) traits		Micromorphological (M_{MIC}) traits	
LL	Leaf length	DTR	Density of trichomes
LW	Leaf width	STD	Stomatal density
LWr	Leaf (length / width) ratio	STL	Stomatal aperture length
PL	Petiole length		
PW	Petiole width		
SLA	Specific leaf area		
NV	Number of secondary veins		
LT	Leaf thickness		
Environmental variables			
Water-related variables		Temperature-related variables	
AI	Aridity index	AMT	Annual mean temperature
PET	Potential of evapotranspiration	TS	Temperature seasonality
AP	Annual precipitation	ATR	Annual temperature range
PS	Precipitation seasonality	MTC	Mean temperature of coldest quarter
PD	Precipitation of driest quarter		

Environmental variables. A matrix of nine environmental variables without collinearity that described the conditions of each sampled population was constructed (Table 1). Seven noncorrelated bioclimatic variables were obtained from WorldClim (Hijmans *et al.* 2005): annual mean temperature, temperature seasonality, annual temperature range, mean temperature of coldest quarter, annual precipitation, precipitation seasonality, and precipitation of driest quarter. Potential evapotranspiration and an aridity index were obtained from the Global Aridity Index and Potential Evapotranspiration Climate Database version 2.0 (Trabucco & Zomer 2009). Potential evapotranspiration relates the evapotranspiration processes and rainfall deficit, and aridity index represents the ratio between precipitation and potential evapotranspiration (high or low values of aridity index correspond to wet or arid conditions, respectively). Latitude was added as a geographic variable. Visualization and manipulation of climatic information was performed using ArcMap® v. 10.2 (ESRI). The overall topographic variables were calculated using Google Earth®.

Univariate analyses. Statistics of each morphological trait were calculated for each of the 39 populations. As a first step, to determine the differences in leaf morphological and functional traits among populations of *Q. mexicana*, we used ANOVA for each of the eight macromorphological and the three micromorphological variables, and a Tukey-Kramer test was conducted for *a posteriori* comparison among populations using R v. 3.6.1 software (R Core Team 2016). Then, data normality was tested using a Shapiro-Wilk test, and Pearson correlations were calculated with R software, using nine bioclimatic variables and latitude as independent variables and eight macromorphological (M_{MAC}) and three micromorphological traits (M_{MIC}) as dependent variables.

Multivariate analyses. We performed discriminant function analysis (DFA) and clustering analysis (CA) to identify leaf morphological groups among populations. First, a DFA was performed based on the morphofunctional traits (M_{MAC}, M_{MIC}, and M_{MOR}), and we used each of the 39 populations as a discriminant factor. Second, from the DFA results, the centroids of each population were obtained, the Euclidian distance among centroids was calculated, and the CA was performed with the Ward method (Ward & Hook 1963). The CA was performed to yield a dendrogram depicting the morphological relatedness among the means of individuals in each population.

From the previously identified groups (named morphogroups), a DFA was performed based on the differences among environmental conditions between morphogroups. Three independent analyses were conducted following the previous procedure: the first considering macromorphological traits (DFA_{MAC}), the second with micromorphological traits (DFA_{MIC}) and the third considering both attribute sets (DFA_{MOR}). This procedure was performed with R software.

To evaluate the contribution of temperature and water availability to leaf morphological variation at a global scale, three redundancy analyses (RDAs) were performed considering the following data sets: a) a complete model including both sets of bioclimatic variables (RDA_{full}: space + climate), b) a partial model considering temperature-related variables controlling the water effect (pRDA1: climate|space), and c) a partial model considering water-related variables controlling the temperature effect (pRDA2: space|climate). Three independent analyses were performed: the first considering macromorphological traits (RDA_{MAC}), the second with micromorphological traits (RDA_{MIC}) and the third considering both traits (RDA_{MOR}). This procedure was performed with the vegan package in R software.

Results

Univariate analyses. All the morphological traits, except for stomatal density, showed significant differences among populations (Figure 2; Appendix 1). After Tukey's test (with at least $P < 0.05$), the morphological traits that had the greatest differences among populations were leaf width, leaf thickness, number of secondary veins and petiole length. In addition, all the morphological traits displayed gradual variation among all the populations (Figure 2).

Among the macromorphological traits, leaf length varied without a regional pattern, whereas leaf width was significantly smaller and the leaf length/width ratio was significantly larger in the northern populations of the Sierra Madre Oriental (Figure 1). Petiole length was significantly longer in some populations in the north of the Sierra Madre Oriental (N1, N2, S1), in Hidalgo (H1, H3), and in the Trans-Mexican Volcanic Belt in Estado de Mexico and Tlaxcala (T3). Significantly wider petioles were detected in a group of populations in the northern Sierra Madre Oriental (N1, N4, N6, S5) and in the southwestern Sierra Madre Oriental (H5, Q4, Q7); narrower petioles were found in the southeastern Sierra Madre Oriental (H7, V1, P4) and parts of the Trans-Mexican Volcanic Belt (M1, P2). The specific leaf area differed in only a couple of populations in the north, and some populations displayed smaller leaf areas. The number of veins was significantly greater in the northern Sierra Madre Oriental and smaller in the southeastern Sierra Madre Oriental (H1, H7, P5, V1) as well as in some populations of the Trans-Mexican Volcanic Belt (P1, T1). The thickest leaves were found in the northern Sierra Madre Oriental and in populations in the southwestern Sierra Madre Oriental (H3, H4, M2). Significantly thinner leaves were found in several populations, predominantly in San Luis Potosí, Puebla, and Querétaro (Appendix 2).

With respect to the micromorphological traits, the populations that displayed the highest density of trichomes were in Puebla (P2, P4), and the lowest density of trichomes was found in populations of the northern Sierra Madre Oriental (N2, N3, N5), as well as populations in Hidalgo (H2, H4). The stomatal aperture length was smaller in most of the populations from Querétaro, as well as some in the north (N3, S2); the largest stomatal aperture was found in populations from the Trans-Mexican Volcanic Belt, significantly differing only in Tlaxcala (T2) (Figure 2; Appendix 2).

In the linear correlation analyses (Table 3; Figure 3), leaf length and specific leaf area displayed similar correlations with the environmental variables; they were positively correlated with latitude, all the temperature variables, potential evapotranspiration, and precipitation of driest quarter, and negatively correlated with the aridity index. Leaf width followed a similar pattern except that it was correlated with precipitation seasonality, and no correlation with the aridity index was found. The leaf length/width ratio was only positively correlated with latitude and negatively correlated with precipitation seasonality. The number of veins and leaf thickness were positively correlated with latitude, temperature seasonality, potential evapotranspiration and precipitation of driest quarter, and the number of veins and leaf thickness were inversely correlated with the aridity index and precipitation seasonality. The number of veins was positively correlated with all the temperature variables. With regard to petiole size, petiole length was not correlated with temperature, but petiole width was negatively correlated with the annual temperature range; with respect to the precipitation variables, petiole length and width were negatively correlated with precipitation seasonality.

