Genetic Variation in *Abies religiosa* for Quantitative Traits and Delineation of Elevational and Climatic Zoning for Maintaining Monarch Butterfly Overwintering Sites in Mexico, considering Climatic Change

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Abstract

Conservation of *Abies religiosa* (sacred fir) within the Monarch Butterfly Biosphere Reserve (MBBR) in Mexico requires adaptive management to cope with expected climatic change, in order to have healthy trees for *Danaus plexippus* overwintering sites in the future. Open pollinated seeds from fifteen *A. religiosa* populations were collected along an elevational gradient (2850-3550 masl; one sampled population every 50 m of elevational difference). Seedlings were evaluated in a common garden test over a period of 30 months. We found significant differences (P < 0.03) among populations in total elongation, final height, date of growth cessation, foliage, stem and total dry weight, as well as frost damage. These differences were strongly associated with the Mean Temperature of the Coldest Month (MTCM; $r^2 = 0.6222$, P = 0.0005). Seedlings originating from lower elevation populations grew more but suffered more frost damage than those from higher elevations. Populations differentiate genetically when they are separated by 364 m in elevation. Such differentiation was used to delineate three elevational/climatic zones for seed collection, with limits defined at: 2650 masl or 9.7 °C of MTCM; 3000 masl or 8.5 °C; 3350 masl or 7.3 °C; and 3700 masl or 6.1 °C. Zonification for seedling deployment aiming to match a suitable climate in year 2030 (after projections using an ensemble of 18 General Circulation Models and a Representative Concentration Pathway 6.0 watts/m²), would have the same MTCM zone limits, but shifted 350 m upwards in elevation. This shift would exceed the highest elevations within the MBBR, necessitating the establishment of *A. religiosa* stands outside the MBBR, to serve as potential future overwintering sites.

Keywords: *altitudinal gradient, growth timing, seed zoning, assisted migration, provenance, common garden, frost damage.*

Introduction

Abies religiosa (Kunth) Schltdl. & Cham. (sacred fir or oyamel) is distributed at high elevations, mostly along the Trans-Mexican Volcanic Belt, of between 2800 and 3500 masl (RZEDOWSKI and RZEDOWSKI, 2005; BENAVIDEZ-MEZA et al., 2011). Their dense and pure stands located between 3000 and 3300 masl serve as the exclusive overwintering sites for the migratory Monarch Butterfly (*Danaus plexippus*), within the Monarch Butterfly Biosphere Reserve (MBBR), on the border between the Michoacán and Mexico states in central-western Mexico. This is the longest distance insect migration known, since the same generation travels from southern Canada to Mexico and then back to Texas (ANDERSON and BROWER, 1996; OBERHAUSER and PETERSON, 2003). Consequently, Mexican overwintering sites are one of the highest priorities for biological conservation in the three countries (Canada, USA and Mexico).

Abies religiosa populations exhibit morphological differentiation for cone size and needle length along the elevational gradient (CASTELLANOS-ACUÑA et al., 2014). This differentiation is likely to have a genetic basis due to the selective force imposed by the climate gradient, as has been demonstrated for several conifers in North America and Mexico (some examples are: REHFELDT 1988; REHFELDT et al., 2014a; 2014b; RUIZ-TALONIA et al., 2014; LOYA-REBOLLAR et al., 2013). At landscape level, it was found for *A. religiosa* that genetic variation in

four amplified fragment length polymorphisms (AFLPs) and three chloroplast microsatellites (cpSSRs) was correlated to environmental and geographic distances, indicating disruptive selection due to environmental differences (MÉNDEZ-GONZÁLEZ et al 2017). Where populations are adapted to local environments, they may become decoupled from suitable climatic habitat due to the effects of climatic change (SÁENZ - ROMERO et al., 2016).

A recent study has illustrated the magnitude of the expected reduction of suitable climatic niche for *A. religiosa*: 69 % by the decade centered on 2030, 88 % for that of 2060 and 97 % for that of 2090. In the Monarch Butterfly Biosphere Reserve (MBBR) of central-western Mexico in particular, the suitable climatic niche for *A. religiosa* will cease to exist by the end of the century (SÁENZ-ROMERO et al., 2012a). Disappearance of suitable climate habitat will be manifested as a severe drought stress, inducing susceptibility to disease and pest attacks, especially in those populations distributed at the lower elevational extreme, or "xeric limit" (MÁTYÁS, 2010), and will eventually cause massive forest decline (BRESHEARS et al., 2005; ALLEN et al., 2010). Trees with signs of decline due to climate-change associated drought stress have already been observed inside the MBBR (SÁENZ-ROMERO et al., 2012a)

Thus, forest tree populations must adapt to these climatic changes at the speed at which global warming is occurring. Their capacity for adaptation will depend on phenotypic variation, seed dispersal, high fecundity, biotic interactions, strength of selection for local adaptation and facilitated migration (AITKEN et al., 2008; FLORIAN et al., 2013). Their possible responses are to adapt by phenotypic plasticity, to migrate by colonization mechanisms, or to perish (AITKEN et al., 2008). Change in the pattern of disturbances, such an increase of frequency and intensity of fires or even more landscape fragmentation, will produce more difficulties in terms of counteracting and adapting to the climatic changes (MILLAR et al., 2007).

Human-assisted movement of tree populations to their suitable climatic habitat, or assisted migration, may represent an opportunity to mitigate the adverse effects of climatic change (SÁENZ-ROMERO et al., 2016). This management strategy consists of using reforestation programs to move natural populations towards sites where the future climate is predicted to be suitable, with the aim of decreasing environmental-climatic pressures and increasing the population resilience (SPITTLEHOUSE and STEWART, 2003).

In order to reduce the risks of maladaptation of the migrated populations, information pertaining to the amount and patterning of genetic differentiation among populations is required, as well as determining the width of the climate niche to which they are adapted. Such information will inform decisions about the climate transfer distance (difference between the climate at seed source and climate of the target planting site; SÁENZ-ROMERO et al., 2017) over which it is safe to move the migrated germplasm. In mountain regions, where the suitable climatic habitat will occur in the future at higher elevations (LENOIR et al., 2008), elevational zonification for matching both contemporary and future projected climate is a useful management tool for matching the contemporary seed source with sites of suitable future climate (RUIZ-TALONIA et

al., 2014). Genetic differentiation among populations along altitudinal gradients can be expressed in common garden tests (REHFELDT 1988; VITASSE et al., 2009)

The objective of this study was to determine whether genetic variation exists among *Abies religiosa* populations originated from an elevational gradient and, if so, to delineate contemporary and future elevational/climate bands for use in a zonification to match contemporary genotypes with future climates. This objective is a prerequisite for the establishment of healthy *A. religiosa* populations that could serve as future overwintering sites for migrating Monarch butterfly populations.

Material and Methods

Seed collection and test establishment

Cones of open-pollinated trees were collected on San Andrés mountain in Michoacán, central-western Mexico. The germplasm was obtained on the northwest slope, along an elevational gradient from 2850 masl to 3550 masl, with one population sampled every 50 m of elevational difference (around 11 randomly selected trees per population), in order to fully sample the natural range of elevational distribution (*Figure 1*). The trees represented by these samples are referred to as populations and the location of each population is called a provenance. We consider the terms altitude and altitudinal as fully equivalent to elevation and elevational, respectively; we will use the latter herein, as suggested by MCVICAR AND KÖRNER (2013).

Table 1

Geographic coordinates and elevational location of 15 sampled *Abies religiosa* populations on San Andrés Mountain, municipality of Hidalgo, Michoacán state, Mexico.

| Population	Elevation (masl)	Latitude (N)	Longitude (W)
1	3,550	19° 48′ 01.0″	100° 36′ 04.7″
2	3,500	19° 48′ 03.6″	100° 36′ 06.8″
3	3,450	19° 48′ 06.3″	100° 36′ 10.3″
4	3,400	19° 48′ 19.0″	100° 36′ 13.5″
5	3,350	19° 48′ 20.2″	100° 36′ 13.8″
6	3,300	19° 48′ 23.8″	100° 36′ 18.6″
7	3,250	19° 48′ 36.1″	100° 36′ 16.3″
8	3,200	19° 48′ 38.0″	100° 36′ 20.6″
9	3,150	19° 48′ 40.9″	100° 36′ 26.4″
10	3,100	19° 48′ 49.0″	100° 36′ 31.2″
11	3,050	19° 48′ 58.7″	100° 36′ 30.6″
12	3,000	19° 49′ 10.1″	100° 36′ 28.4″
13	2,950	19° 49′ 15.0″	100° 36′ 05.8″
14	2,900	19° 49′ 23.0″	100° 35′ 54.3″
15	2,850	19° 49′ 38.6″	100° 35′ 35.0″
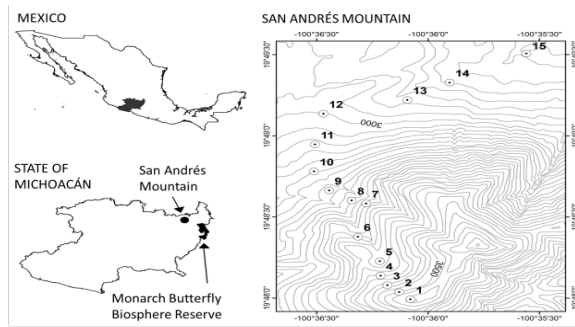
Figure 1
Geographic location of 15 sampled Abies religiosa populations on San Andrés Mountain, municipality of Hidalgo, Michoacán state, Mexico.