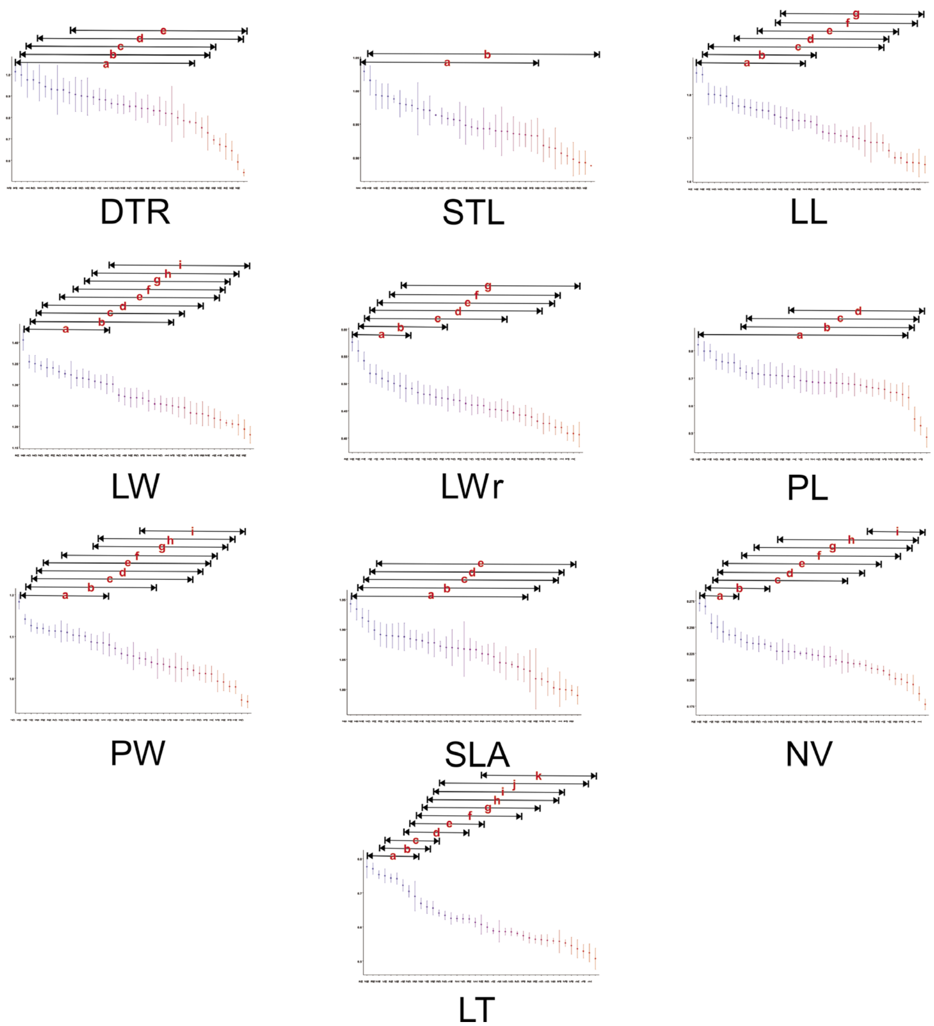

Figure 2. Summary of the Tukey-Kramer post-hoc tests for the differentiation of micro- and macromorphological traits among populations of *Quercus mexicana*. Lines above populations indicate no significant differences (at $P < 0.05$) and only the results with a significance $P < 0.001$ are presented. Abbreviations of leaf morphological traits as indicated in Table 1.

Among the micromorphological characters, the density of trichomes showed a negative correlation with latitude, temperature seasonality, precipitation of driest quarter, and potential evapotranspiration but a positive correlation with the aridity index. Stomatal density was correlated only with potential evapotranspiration, while stomatal aperture length was negatively correlated with latitude, potential evapotranspiration, precipitation of driest quarter, and three of the temperature variables (Table 2).

Table 2. The results of ANOVA for the differentiation of leaf morphological traits among populations of *Quercus mexicana*. Abbreviations of leaf morphological traits as indicated in Table 1. Degrees of freedom (df), sum of squares (ss), mean squares (ms) and *F*-values are indicated (*** $P < 0.001$ and * $P < 0.05$).

	df populations /df residuals	ss populations / ss residuals	ms populations / ms residuals	*F* value
DTR	38 / 126	1.953 / 1.741	0.05139 / 0.01381	3.72***
STD	38 / 126	0.471 / 1.026	0.01239 / 0.00814	1.522*
STL	38 / 126	0.194 / 0.241	0.0051 / 0.00191	2.671***
LL	38 / 329	1.088 / 1.358	0.02862 / 0.00412	6.933***
LW	38 / 329	1.018 / 1.286	0.02679 / 0.0039	6.855***
LWr	38 / 329	0.579 / 0.859	0.01524 / 0.00261	5.836***
PL	38 / 329	1.571 / 4.128	0.04134 / 0.01255	3.295***
PW	38 / 329	1.115 / 1.163	0.02933 / 0.00353	8.298***
SLA	38 / 329	0.512 / 1.422	0.01349 / 0.00432	3.121***
NV	38 / 329	0.145 / 0.147	0.00381 / 0.00044	8.515***
LT	38 / 329	2.047 / 1.178	0.05386 / 0.00358	15.04***

Table 3. Results of Pearson's correlations between environmental variables and leaf morphological traits. Abbreviations of leaf morphological traits as indicated in Table 1. Significant values: *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$.

		Micromorphological traits			Macromorphological traits							
		DTR	STD	STL	LL	LW	LWr	PL	PW	SLA	NV	LT
	LAT	-0.41***	0.05	-0.18*	0.16**	-0.04	0.29***	0.12*	0.24***	0.12*	0.49***	0.44***
Precipitation	AI	0.26***	-0.10	0.23**	-0.15**	-0.09	-0.07	-0.10*	0.01	-0.13*	-0.36***	-0.18***
	PET	-0.36***	0.17*	-0.19*	0.21***	0.15**	0.08	0.15**	0.17**	0.18***	0.32***	0.31***
	AP	0	0	-0.11	0.04	0.09	-0.07	-0.15**	0.13*	0.07	-0.08	-0.02
	PS	0.20**	0.05	-0.02	0.07	0.21***	-0.23***	-0.11**	-0.18***	0.07	-0.19***	-0.31***
	PD	-0.29***	-0.02	-0.21**	0.16**	0.09	0.08	-0.05	0.30***	0.14**	0.22***	0.25***
Temperature	AMT	-0.14	0.09	-0.22**	0.39***	0.39***	-0.02	0.01	-0.04	0.20***	0.22***	0.04
	TS	-0.34***	0.1	-0.25***	0.31***	0.26***	0.07	0.07	0.13*	0.23***	0.39***	0.26***
	ATR	0.06	0.08	-0.05	0.11*	0.16**	-0.05	0	-0.19***	0.11*	0.17**	0.02
	MTC	-0.06	0.08	-0.20*	0.38***	0.39***	-0.05	0	-0.09	0.18***	0.14**	-0.03

The highest correlation scores with temperature variables were observed for annual mean temperature and temperature seasonality in relation to leaf length, leaf width, density of trichomes and number of veins. On the other hand, the water availability variable with the highest correlation scores was potential evapotranspiration in relation to the density of trichomes and number of veins.

Multivariate analyses. DFA of micromorphological traits (DFA_{MIC}) explained 51.3 % of the variation for the first discriminant function (DF1), and 30.5 % for the second discriminant function (DF2, Figure 4A). For the DFA_{MAC}, DF1 explained 42.4 % of the variation, and DF2 explained 24.1 % (Figure 4B). In the combined dataset, DFA_{MOR}, the DF1 explained 30 % and DF2 explained 23.3 % of the differentiation among populations (Figure 4C). All the axes were significant according to Wilk's lambda (Figure 4). From the three analyses, the highest percentage of accurate classification was obtained with DFA_{MOR} (68.1 %). In the three DFAs, the populations from northern Sierra Madre Oriental together with others from Hidalgo formed a separate group (Figure 4). In the DFA_{MIC}, the density of trichomes was the trait that contributed most to explaining the DF1, while stomatal length aperture explained the variation in DF2. In DFA_{MAC} and DFA_{MOR}, leaf thickness and leaf length presented the highest scores in discriminating populations.