Seedlings were grown for nine months in 380 cm² plastic containers in a forest nursery shade-house, arranged under a randomized complete block design, at the Instituto de Investigaciones en Ecosistemas y Sustentabilidad, Universidad Nacional Autónoma de México, Morelia, in Michoacán state, Mexico. The seedlings were then transplanted in a common garden test, consisting of two rectangular wooden-framed raised beds (each 12.3 m long, 1.50 m width, 0.6 m tall). These wooden structures were filled with a 20-cm layer of coarse extrusive volcanic gravel for improved drainage (particle size: 28.4–37.3 mm), overlaid with a 40 cm layer of loamy soil mixed with dry chopped tree leaves and branches (imitating a forest soil) as a substrate. The test was covered by a 35 % shade net. Seedlings were placed in a randomized complete block design, with six blocks, 15 populations, in plots of six-seedlings in a row, spaced at 0.25 m within plots that were 0.25 m apart. There was a total of 36 seedlings per provenance and a total of 540 seedlings for the experiment. The first and the last plot of each wooden structure were flanked by a row of randomly selected seedlings in order to minimize the edge effect. The test was conducted at the Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán (101° 15′ 01.10″ W, 19° 41′ 22.60″ N, 1900 m a.s.l., mean annual temperature 17.0 °C, average annual precipitation 881 mm). Plants were irrigated as required to avoid water stress.

Starting from the 1st January 2015, when the seedlings were nineteen months old (from seed germination), and prior to initiation of shoot elongation, seedling height (mm) was evaluated every 21 days and then every ten days during the subsequent period of most rapid shoot elongation. Measurements were taken over a period of ten months (January to October, 2015). Height was measured with a ruler supported by an aluminum bar in order to standardize the measurements.

In December 2015, when the trees were two years and six months old, the aerial part was harvested on four of the six blocks in order to determine dry weight. Needles, branches and stem were separated and dried in an oven (Economy Oven Precision-Thermo Electron Corporation®, USA) at 60 °C for 72 hours. Dry weight was measured using a EQ-5/10-Torrey® balance. Total aerial dry weight per plant was estimated by summing the dry weight values of the needles, branches and stem.

We estimated frost resistance by inducing frost damage in the lab, on two blocks that had not been harvested, during January to March 2016 (before bud elongation), using the methodology of SÁENZ-ROMERO and TAPIA-OLIVARES (2008). The experiment consisted of placing two branches from each seedling (cut at 5 cm from the branch bud; harvested between 7 and 8 am the day after watering to field capacity, in order to prevent any drought stress) into a refrigerator (samples inside a sealed plastic bag to prevent rapid dissection, and inside a cardboard box to buffer the speed of temperature change) at 4 °C for 2 hours and then in a freezer (Torrey®, USA) with a modified manual-controlled thermostat. Temperature was then decreased slowly (descending 3 °C per hour) until reaching a target temperature (-20 °C). The target temperature was determined beforehand from the results of pilot tests and selected in order to achieve 30 to 70 % damage (ANEKONDA et al., 2000), avoiding too low a temperature that would cause close to 100 % damage to all of the samples, or a temperature that was too high to cause any damage. The temperature was then raised back to 0 °C at the same slow rate. The branches were left to reach room temperature overnight and then left on a lab bench for 48 hours in order to express tissue damage. Each of ten needles around the bud were visually evaluated, using a score of 0 to denote no damage (although partially dehydrated due to harvesting and the time in process, but of normal shape and green color), 0.5 to denote intermediate damage (needles moderately twisted, a mix of dark green and brownish color) and 1 to denote full damage (needles severely dehydrated, twisted and of brownish color). The scores for all needles were summed to attain an index score per branch of 0 = no damage to 10 = 100 % damage.

Data analysis
A logistic growth function for total height was fitted for each individual seedling, using PROC NLIN of SAS (2004) and the following model (SÁENZ-ROMERO et al., 2012b, after REHFELDT and WYKOFF, 1981):

$$Yi = \frac{1}{1 + e^{\left(\beta_0 + \beta_1 X + \frac{\beta_2}{x}\right)}}$$

Where Y_i = observation of total height on the ith seedling; β_0, β_1 and β_2 = regression parameters and X = measurement date (Julian day).

Regression parameters (β_0, β_1 and β_2) were used to estimate a growth curve of predicted values for each individual tree, using the model:

$$P_i = (1/(1 + e^{(\beta_0 + \beta_1 X \beta_2 \ (1/X))})Z$$

Where P_i = predicted growth (total height) for the ith seedling; β_0, β_1 and β_2 = regression parameters; X = measurement date (Julian day) and Z = total elongation (mm).

The predicted growth curve for each seedling was used to estimate: Total elongation, defined as the difference between

the final measurement of 28 month old minus 19 month old seedling height (e.g. the growth that occurred during the growing period of the second year of life); the day at which 2 mm of growth had been attained by each seedling, which is the start of the growth period in Julian days; the day at which all but 2 mm growth had been attained, i.e., the end or cessation of the growth period in Julian days; the rate of elongation between 20 % and 80 % of total elongation, i.e., the maximum growth rate; and the number of days between the start and growth cessation, i.e., the duration of growth period.