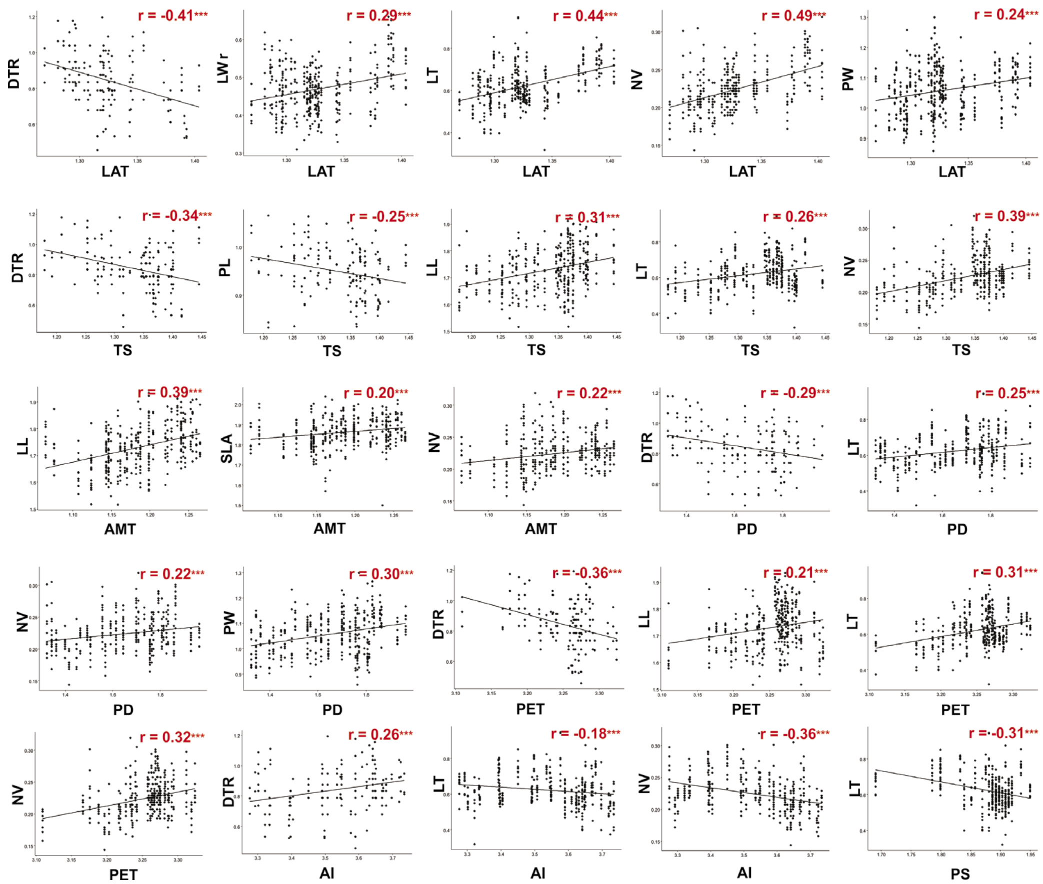

Figure 3. Graphs of Pearson's correlations between leaf morphological traits and environmental variables. Only significant correlations are presented. Abbreviations of leaf morphological traits as indicated in Table 1.

The cluster analyses allowed us to identify five groups for micromorphological traits and three groups for macromorphological traits and another three for all the morphological traits (Figure 4). The first group of the cluster with all the morphological traits comprised populations from the northern of Sierra Madre Oriental and Altiplano and southern Sierra Madre Oriental regions (Figure 4C). The traits that characterized this first group were thin leaves (range 0.17 to 0.22 mm) and wide petioles (1.31 to 1.58) (DFA_{MOR}, Figure 4C). The second group included populations from the Trans-Mexican Volcanic Belt, the southeastern Sierra Madre Oriental and one in the north. This group was characterized by high density of trichomes (8.4 to 10.56 per mm^2), large stomatal aperture (8.59 to 11.09 mm^2), and short (4.35 to 5.26 cm) and narrow (1.55 to 1.86 cm) leaves (DFA_{MOR}, Figure 4C). In the third group, most of the populations were located on the western slope of the southern Sierra Madre Oriental, in San Luis Potosí and some in the northernmost distribution (Figure 4C). The traits that characterized this group were long (5.67 to 7.29 cm) and wide (1.87 to 2.63 cm) leaves with low density of trichomes (3.5 to 7.49 per mm^2).

DFA based on environmental variables for DFA_{MIC}, explained 56.1 and 28.9 % of the variance via the first two discriminant functions, respectively; only the first function was significant (Figure 4A). The contribution of precipitation seasonality to the first discriminant axis was the most important for separating the populations; however, the groups were not recovered according to the morphofunctional traits, with 59 % of them correctly classified. For DFA_{MAC}, the first two discriminant functions explained 62.7 and 37.3 % of the variance, and neither of them was significant. The analysis that best explained the variance of the discriminant functions was DFA_{MOR}, with 78.8 and 21.2 % for the first two discriminant functions, respectively. However, only the first function was significant. The climatic variables of temperature seasonality and potential evapotranspiration were the most important for discriminating among populations according to DF1, and these variables characterized the group of populations of the western slope of the Sierra Madre Oriental. The northern Sierra Madre Oriental group was better explained by the aridity index; the Trans-Mexican Volcanic Belt + southeastern Sierra Madre Oriental group was better explained by negative scores of annual precipitation (Figure 4C).