These growth variables, as well as dry weight and frost damage, were used in an analysis of variance to test the significance of differences among populations, using PROC GLM of SAS (SAS, 2004). PROC VARCOMP, METHOD=REML (SAS, 2004) was used to estimate the ratio of the variance component to total variance. These analyses used the following statistical model:

$$Y_{ijk} = \mu + B_i + P_j + B_i * P_j + \varepsilon_{ijk}$$

Where Y_{ijk} = observation on the th seedling of the jth population of the ith block, μ = overall mean, B_i = effect of ith block, P_j = effect of the jth population, B_i*P_j = interaction of population by block and ε_{ijk} = error term; $i = 1…b, j = 1… t$, and $k = 1… n$, which are the number of blocks, populations and seedlings per plot, respectively.

The relationship between the environmental gradient (along the elevational transect) of the seed sources and the genetic variation among populations was assessed by regressing the means per population of each of the variables that were found to be significant in the ANOVA against the seed source elevation and then also against each one of 15 climatic variables representing the environmental gradient. The climatic variables were: Mean Temperature of Coldest Month (MTCM, °C); Frost Free Period (FFP, days); Degree Days above 5 °C in the Frost Free season (GSDD5); Julian date of the first freezing date of autumn (FDAY, Julian day). These climatic variables have been shown to be important as primary factors controlling plant distribution (REHFELDT, 2006). Climatic values were estimated for the 15 *A. religiosa* provenances for the contemporary climate (average 1961-1990). Climatic values were obtained from spline climate surfaces fitted from monthly average temperatures (mean, maximum and minimum) and monthly precipitation values obtained from numerous weather stations (SÁENZ-ROMERO et al., 2010). Regression analysis was also conducted between the first principal component, obtained from those quantitative traits that were significantly different among populations and had a significant association with the elevational gradient (using PROC PRINCOMP of SAS, 2004), against seed source elevation and climate.

Regression analysis was conducted by testing elevation or one climatic variable at a time, since there was a strong correlation among climatic variables. Regression used a linear and quadratic model with PROC REG (SAS, 2004).

The linear model was:

$$Y_{ij} = \beta_0 + \beta_1 X_i + \varepsilon_{ij}$$

Where Y_{ij} = population mean, β_0 = intercept, β_1 = slope, X_i = value of elevation or a climatic variable of the ith population origin, ε_{ij} = error.

The quadratic model was:

$$Y_{ij} = \beta_0 + \beta_1 X_i + \beta_2 X_i^2 + \varepsilon_{ij}$$

Where Y_{ij} = population mean, β_0 = intercept, β_1 = slope, β_2 = regression parameter, X_i = value of elevation or climatic variable of the ith population origin, ε_{ij} = error.

Seed zoning

The elevational seed zones that were based only on morphological variation and proposed by CASTELLANOS-ACUÑA et al., (2014) were delineated once again, but this time based on genetic differentiation among populations.

Since there is a strong association between climatic values and elevation, climatic fixed seed zones were delimitated using both of the criteria (elevational and climate intervals that made two populations significantly different). We know that climate is known to be the selective force; the use of elevation is easier than climatic values in terms of seed zone delimitation in reforestation operations.

Thus, differentiation along the cline was interpreted relative to the least significant difference (LSD, α=0.20) that must separate populations before there can be reasonable certainty of genetic differentiation (REHFELDT, 1993). This LSD was translated to elevational values and then to climatic values. First, we used the LSD values of only those quantitative traits that presented significant differences among populations as well as a significant association with the elevational cline. The contemporary seed zoning was then delineated with the average of the equivalence in elevational difference that represents the LSD for each quantitative trait, after fitting a regression between the mean value of each trait per population and the elevation of provenance. We then found the equivalence in climate of this seed zoning for the first principal component to the corresponding climatic values, fitting a regression between the mean value per population of the first principal component and the climatic values of the provenance.

Estimation of future climatic variables

Point estimates of the forecasted climate for each provenance for the decade centered on the year 2030 were obtained by interrogating the spline climatic surfaces fitted with outputs of an ensemble of 17 Global Circulation Models and an intermediate Representative Concentration Pathway (RCP, 6.0 watts/m²), available at http://charcoal.cnre.vt.edu/climate/customData/ (CROOKSTON and REHFELDT, 2016).

Estimation of future seed zones

The assumption is that natural populations have adapted evolutionarily to the contemporary climate (average 1961-1990),

and the current climate has already been affected by global warming.

In order to promote adaptive management to reduce the potential impacts of climate change on *A. religiosa* populations, we have produced simple guidelines that can be applied in land use management to realign contemporary populations to inhabit the same climate in the decade centered on the year 2030 that they inhabit today. The guidelines consisted of delineating elevational seed zoning, defined under the contemporary climate, to be used for seed collection, then producing seedlings in a nursery, and finally deployment (at present) in the sites where the climate for which they are adapted will occur in the decade centered on the year 2030. Such deployment sites will be defined under a future projected climate, assuming that the existing phenotypic plasticity will be enough to enable the deployed seedlings to survive the contemporary climate (colder today at higher elevations) in order to grow, successfully compete and reach healthy, adapted adulthood by the decade centered on the year 2030.