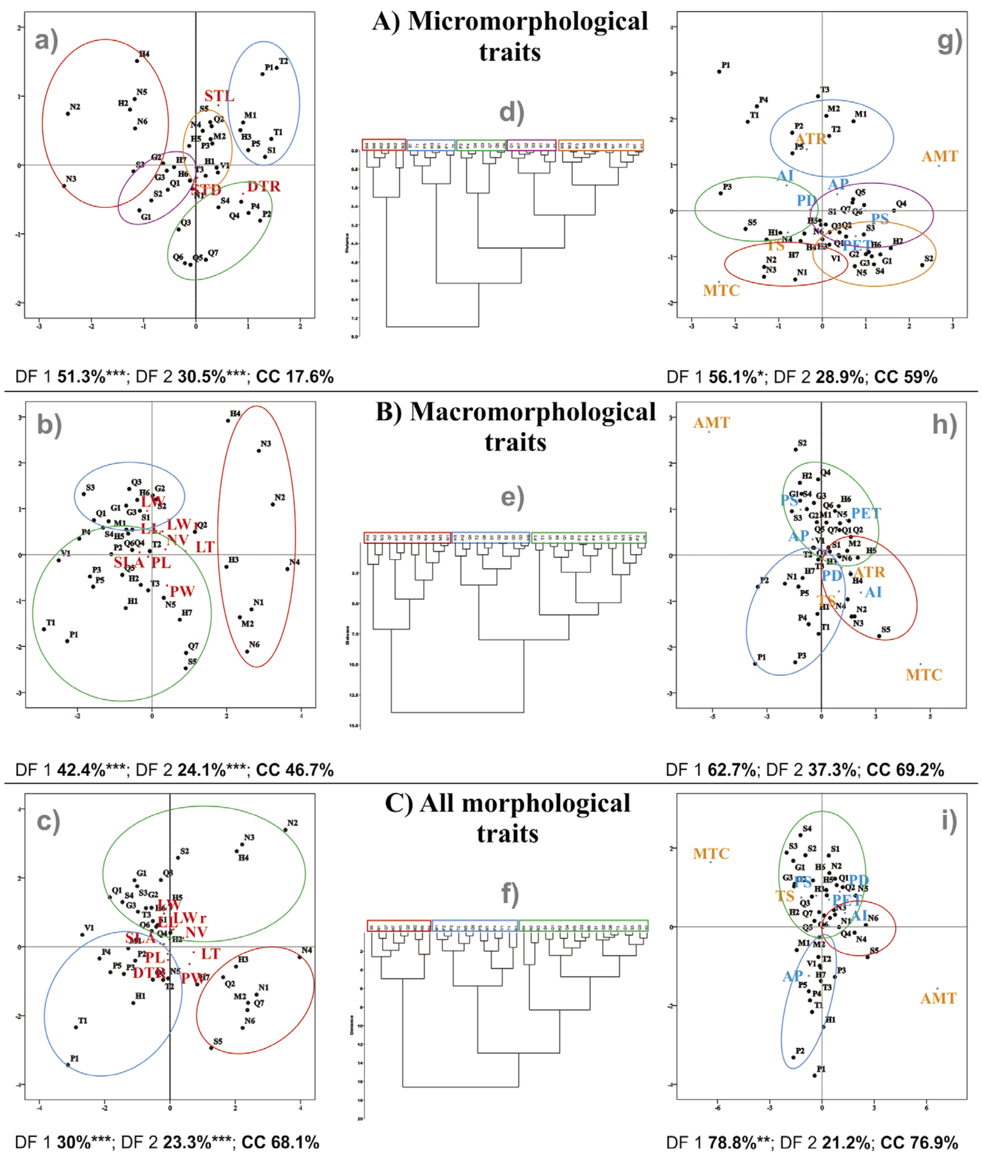

Figure 4. Discriminant function analyses (DFA) and clustering analyses (CA) for A) micromorphological traits, B) macromorphological traits and C) combined both datasets. DFA based on morphological traits (left: letters a, b, c), groups identified in the CA (center: d, e, f) and DFA based on environmental differences (right: g, h, i) are presented. Percentage of explained variation of the first two discriminant functions and CC represent the percentage of correctly classified cases in DFA. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ Abbreviations of location as shown in Figure 1 (described in Appendix 1) and of morphological traits indicated in Table 1.

In the redundancy analyses, temperature-related variables explained 2.83 % (RDA$_{MIC}$; $P > 0.05$), 4.77 % (RDA$_{MAC}$: $P < 0.001$) and 5.45 % (RDA$_{MOR}$: $P < 0.001$) of leaf variation, with significance in the last two analyses. Water-related variables significantly explained 5.0 % (RDA$_{MIC}$; $P < 0.05$), 7.47 % (RDA$_{MAC}$: $P < 0.001$) and 9.84 % (RDA$_{MOR}$: $P < 0.001$) of the leaf variation. The interaction of temperature and water variables significantly explained 18.06 % (RDA$_{MIC}$; $P < 0.001$), 17.01 % (RDA$_{MAC}$: $P < 0.001$) and 20.83 % (RDA$_{MOR}$: $P < 0.001$) of the leaf variation (Appendix 3). For the full RDA, the most important and significant environmental variable in RDA$_{MIC}$ was precipitation of driest quarter; for RDA$_{MAC}$ and RDA$_{MOR}$ the most important variables were precipitation seasonality, precipitation of driest quarter and the aridity index (Appendix 3). When plotting the full redundancy analysis results, precipitation seasonality and the aridity index were positively correlated with the density of trichomes. Potential evapotranspiration, precipitation of driest quarter, and temperature seasonality were negatively correlated with petiole width and leaf thickness (Figure 5).

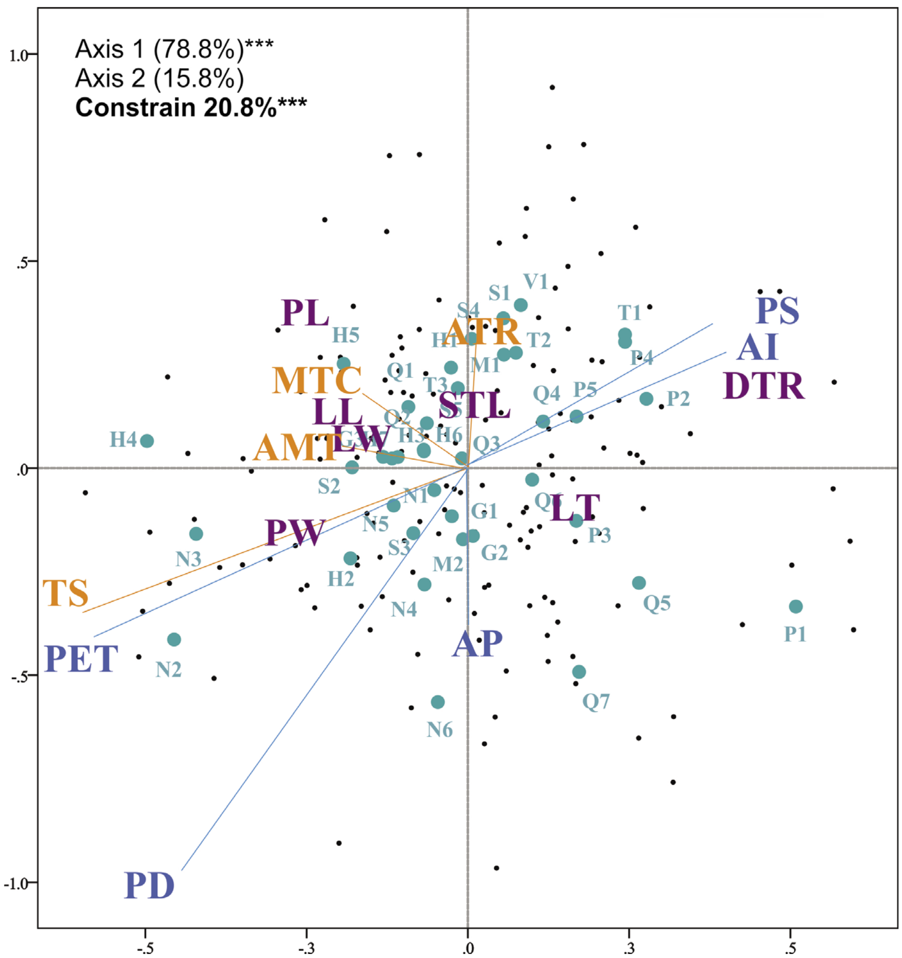

Figure 5. Full redundancy analysis with the complete data set of morphological and environmental variables (RDA$_{MOR}$). Purple letters represent morphological traits, orange letters represent temperature variables and blue letters represent precipitation variables. Dots in green indicate the populations of *Quercus mexicana* (abbreviations of localities are described in Appendix 1). Percentage of explained variation of each axis at *** $P < 0.001$.

Discussion

Leaf morphological variability. Populations of *Q. mexicana* showed variable leaf traits along their distribution, with groups of similar traits, although some populations with similar traits are not geographically proximal. Variation in leaf traits over a wide distribution area and along environmental gradients has been detected in *Q*. *rugosa* (Uribe-Salas *et al.* 2008), *Q*. *elliptica* (Maya-García *et al.* 2020) and *Q*. *deserticola* (Rodríguez-Gómez *et al.* 2018). Leaf morphological variation at the landscape level has also been observed in *Q*. *castanea* (Lara-De la Cruz *et al.* 2020).

Specifically, the northern Sierra Madre Oriental populations showed the greatest differences and were more consistent with the recovered groups; in the correlation analyses, these populations showed most of the significant differences in leaf trait variation. Indeed, latitude was positively correlated with most of the traits evaluated. A correlation with a latitudinal trend has also been found in *Q*. *rugosa* (Uribe-Salas *et al.* 2008), in which shorter leaves were present in its northern distribution. The most important traits for differentiating the northern Sierra Madre Oriental group from the rest of the populations were leaf thickness and petiole width. Leaf thickness was the most significantly different trait among the populations; this trait was also the most differentiated for *Q*. *castanea* populations at the landscape level (Lara-De la Cruz *et al.* 2020).

Relationships with environmental factors. In *Q*. *mexicana*, all the leaf traits showed correlations with variables of precipitation and temperature, except for stomatal density. In several studies, leaf variability and geographical differentiation of *Quercus* species have also been correlated with environmental variables (*e.g*., Uribe-Salas *et al.* 2008, Rodríguez-Gómez *et al.* 2018, Maya-García *et al.* 2020).

In this study, leaf thickness was correlated with temperature seasonality, indicating its association with extreme temperatures, which coincides with the northern Sierra Madre Oriental populations, and thinner leaves were associated with less arid and more seasonal environments, coinciding with the Trans-Mexican Volcanic Belt and southeastern Sierra Madre Oriental populations (Figure 2). Lara-De la Cruz *et al.* (2020) also found thinner leaves at drier sites in *Q*. *castanea* at the landscape level. Contrary to our findings, in *Q*. *elliptica*, thickness was positively correlated with precipitation seasonality (Maya-García *et al*. 2020). Higher tissue density and thickness are correlated with higher water content (Vendramini *et al.* 2002). At sites with lower water availability, leaves are generally characterized by higher tissue density (de la Riva *et al.* 2016). Thus, a higher tissue density is associated with more successful performance under water-limited conditions (Harzé *et al.* 2016), which would explain the greater thickness and petiole width characterizing the northern Sierra Madre Oriental populations.

The largest specific leaf area of *Q. mexicana* was identified at drier sites with higher evapotranspiration and at higher latitudes. This result contrasts with the expectations of a correlation between specific leaf area and rainfall (Gouveia & Freitas 2009). Commonly, a decrease in specific leaf area has been associated with drier sites (Aranda *et al.* 2014) and an increase in temperature (Lee *et al.* 2005) in different oak species. The specific leaf area of *Q. mexicana* was also correlated with temperature variables. In other plant species, high temperature has been shown to decrease water use efficiency but increases specific leaf area (Meier & Leuschner 2008). Similarly, in *Q. elliptica*, specific leaf area was associated with drier conditions (Maya-García *et al.* 2020).

The number of secondary veins showed significant differentiation among populations and a significant correlation with all the environmental variables. Vein density shows a high inter- and intraspecific variation, and a very diverse responses to a combination of environmental traits (Zhu *et al.* 2012). For instance, higher vein density has been associated with higher sun exposure (Sack & Frole 2006) and drier soils for some species (Dunbar-Co *et al.* 2009). In other species, higher vein density is found under more humid conditions (Zhu *et al.* 2012). For *Q. mexicana*, the correlations suggested that leaves with a larger number of secondary veins were associated with humidity and less marked precipitation seasonality, but leaves with a larger number of secondary veins were also associated with higher potential evapotranspiration and more marked temperature seasonality.

Among the micromorphological traits, the density of trichomes was the trait that showed the highest discrimination coefficient among populations of *Q. mexicana*. Populations with the highest trichome density were identified at lower latitudes, at sites with marked precipitation seasonality and less evapotranspiration, characterizing the populations of the southern Sierra Madre Oriental and Trans-Mexican Volcanic Belt (Figures 2, 3). Patterns of trichome density observed in other plant species indicate that greater density is associated with drier conditions (Pérez-Estrada *et al.* 2000, Benz & Martin 2006) or lower temperatures (Agrawal *et al.* 2004). Our results showed an inverse pattern of trichome density; for *Q. mexicana* lower trichome density characterized populations in the northern Sierra Madre Oriental located at sites with drier conditions and extreme high temperatures.

Although stomatal size and its density are relatively plastic traits (Richardson *et al.* 2001), we did not find significant differences among populations in stomatal density, nor did stomatal size or density show significant correlations with most of the environmental variables; only a significant correlation with potential evapotranspiration was found, as has been shown in other oak species (Nóbrega & Pereira 1992). Stomatal aperture length correlations with environmental variables indicate that larger stomata are associated with more humid conditions with less temperature seasonality. A small aperture length can provide a reduction in total pore area, facilitating faster closure in response to drier environments (Lawson & Blatt 2014), which would explain the presence of a larger stomatal aperture length in populations of the Trans-Mexican Volcanic Belt and southeastern Sierra Madre Oriental, where the conditions are more humid than those of the northern Sierra Madre Oriental.

On the other hand, the relationships that are established between micro- and macromorphological traits and environmental variables are very interesting and can be summarized by the RDA. First, the densities of trichomes and stomata are better explained by the seasonality of temperature and the availability of water. The size of the leaves seems to respond to changes in temperature; larger leaves and wider petioles are found in places with extreme temperature conditions, and the seasonality of temperature seems to be a key variable in the morphological variation and differentiation of *Q. mexicana* populations. Global trends indicate that leaves are generally smaller at drier and warmer sites, or at colder and wetter sites (Wright *et al.* 2017). The presence of larger leaves under drier or colder conditions is possible because of the thermoregulatory capability of leaves (Meier & Leuschner 2008, Michaletz *et al.* 2016): larger leaves can warm up fast on colder mornings, allowing better photosynthetic performance (Wright *et al.* 2017).

Even though we found that morphology was significantly correlated with environmental variables, the RDAs explained a relatively low, although significant percentage of the variation, specifically, when temperature and water variables were considered separately. This finding may be due to multiple factors, such as weak selection, extensive gene flow among populations (Riordan *et al.* 2016) or the effect of other environmental variables on populations, such as geomorphology or soil types (Wright *et al.* 2017). The global patterns of leaf change in relation to climatic variables also explain a small but significant amount of the variation, according to the evaluation of Wright *et al.* (2017).

To better understand the observed relationship between morphology and environmental variation and to assess if any of the aforementioned factors affect this relationship, it is necessary to assess the extent of genetic differentiation among the *Q. mexicana* populations. For example, in *Q. deserticola*, leaf variation is correlated with the environmental gradient of the Trans-Mexican Volcanic Belt but not with the genetic variation of the populations; phenotypic plasticity, common ancestry or adaptative differences could explain the environmental responses of the leaf variation (Rodríguez-Gómez *et al.* 2018).

Potential impacts of climate change. The morphological variability and differentiation among populations indicate species adaptability to environmental changes. Our results show a wide range of responses of *Q. mexicana* populations given the high environmental diversity of the distribution range of the species. Some trait variations seem to be restricted to specific climatic conditions or geographical areas, while other traits vary throughout the range without a specific pattern.

In Mexico, oak species have been moderately impacted by recurring climate changes caused by decreases in temperature coupled with conditions of moderated humidity throughout the Tertiary and Quaternary (Graham 1993). Palynological records indicated recurrent expansion–contraction changes in the communities of temperate plants during the Pleistocene (1.8 Ma to 10,000 years ago) in central Mexico (Lozano-García & Vázquez-Selem 2005). These outcomes suggested that species must have survived Pleistocene glaciations in areas with favorable climates at lower altitudes in the mountain major ranges of Mexico, and migration to higher elevations would have occurred during warm periods (Toledo 1982, Metcalfe *et al.* 2010). The effects of these climatic fluctuations on oak species in Mexico lead to more diversity and less genetic structure but larger historical population sizes and a complex exchange among species (González-Rodríguez *et al.* 2004, Tovar-Sánchez *et al.* 2008).