We delineated future seed zones using as limits the same values of the most relevant climate variable used to define the contemporary seed zone limits. We chose the best climatic variable to predict the population average growth performance, using the first principal component derived from the growth variables under the contemporary climate. To find the future equivalence in elevation of that climate variable, we fitted a regression (PROC REG of SAS, 2004) between that climatic variable, projected for the decade centered on the year 2030 for the ensemble of Model Scenarios and under the RCP 6.0 w/m², and the elevation of each population

Results

Differences among populations

There were statistically significant differences among provenances in terms of total elongation ($P = 0.0167$), final height ($P = 0.0011$), date of growth cessation ($P = 0.0018$), foliage dry weight ($P = 0.0096$), stem dry weight ($P = 0.0152$), total dry weight ($P = 0.0103$) and frost damage ($P = 0.0103$). The contribution to total variance of the differences among populations ranged from 6 % for foliage dry weight to 63 % for frost damage) (*Table 2*).

In general, there were no significant differences among blocks or interactions between blocks by population, apart from the date of growth cessation (blocks: $P = 0.0001$), duration of growth (blocks: $P = 0.0002$; interaction: $P = 0.0269$) and frost damage (blocks: $P = 0.0001$; interaction: $P = 0.0002$), demonstrating that the environmental conditions in which the trees grew in the common garden test were relatively homogeneous (*Table 2*).

There was, when fitting a regression, a significant association between the seed source elevation and mean seedling height per population (coefficient of determination $r^2 = 0.4061$, $P = 0.0103$), total elongation ($r^2 = 0.4416$, $P = 0.0067$. Figure 2a), growth cessation ($r^2 = 0.5470$, $P = 0.0016$)], foliage dry weight (lineal: $r^2 = 0.2908$, $P = 0.0366$) and with a quadratic model for frost damage ($r^2 = 0.8684$, $P < 0.0001$. *Figure 2b*).

Table 2

Percentage contribution to total variance (%) and significance (P) after an analysis of variance for quantitative traits for 15 populations of *Abies religiosa*.

| Trait	Population		Block		Population*Block		Error
	%	P	%	P	%	P	%
Seedling height	12.9	0.0011	0.0	0.7543	10.8	0.104	76.3
Total elongation	7.0	0.0167	0.0	0.6935	6.0	0.0564	87.0
Start of growth	0.0	0.9785	3.0	0.2662	0.0	0.2102	97.0
Growth cessation	8.0	0.0018	8.5	0.0001	0.7	0.1095	82.0
Duration of growth	2.3	0.0916	13.5	0.0002	3.7	0.0269	80.0
Rate of elongation	0.0	0.5523	1.9	0.2426	11.5	0.0155	86.6
Foliage dry weight	5.9	0.0096	0.0	0.5801	6.1	0.0774	87.6
Stem dry weight	5.4	0.0152	0.0	0.5868	5.0	0.1817	89.6
Total dry weight	5.9	0.0103	0.0	0.6718	5.9	0.1232	88.2
Frost damage	62.9	0.0130	7.21	<0.0001	7.4	0.0002	22.6
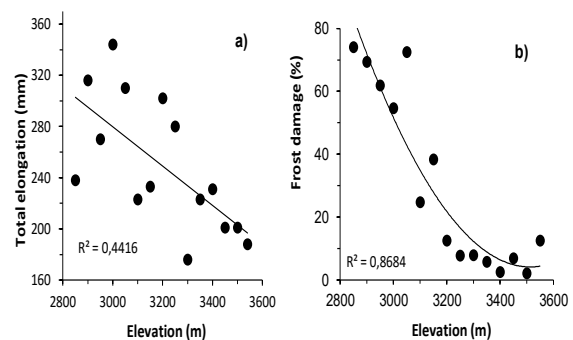
Figure 2

Regression of means per population in 15 populations of *A. religiosa* collected at Cerro de San Andrés, Michoacán, Mexico, against the provenance elevation (masl), for: a) Total elongation. b) Mean of frost damage (-20 °C).

Principal component analysis was conducted only with traits that presented 1) significant differences between populations and 2) a significant association with the elevational cline (representing the environment gradient). These traits were: total elongation, final height, date of growth cessation, dry weight of foliage and frost damage. The resulting first principal component (PRIN1) had an eigenvalue comprising 76 % of the total variation. Consecutive analysis was therefore conducted using only PRIN1 as a variable representing the variation across the traits.