A recent assessment of the climatic trends between 1910 and 2009 in Mexico (Cuervo-Robayo *et al.* 2020) indicates that warmer and drier conditions can be expected in the species distribution area in the future. The Mexican Transition Zone, which includes the Sierra Madre Oriental and the Trans-Mexican Volcanic Belt, showed an increase of 0.03 °C in the annual mean temperature between 1950 and 2009 and a decrease of 12 mm in annual rainfall. If these trends continue, it is probable that populations of *Q. mexicana* adapted to drought or extreme temperatures will respond better to these climate changes. However, populations with morphological traits best adapted to humid conditions could be negatively affected.

Morphological and functional leaf traits show a plastic response in plants. In *Q. mexicana*, apart from plasticity, other factors could influence the variation in these traits. In the case of this endemic species, populations could face pressures from future climate changes, and a reduction in phenotypic plasticity and, consequently the genetic diversity of the species can be expected. Integrating data on the genetic structure of the species could provide a complete perspective on the future outcome for this endemic oak.

Acknowledgements

This paper was improved by the revisions of two anonymous reviewers. We thank Víctor Rocha Ramírez, Esther Aguilar, Rafael Aguilar Romero, Celia Sanginés and Cristopher Ortega for technical assistance in the field work. This paper constitutes a partial fulfillment of the Graduate Program in Biological Sciences of the National Autonomous University of Mexico (UNAM) by VSA. VSA received a Ph.D. scholarship 348092 from CONACyT. This project was supported by PAPIIT/PAPIME-DGAPA (UNAM) project RV201015, IA208218, PE216619 and CONACYT 240136.

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Associate editor: Monserrat Vázquez Sánchez

Author contributions: KO, VSA and ATM designed the study; VSA and ATM performed the fieldwork and laboratory analysis; VSA, ATM, CGA and KBHE conducted the statistical analyses; KO and ATM supervised the whole project; and KO, VSA, ATM, KBHE and CGA wrote the manuscript.

Appendix 1. Localities of populations of *Quercus mexicana* sampled for this study.

Population	Name of the locality	Elevation (m)	Latitude	Longitude	Aridity index
G1	Palomas, Guanajuato	1,860	21.39640	-99.89830	0.2239
G2	Xichú, Guanajuato	1,939	21.29510	-99.88830	0.2151
G3	Atarjea, Guanajuato	2,119	21.18930	-99.89180	0.3316
H1	Mineral del Chico, Hidalgo	2,806	20.14250	-98.69390	0.3923
H2	Cardonal, Hidalgo	2,364	20.67260	-99.14310	0.2873
H3	Cerro Prieto, Hidalgo	2,114	20.80370	-99.19970	0.3069
H4	Jacala, Hidalgo	1,921	20.91900	-99.20940	0.3641
H5	La Colorada, Hidalgo	1,677	21.02410	-99.13110	0.3906
H6	Zimapán, Hidalgo	2,132	20.79380	-99.32900	0.2483
H7	Apulco, Hidalgo	2,187	20.29770	-98.34530	0.3779
M1	Magú, Estado de México	2,396	19.68500	-99.31750	0.3043
M2	Jilotepec, Estado de México	2,512	19.92820	-99.55720	0.3487
N1	Laguna de Sánchez, Nuevo León	1,960	25.32250	-100.25840	0.2634
N2	Bosque Escuela, Nuevo León	1,619	24.70690	-99.86110	0.2232
N3	Pablillo, Nuevo León	2,131	24.59260	-99.97700	0.2592
N4	Puerto las Ánimas, Nuevo León	2,335	24.46710	-99.91170	0.2876
N5	San Josecito, Nuevo León	2,577	23.92670	-99.96240	0.2572
N6	Peña Nevada, Nuevo León	2,637	23.82380	-99.87980	0.2939
P1	Nicolás Bravo, Puebla	1,836	18.60450	-97.29620	0.3124
P2	El Seco, Puebla	2,480	19.11220	-97.57540	0.2399
P3	Tetela de Ocampo, Puebla	2,052	19.81000	-97.76480	0.3761
P4	Chignahuapan, Puebla	2,439	19.77470	-98.16360	0.2426
P5	Cuauhtémoc, Puebla	2,373	19.80540	-98.08140	0.2512
Q1	Landa de Matamoros, Querétaro	1,688	21.25060	-99.18750	0.4726
Q2	El Madroño, Querétaro	1,853	21.27930	-99.17000	0.5083
Q3	Pinal de Amoles, Querétaro	2,122	21.15440	-99.60800	0.3444
Q4	Cerro Pinguical, Querétaro	2,553	21.12510	-99.67830	0.4153
Q5	Cadereyta, Querétaro	2,289	20.87640	-99.59540	0.3917
Q6	San Joaquín, Querétaro	2,406	20.93230	-99.55560	0.4311
Q7	Cerro Boludo, Querétaro	2,255	20.92960	-99.52780	0.4379
S1	Guadalcázar, San Luis Potosí	1,999	22.66740	-100.41700	0.2014
S2	Valle de los Fantasmas, San Luis Potosí	2,340	22.09620	-100.65930	0.1411
S3	San Luis Potosí, San Luis Potosí	2,046	22.05520	-100.58100	0.1760
S4	Armadillos de los Infantes, San Luis Potosí	1,920	22.18840	-100.68790	0.1485
S5	Real de Catorce, San Luis Potosí	2,876	23.60610	-100.86710	0.1491
T1	Lázaro Cárdenas, Tlaxcala	2,460	19.55080	-97.96310	0.2489
T2	El Verde, Tlaxcala	2,296	19.33060	-98.45980	0.2634
T3	Atotonilco, Tlaxcala	2,389	19.38920	-98.45260	0.2766
V1	Agua Bendita, Veracruz	2,124	20.36910	-98.51540	0.4273

Appendix 2. Means and standard deviations of all morphological traits of the 39 populations of *Quercus mexicana*. Abbreviations of the populations as in Appendix 1 and for morphological traits as indicated in Table 1.

Pop	DTR (No/mm²)	STD (No/mm²)	STL (mm)	LL (cm)	LW (cm)	LWr	PL (cm)	PW (cm)	SLA (g/cm²)	NV	LT (mm)
G1	5.794 / 1.275	392.604 / 66.872	8.028 / 1.808	5.5921 / 1.1148	2.0354 / 0.4142	2.846 / 0.422	0.4825 / 0.1332	0.1087 / 0.0404	69.823 / 7.842	13.156 / 1.021	0.28 / 0.048
G2	5.998 / 0.57	311.902 / 73.401	8.381 / 1.687	6.0126 / 1.0175	2.0986 / 0.3844	2.982 / 0.498	0.4863 / 0.1173	0.1125 / 0.0329	75.895 / 16.7	13.36 / 1.588	0.241 / 0.044
G3	5.907 / 1.812	316.264 / 62.076	8.887 / 1.706	6.313 / 1.0589	2.2476 / 0.3502	2.861 / 0.396	0.5968 / 0.1608	0.1302 / 0.0436	77.638 / 12.211	12.889 / 1.655	0.265 / 0.044
H1	8.009 / 1.704	343.529 / 50.763	9.004 / 1.47	5.2668 / 1.0665	1.6786 / 0.2913	3.273 / 0.471	0.697 / 0.2023	0.1263 / 0.0487	80.94 / 16.298	11.64 / 1.258	0.279 / 0.076
H2	3.992 / 2.098	344.308 / 80.844	9 / 1.909	5.6735 / 1.1733	1.9035 / 0.2981	2.916 / 0.422	0.5443 / 0.1892	0.1333 / 0.0368	77.037 / 18.435	12.14 / 1.212	0.256 / 0.039
H3	7.907 / 1.329	407.872 / 79.619	9.749 / 2.017	5.6151 / 1.1343	1.9089 / 0.4095	2.899 / 0.349	0.5906 / 0.1501	0.1289 / 0.0481	71.087 / 12.748	12.778 / 1.223	0.183 / 0.03
H4	4.544 / 1.113	321.717 / 52.415	9.718 / 1.608	7.2954 / 1.5236	2.631 / 0.5565	2.789 / 0.343	0.5626 / 0.2217	0.1216 / 0.0513	78.499 / 24.887	12.88 / 1.662	0.174 / 0.05
H5	6.543 / 2.955	318.446 / 62.363	9.084 / 1.897	6.3986 / 1.1821	2.2187 / 0.4054	3.055 / 0.472	0.5506 / 0.2044	0.1422 / 0.0327	79.438 / 14.978	11.98 / 1.237	0.279 / 0.053
H6	7.634 / 2.281	381.698 / 75.949	8.704 / 1.45	6.1099 / 1.0355	2.1641 / 0.3368	2.926 / 0.355	0.499 / 0.1399	0.1197 / 0.0408	78.478 / 12.523	12.6 / 1.784	0.246 / 0.036
H7	6.362 / 1.113	356.252 / 118.8	8.541 / 1.764	5.1383 / 1.0352	1.7956 / 0.3941	3.049 / 0.455	0.5152 / 0.1709	0.1353 / 0.0317	71.937 / 14.969	11.422 / 1.469	0.225 / 0.039
M1	8.452 / 3.752	359.887 / 27.804	9.79 / 2.097	5.2381 / 0.8897	1.6285 / 0.2252	3.337 / 0.51	0.6068 / 0.1813	0.0945 / 0.0359	76.664 / 14.762	12.6 / 2.06	0.241 / 0.037
M2	7.157 / 0.94	376.246 / 76.106	9.361 / 1.455	4.7339 / 0.7143	1.6869 / 0.3392	3.086 / 0.501	0.4739 / 0.1391	0.131 / 0.038	83.758 / 14.952	13.72 / 1.565	0.182 / 0.039
N1	7.225 / 1.413	403.51 / 42.936	8.713 / 1.908	5.2196 / 0.9576	1.6703 / 0.3362	3.324 / 0.481	0.5582 / 0.1353	0.1378 / 0.0349	72.426 / 31.906	14.174 / 2.224	0.2 / 0.033
N2	3.522 / 0.513	336.258 / 54.492	8.713 / 1.525	6.4937 / 1.423	1.8954 / 0.3869	3.516 / 0.468	0.46 / 0.1452	0.1303 / 0.0322	87.066 / 15.736	14.378 / 1.898	0.173 / 0.032
N3	3.976 / 0.797	327.17 / 31.608	8.838 / 1.759	7.1483 / 1.4268	2.0479 / 0.3813	3.681 / 0.602	0.6563 / 0.1943	0.1265 / 0.0332	79.016 / 17.047	15.26 / 1.882	0.192 / 0.034
N4	6.816 / 0.862	327.17 / 60.13	9.221 / 1.797	5.5534 / 0.9871	1.5976 / 0.3244	3.789 / 0.564	0.4939 / 0.1277	0.1354 / 0.0421	78.437 / 13.922	15.58 / 1.885	0.185 / 0.034
N5	4.771 / 0.862	303.541 / 50.41	9.185 / 1.87	4.6277 / 0.7657	1.5542 / 0.2805	3.076 / 0.326	0.5206 / 0.1175	0.0995 / 0.0274	66.186 / 8.777	13.275 / 1.485	0.234 / 0.033
N6	4.998 / 0.704	310.812 / 51.5	8.716 / 1.475	4.4915 / 0.9527	1.6378 / 0.3608	3.014 / 0.588	0.4575 / 0.193	0.1343 / 0.0368	76.611 / 10.096	13.471 / 1.689	0.179 / 0.032
P1	8.634 / 1.862	239.925 / 45.752	11.095 / 2.518	4.3556 / 1.1594	1.721 / 0.304	2.642 / 0.298	0.3193 / 0.1053	0.1143 / 0.0407	69.257 / 11.535	10.629 / 1.477	0.307 / 0.068

Pop	DTR (No/mm²)	STD (No/mm²)	STL (mm)	LL (cm)	LW (cm)	LWr	PL (cm)	PW (cm)	SLA (g/cm²)	NV	LT (mm)
P2	10.565 / 2.683	341.711 / 59.199	8.642 / 2.259	5.0959 / 0.6614	1.7479 / 0.4473	3.186 / 0.603	0.4592 / 0.1244	0.1038 / 0.0383	67.532 / 18.844	12.88 / 2.201	0.291 / 0.058
P3	7.498 / 1.879	287.183 / 76.702	9.197 / 1.663	5.1335 / 1.2579	1.8028 / 0.4058	2.97 / 0.331	0.4702 / 0.1653	0.1159 / 0.0568	76.323 / 19.804	12.218 / 1.031	0.301 / 0.076
P4	10.224 / 2.769	285.729 / 93.624	8.59 / 2.134	5.0986 / 0.6526	2.0504 / 0.3115	2.582 / 0.349	0.48 / 0.1326	0.0996 / 0.0306	63.133 / 6.629	11.86 / 1.107	0.283 / 0.04
P5	9.031 / 3.402	370.793 / 32.105	9.591 / 2.099	4.9722 / 1.0411	1.8652 / 0.415	2.703 / 0.423	0.5033 / 0.1458	0.1075 / 0.0347	64.033 / 8.813	11.222 / 1.46	0.275 / 0.047
Q1	6.702 / 1.323	261.736 / 41.955	8.233 / 1.319	5.896 / 1.2316	2.2709 / 0.4319	2.646 / 0.33	0.5075 / 0.1531	0.1191 / 0.036	77.892 / 15.627	12.24 / 1.08	0.281 / 0.055
Q2	6.816 / 1.879	287.183 / 46.61	9.502 / 1.834	5.9082 / 1.0694	2.2256 / 0.4285	2.773 / 0.388	0.516 / 0.1956	0.1322 / 0.0413	71.042 / 13.507	12.675 / 1.095	0.214 / 0.067
Q3	7.384 / 1.799	338.076 / 112.281	8.274 / 1.787	6.3867 / 1.3424	2.1795 / 0.5791	3.018 / 0.437	0.5319 / 0.1508	0.1169 / 0.0389	80.274 / 14.235	13.311 / 1.474	0.264 / 0.049
Q4	8.52 / 2.683	334.441 / 45.578	8.731 / 1.624	5.8818 / 1.0165	2.1336 / 0.296	2.863 / 0.398	0.5375 / 0.1541	0.1376 / 0.055	70.084 / 14.625	12.275 / 1.664	0.271 / 0.047
Q5	8.588 / 4.246	390.423 / 37.303	8.046 / 1.46	4.4205 / 0.8421	1.7337 / 0.4223	2.693 / 0.358	0.3776 / 0.1395	0.092 / 0.0296	74.837 / 7.653	12.86 / 1.485	0.235 / 0.036
Q6	7.952 / 2.184	399.875 / 45.578	7.749 / 1.366	5.4635 / 1.0438	1.8706 / 0.2935	3.004 / 0.425	0.4703 / 0.0997	0.1114 / 0.0532	74.726 / 11.791	13.267 / 1.195	0.262 / 0.044
Q7	8.747 / 2.086	359.887 / 81.319	8.007 / 1.832	4.9914 / 1.007	1.8092 / 0.306	2.917 / 0.436	0.3523 / 0.1191	0.1585 / 0.0442	75.112 / 16.535	12.709 / 1.329	0.242 / 0.041
S1	10.769 / 3.388	355.525 / 47.911	9.106 / 1.818	5.9741 / 1.2052	1.909 / 0.4515	3.235 / 0.478	0.6536 / 0.1943	0.1129 / 0.0443	75.046 / 13.3	13.76 / 1.117	0.263 / 0.043
S2	6.134 / 1.493	359.887 / 68.974	8.529 / 1.774	6.0559 / 1.3826	2.1064 / 0.4883	2.8 / 0.482	0.5327 / 0.1677	0.1134 / 0.0475	89.336 / 14.586	13.92 / 2.078	0.225 / 0.048
S3	5.725 / 2.486	296.634 / 42.518	8.452 / 1.359	5.7303 / 1.3163	2.2946 / 0.443	2.731 / 0.424	0.5046 / 0.1856	0.112 / 0.044	83.732 / 12.361	12.78 / 1.183	0.291 / 0.086
S4	8.52 / 2.467	361.705 / 73.455	8.62 / 1.695	5.7973 / 1.2058	2.1037 / 0.4714	2.817 / 0.385	0.5975 / 0.1783	0.1134 / 0.0333	73.031 / 11.168	12.44 / 1.248	0.277 / 0.048
S5	7.157 / 2.101	321.717 / 61.595	9.493 / 1.686	4.4635 / 0.8355	1.7054 / 0.3208	2.863 / 0.476	0.533 / 0.1079	0.1384 / 0.0461	62.188 / 8.621	11.4 / 1.074	0.217 / 0.033
T1	9.656 / 2.338	332.623 / 27.373	9.767 / 1.769	4.556 / 0.688	1.8031 / 0.33016	2.582 / 0.338	0.5165 / 0.1886	0.1096 / 0.0372	63.739 / 9.719	10.06 / 1.132	0.324 / 0.087
T2	10.338 / 3.842	287.183 / 57.982	10.584 / 1.733	5.5196 / 0.8632	1.8148 / 0.2922	3.189 / 0.4	0.5074 / 0.1496	0.1142 / 0.0265	72.943 / 9.799	11.92 / 1.259	0.242 / 0.044
T3	7.498 / 1.181	351.163 / 74.527	9.068 / 1.584	5.1321 / 1.6556	1.8176 / 0.3127	3.145 / 0.544	0.5361 / 0.1211	0.1246 / 0.0332	75.752 / 28.49	12.31 / 1.906	0.257 / 0.073
V1	8.406 / 2.538	270.824 / 92.13	8.401 / 1.57	5.0751 / 1.2306	2.0387 / 0.4175	2.58 / 0.395	0.5141 / 0.1558	0.1025 / 0.0326	64.196 / 11.755	11.114 / 1.623	0.301 / 0.054