The average of the first principal component values per population had a strong and significant association with all climatic variables, as indicated by the regression analysis (see example in *Figure 3*), where positive and larger values of PRIN1 corresponded to larger seedlings, with larger values of

seedling height, late growth cessation and high dry foliage weight, although with less resistance to frost damage; negative values corresponded to smaller seedlings, with smaller seedling height, lower total elongation, earlier growth cessation and lower dry foliage weight, although with high resistance to frost damage (*Figure 3a*). We obtained the best fit when regressing (using a linear model) against degree days >5 °C of the growing season (GSDD5, r^2 = 0.6383, *P* = 0.0004), difference in Julian day of the first frost of autumn (FDAY, r^2 = 0.6370, *P* = 0.0004), mean temperature of the coldest month (MTCM, r^2 = 0.6222, *P* = 0.0005) (*Figure 3a*); Frost Free Period (FFP, r^2 = 0.6206, *P* = 0.0005) and Growing Season Precipitation, April to September (GSP, quadratic model: r^2=0.6813, *P* = 0.0010) of the provenances.
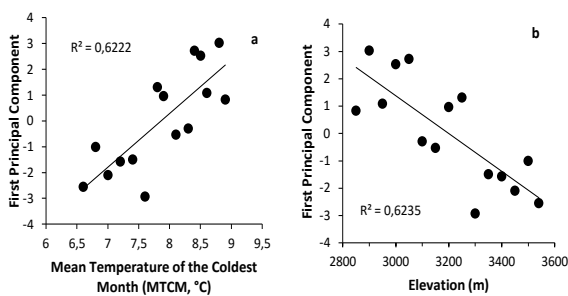
Figure 3
Mean by population of Principal component (PRIN 1) regressed against: a) Mean Temperature of Coldest Month (MTCM, r2=0.6222, P=0.0005) and b) Elevation of origin of the population (masl; r2=0.6235, P=0.0005).

The pattern of genetic differentiation among populations presented a significant association with elevational cline, with populations originated at high elevations producing shorter seedlings (negative values or PRIN 1) and low-elevation populations presenting larger seedlings (positive values of PRIN 1; lineal model r^2 = 0.6235, *P* = 0.0005) (*Figure 3b*). This result can be explained by the very strong association between the provenance elevation and climate values. Elevation was always highly significant when regressed against mean temperature of the coldest month (r^2 = 0.9886, *P* <0.0001), degree days <5 °C (GSDD5, r^2 = 0.9891, *P* <0.0001) and difference in Julian day of the first frost of autumn (FDAY, r^2 = 0.7141, *P* <0.0001), Frost Free Period (FFP, r^2 = 0.7659, *P* <0.0001), as well as the growing season precipitation, April to September (GSP, r^2 = 0.9186, *P* = 0.0001) of the provenances.

Re-delineation of seed zoning without considering climate change

Of the quantitative traits that had significant differences among populations and that were also associated with the elevational cline, the least differences that made two populations significantly different were those of Seedling height (LSD = 108.2 mm), Total elongation (66.0 mm), Growth cessation (17 days), Foliage dry weight (23.8 g) and Frost damage (16.5 %).

Translating such LSD to an elevational interval that made two populations of *A. religiosa* genetically different, we found

that populations differ where they are separated by 363 meters of elevational difference for Seedling height, 427 m for Total elongation, 355 m for Growth cessation, 542 m for Foliage dry weight and 133 m for Frost damage. The average of these values was 364 m of elevational difference, which was rounded down to 350 m for management simplification purposes.

We found the equivalence in climate of the 350 m in elevational difference for the mean temperature of coldest month (MTCM), which was a good predictor of the principal component values for growth traits as indicated before, and is also a variable easily measured and interpreted by forest managers. We returned to the LSD for each quantitative trait (significant both among populations and in terms of its association with the elevational cline) and found its equivalence for Seedling height (1.2 °C of MTCM), Total elongation (1.4 °C), Growth cessation (1.2 °C), Foliage dry weight (1.6 °C) and Frost damage (0.5 °C). These values averaged 1.18 °C of MTCM, which was rounded up to 1.2 °C of MTCM to simplify the climatic seed zoning. In summary, seed zoning can be carried out in intervals of either 350 m elevational difference or 1.2 °C of difference in the mean temperature of coldest month (MTCM).

Considering that the elevational natural distribution of *A. religiosa* populations ranges from approximately 2600 masl to a maximum of 3700 masl, seed zones were established using this elevational range of distribution, producing three different elevational zones. We began with the mean temperature of coldest month value predicted for the population that occurs at the lowest elevation (9.7 °C of MTCM) and delimited the fixed zone climatic interval limits every 1.2 °C of difference in the mean temperature of the coldest month. This produced three fixed zones: I, 9.7 to 8.5 °C of MTCM, II, 8.5 to 7.3 °C of MTCM and III, from 7.3 to 6.1 °C of MTCM. We then found the equivalence of these zones in terms of in elevational limits, thereby establishing zones I (2650 to 3000 masl), II (3000 to 3350 masl) and III (3350 to 3700 masl).

Finally, in order to have the equivalence of the zoning for other climatic variables, we translated back the fixed zone climatic interval limits to frost free period (FFP), degree days >5 °C of the growing season (GSDD5) and Julian day of the first frost of autumn (FDAY), using the respective regression parameters of the association between elevation and each of the other climatic variables that had shown highly significant association with the principal components. The zoning results are summarized on *Table 3*, where the fixed zone climatic intervals are defined by ranges of 14 days of FFP, 323 °C GSDD5 and 6 days of FDAY.