Appendix 3. Partial redundancy analyses for micromorphological traits (RDA_{MIC}), macromorphological traits (RDA_{MAC}) and both set of traits (RDA_{MOR}) including temperature-related variables (pRDA1) and water availability-related variables (pRDA2); and full redundancy analysis (fullRDA) including all environmental variables. Total and constrained variance (λ), and *F* statistic ($P < 0.001$***, $P < 0.01$** and $P < 0.05$*) of axes 1 and 2 and environmental variables included in the analysis and respective R^2adj. Percentage of constrained variance (λ%) indicates the proportion of morphological variation explained by the environmental variables. Those variables or axes with higher statistical significance are in bold. Abbreviations of morphological traits as indicated in Table 1.

		RDA_{MIC}			**RDA_{MAC}**			**RDA_{MOR}**		
		λ	**%**	***F***	**λ**	**%**	***F***	**λ**	**%**	***F***
pRDA1 (Temperature)	Total	0.0343	100		0.0534	100		0.0864	100	
	Constrained	0.001	2.83	1.3384	0.0025	**4.77**	**6.45*****	0.0047	**5.45**	**2.63*****
	R^2adj	0. 007			0.038			0.035		
	Axis 1	0.0008	87.17	4.69	0.0018	**72.29**	**14.88*****	0.0037	**78.08**	**8.22*****
	Axis 2	0.0001	0.97	0.52	0.0006	22.13	4.56*	0.0007	15.81	1.67
	Cumulative	0.0009	96.85		0.0024	94.42		0.0044	93.89	
	AMT	0.0001	12.41	0.66	0.0012	**48.14**	**9.91*****	0.0026	**54.99**	**5.79*****
	TS	0.0003	30.44	1.63	0.0004	17.01	3.50**	0.0006	12.26	1.29
	ATR	0.0004	44.93	2.40	0.0005	21.64	4.45**	0.001	21.67	2.28*
	MTC	0.0001	12.21	0.65	0.0003	13.21	2.72*	0.0005	11.07	1.17
pRDA2 (Water availability)	Total	0.0343	100		0.0534	100		0.0864	100	
	Constrained	0.0017	**5.00**	**1.89***	0.004	**7.47**	**6.45*****	0.0085	**9.84**	3.80***
	R^2adj	0.024			0.063			0.074		
	Axis 1	0.0011	62.51	5.99	0.0027	**66.21**	**21.35*****	0.0059	**69.55**	**13.23*****
	Axis 2	0.0005	28.94	2.77	0.001	24.33	7.85**	0.0015	17.8	3.38
	Cumulative	0.0016	91.45		0.0037	90.55		0.0074	87.35	
	AI	0.0005	27.21	2.58	0.0014	**34.53**	**11.13*****	0.0032	**38.08**	**7.24*****
	PET	0.0007	**39.36**	**3.72***	0.0007	**17.54**	**5.66*****	0.0016	18.7	3.56**
	AP	0.0005	28.3	2.67	0.0009	**23.65**	**7.63*****	0.0019	22.95	4.36**
	PS	0.0000	1.71	0.16	0.0006	14.36	4.63**	0.0006	7.52	1.43
	PD	0.0000	3.39	0.32	0.0004	9.88	3.19*	0.0011	1.27	2.43*

		RDA_{MIC}			**RDA_{MAC}**			**RDA_{MOR}**		
		λ	**%**	***F***	**λ**	**%**	***F***	**λ**	**%**	***F***
Full RDA (Temperature + Water)	Total	0.0343	100		0.0534	100		0.0864	100	
	Constrained	0.0062	**18.06**	**3.80*****	0.0091	**17.01**	**8.15*****	0.018	**20.83**	**4.47*****
	R^2adj	0.133			0.149			0.162		
	Axis 1	0.0051	**83.01**	**29.46*****	0.0047	**51.25**	**37.71*****	0.0104	**58.02**	**23.36*****
	Axis 2	0.0007	11.79	4.18	0.0026	**28.96**	**21.31*****	0.0031	17	6.85**
	Cumulative	0.0058	94.8		0.0073	80.22		0.0135	75.03	
	AMT	0.0001	1.94	0.66	0.0012	*13.51*	9.91***	0.0026	14.4	5.79***
	TS	0.0001	1.91	0.65	0.0004	4.77	3.5**	0.0006	3.21	1.29
	ATR	0.0004	7.04	2.40	0.0005	6.07	4.45**	0.001	5.67	2.28*
	MTC	0.003	4.77	1.63	0.0003	3.71	2.71*	0.0005	2.89	1.17
	AI	0.0005	8.78	3.00	0.0014	**15.58**	**11.43*****	0.0031	**17.04**	**6.86*****
	PET	0.001	15.63	5.34**	0.0008	8.56	6.28***	0.0022	12.35	4.97***
	AP	0.0000	0.49	0.17	0.0006	6.13	4.50***	0.0008	4.61	1.86
	PS	0.0011	17.82	6.09**	0.002	**21.82**	**16.01*****	0.03	**16.65**	**6.7*****
	PD	0.0026	**41.59**	**14.21*****	0.0018	**19.84**	**14.56*****	0.0042	**23.16**	**9.33*****
WATER ∩ TEMP		**0.0035**	**10.23**		**0.0025**	**4.76**		**0.0048**	**5.53**	