Seed zoning considering climate change for realigning populations to year 2030

Future fixed elevational zone intervals limits were estimated based on prediction of the elevation at which these will occur in the decade centered on the year 2030 (under intermediate RCP 6.0 watts/m^2 scenario), the same values of the mean of temperature of coldest month (MTCM) that defined the limits of the contemporary fixed intervals limits (as in *Table 3*). The estimated limits of three fixed seed zones shifted to a higher elevation 350 m (compared to the contemporary zonification of *Table 3*), as a result of upwards displacement on the

Table 3

Climatic and elevation limits of three seed zones for *A. religiosa* in a contemporary climate.

	Elevation				Mean Temperature of the Coldest Month (MTCM)				Frost Free Period (FFP)			
	Limits (m)		Range (m)	Intervall (±)	Limits (°C)		Range (°C)	Intervall (±)	Limits (days)		Range (days)	Intervall (±)
Zone	Lower	Upper			Lower	Upper			Lower	Upper		
I	2650	3000	350	175	9.7	8.5	1.2	0.6	179	165	14	7
II	3000	3350	350	175	8.5	7.3	1.2	0.6	165	151	14	7
III	3350	3700	350	175	7.3	6.1	1.2	0.6	151	137	14	7

(Continuation of Table 3)

	Elevation masl				First Day of Freezing of Autumn (FDAY)				Degree days >5° C of the) Growing Season (GSDD5)			
	Limits (m)		Range (m)	Intervall (±)	Limits (Julian Day)		Range (day)	Intervall (±)	Limits (°C))		Range (°C)	Intervall (±)
Zone	Lower	Upper			Lower	Upper			Lower	Upper		
I	2650	3000	350	175	294	288	6	3	1781	1458	323	161.5
II	3000	3350	350	175	288	282	6	3	1458	1135	323	161.5
III	3350	3700	350	175	282	276	6	3	1135	812	323	161.5

mountains of the mean temperature of coldest month. Thus, the future limits will be: Zone I_{2030}, 3000 to 3350 masl; Zone II_{2030}, 3350 to 3700 masl; Zone III_{2030} 3700 to 4050 masl (*Table 4*). It is important to realize that the mean temperature of the coldest month values that defined the zone intervals limits for future zonification (*Table 4*) are the same temperature values as those for the contemporary zones (*Table 3*), but will occur in the future 350 m higher in elevation. This upward shift can be visualized in Figure 4.

Note for the future seed zones (*Table 4*), that the upper limit of Zone III_{2030} exceeds the elevation of the summits of the Biosphere Reserve of Monarch Butterfly, which are between 3550 and 3600 m in elevation.

Figure 4

Provenance mean temperature of coldest month for climate contemporary and for the decade centered on the year 2030 (RCP 6.0 watts/m2), plotted against provenance elevation (masl). Arrow indicates upward elevational movement required (350 m of elevational difference) to match a given mean temperature of coldest month value to which the population is adapted at present, but where this temperature will occur at a higher elevation in year 2030.

Discussion

Differentiation among populations

The significant differentiation among populations in terms of quantitative traits, as well as the strong association with the elevation and climate of the provenance, indicate the existence of a strong genetic differentiation between populations along the environmental (elevational and climatic) cline, probably as a result of selective environmental pressure. As has been demonstrated in other North American conifer populations along elevational gradients (REHFELDT 1988; 1993), seedlings originating from lower elevations present higher growth potential but lower resistance to frost damage than those from higher elevations, which have lower growth potential but higher resistance to frost damage. Our results also coincide with
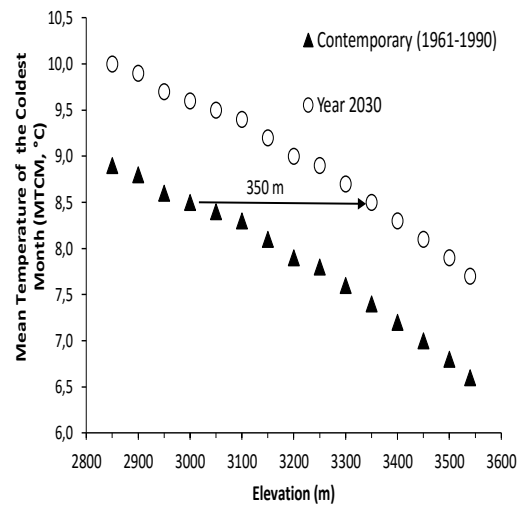
Table 4
Climatic and elevation limits of three fixed seed zones for *A. religiosa* projected for the decade centered on year 2030.

Zone$_{2030}$	Elevation masl				Month MTCM			
	Limits		Range (m)	Interval (±)	Limits		Range (m)	Interval (±)
	Lower	Upper			Lower	Upper		
I$_{2030}$	3000	3350	350	175	9.7	8.5	1.1	0.5
II$_{2030}$	3350	3700	350	175	8.5	7.4	1.1	0.5
III$_{2030}$	3700	4050	350	175	7.4	6.2	1.1	0.5

the pronounced elevational and climatic genetic differentiation pattern found in other Mexican conifers, *e.g. P. pseudostrobus* (SÁENZ-ROMERO et al., 2012b), *P. hartwegii* (LOYA-REBOLLAR et al., 2013) and *P. patula* (RUIZ-TALONIA et al., 2014). Most of those clines have been demonstrated in young seedlings in common garden tests, but there is also robust evidence of a strong age-age correlation between young seedlings grown in common garden tests with growth at older ages at field sites, when the latter are placed in optimal sites; see for example REHFELDT et al. (2004).

The driving force of selective pressure seems to be temperature rather than precipitation. This is indicated by the fact that the quantitative traits were strongly differentiated among populations with a pronounced elevational pattern of variation (Seedling height, Total elongation, Growth cessation, Foliage dry weight, and Frost damage) when synthetically expressed as the first principal component, and were more strongly associated with temperature variables: degree days >5 °C of the growing season (GSDD5; r^2 after a linear regression = 0.64), Julian day in which the first frost of autumn occurs (FDAY; r^2 = 0.63), mean temperature of coldest month (MTCM; r^2 = 0.62), and Frost Free Period (FFP; r^2 = 0.62). The association with precipitation variables was more moderate; for example, with Mean Annual Precipitation (MAP; r^2 = 0.58) or Growing Season Precipitation (GSP; r^2 = 0.58). Thus, the *Abies religiosa* population appears to modify its genetic structure as an adaptation to the stress imposed by temperature, perhaps in extremely cold temperatures, as suggested by MTCM, FDAY and FFP.

Use of zonification

The zonification developed based on the least significant difference that made two populations differ genetically for relevant quantitative traits is two-fold, such that forest managers can choose to use either elevation or climatic values to decide seed and seedling movements. The elevational interval that delineates the new zonification, 350 m of elevational difference, is broader in range than previously utilized elevation intervals (250 m of elevational difference), the delimitation of which was based solely on morphological evaluation (longitude of cones and needles; CASTELLANOS-ACUÑA et al., 2014).

The suggested procedure for the use of zonification under the contemporary climate for ecological restoration and conservation while ignoring the potential effects of climatic change, would be to: (i) take seeds of a given fixed seed zone to produce seedlings in nursery and then (ii) reforest sites of the same seed zone or in sites at ± 175 m in elevation or ± 7 days of frost free period or ± 3 days of FDAY or ± 323 °C of GSDD5, relative to the site of seed collection.

In order to realign the genotypes to the climate for which they are adapted and which is predicted to occur at higher elevation by year 2030, assuming that the mean temperature of coldest month is a reliable indicator of the climate suitable for a given population, it will be necessary to collect seeds from contemporary seed Zone I (*Table 3*), produce seedlings in the nursery and then deploy these seedlings in the corresponding and climatically equivalent future Zone I$_{2030}$ (*Table 4*). This will imply an average upwards shift of 350 m.

The need for assisted migration

The projected reductions of 62 % in the climate niche of *Abies religiosa* by the decade centered on the year 2030, 88 % by 2060 and 96 % by 2090, represent a serious threat to the natural population of this pine species (SÁENZ-ROMERO et al., 2012a). Human intervention is likely to be required in order to adapt the populations to the future climate (TCHEBAKOVA et al., 2005; AITKEN et al., 2008; SÁENZ-ROMERO et al., 2012a). Assisted migration of *A. religiosa* populations requires a massive reforestation program (SÁENZ-ROMERO et al., 2016). However, if the shift is not undertaken now, the forests are likely to continue the gradual process of decline because of the everincreasing mismatch between the populations and the climate to which they are adapted (SÁENZ-ROMERO et al., 2012a; LÓPEZ-GÓMEZ et al., 2015).

Assisted migration, along with other ex-situ conservation approaches, would be necessary in order to conserve gene pools of contemporary populations. Nevertheless, controversy persists regarding assisted migration and its effects on longterm biotic interactions (BUCHAROVA, 2017) since these interactions and long-term responses to changing climates are extremely complex and difficult to predict (SUTTLE et al., 2007)

There is solid evidence that natural migration is already occurring, but at a speed far below that required to maintain the match between tree populations and their suitable climatic habitat (LENOIR et al., 2008). Unfortunately, *A. religiosa* individuals have a very slow growth rate. It is a species with a long life history and sexual maturity is probably reached at 17 to 25 years (RZEDOWSKI et al., 2005). Furthermore, individuals of this species present low fecundity, regardless of the degree of land fragmentation. All of these factors suggest that the populations may be unable to migrate by natural means at the speed necessary to remain within suitable habitat.

A movement of more than 350 m in elevation may invite the risk of frost damage at present (SÁENZ-ROMERO and TAPIA-OLIVAREZ, 2008). One solution to this problem is the use of nurse plants, such as *Lupinus elegans*, *Lupinus montanus* or other local shrub species, which could act to protect the migrated *A. religiosa* plants from frost damage (BLANCO-GARCÍA et al., 2011). It would be highly recommendable to plant these nurse plants at least one year prior to planting the *Abies religiosa* seedlings.

Acknowledgements

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