Chapter 6

# Genetic Variability of Mountain Pine (*Pinus hartwegii* Lindl) in the Protection of Flora and Fauna Area Nevado de Toluca

Rosa Laura Heredia-Bobadilla,
Guadalupe Gutiérrez-González,
Amaury-M. Arzate-Fernández and
Sergio Franco-Maass

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.79860

**Abstract**

Mountain pine (*Pinus hartwegii* Lindl) is one of the most abundant conifers in the Protection of Flora and Fauna Area Nevado de Toluca in central Mexico; this natural protected area is threatened by urbanization; this has been manifested in forest health; there has been an increase in forest parasites like bark beetles and dwarf mistletoes, making necessary improve forest management and conservation, hence our objective was to study the genetic diversity of mountain pine under the attack of parasites and to generate information that could be used to improve strategies of conservation of these forests. We classified sampled trees into four categories according to the type of parasite present in a tree (bark beetle: BB; dwarf mistletoe: DM; bark beetle and dwarf mistletoe: BM and non-attacked trees or healthy trees: HT). Genetic diversity was low in comparison with other pine species, but we observed an interesting issue: trees attacked by bark beetle and dwarf mistletoe had higher levels of heterozygosis: $He_{nc}$ = 0.1924 and $He_{nc}$ = 0.1993, respectively. These results suggest that trees with bark beetle and dwarf mistletoes may have higher genetic variability and are a highly valuable genetic resource for mountain pine.

**Keywords:** mountain pine, genetics, bark beetle, dwarf mistletoe, conservation

## 1. Introduction

Forests of the Trans-Mexican Volcanic Belt (TMVB) physiographic region are among the most threatened areas in Mexico. The TMVB region encloses a large number of natural protected areas (NPA), the Protection of Flora and Fauna Area Nevado de Toluca (PFFANT) is one of them. The closeness of this NPA to large cities entails environmental pressures that includes the presence of human settlements, illegal logging, introduction of exotic species and the growing incidence of forest parasites like bark beetles and dwarf mistletoes [1–3].

PFFANT is formed by conifer forests, with the genera *Pinus* and *Abies* being the most representative of this zone [4, 5]. *Pinus hartwegii* Lindl. is forming large forest stands and is the pine species which grows at the highest altitudes in the PFFANT [6], unfortunately is affected by dwarf mistletoes (*Arceuthobium* spp.) and bark beetles (*Dendroctonus* spp.). The incidence of these parasites is growing, probably because of climate change and deforestation [1, 7–9].

México ranks fourth in terms of deforestation, with approximately 670,000 ha/year causing losses in genetic diversity and changes in locally adapted populations for example giving place to an increase of parasites populations [10–13]. Results of forest health diagnoses have suggested that bark beetles are a group of parasites that have affected large extension of forests in Mexico (40.5%), followed by parasitic plants (38.7%), both of them leaving negative consequences in the forest, like high mortality rates of trees affected [11, 14, 15].

Bark beetles grow under the cortex and induce weakening of the tree, the construction of galleries and the inoculation of a staining fungi which is carried by female beetle results death [14]. In the PFFANT, *Dendroctonus adjunctus* is affecting *P. hartwegii* [6, 14]. Dwarf mistletoes are obligate heterotrophic plants that acquire all their water and nutrients from their host and can significantly inhibit its growth causing permanent deformation of the stem and crown. This parasite weakens trees in such a way that they become more susceptible to attack by insects, particularly bark beetles. In the PFFANT, *P. hartwegii* is the host of *Arceuthobium vaginatum* and *A. globosum* [6, 8, 15]. Bark beetles and dwarf mistletoes epidemics can lead to shifts in forest, forest successional trajectories and susceptibility to future disturbances [16–18].

Forest trees are key drivers of terrestrial biodiversity because they function as a carbon sink, preserve the water quality and regulate climate [19, 20]; genetic variability studies are crucial to understand the basic biology of these organisms and to obtain insights on evolution, disease resistance and conservation genetics [21–23]. In conifer species for example, gene flow is mediated by three types of genomes with contrasting inheritance: nuclear (biparental), mitochondrial (maternal) and chloroplast (paternal) this particularity opens avenues to the study of conifer DNA polymorphism, the study of genetic variability with these three types of markers allow making inferences on the distribution of genetic resources and habitat connectivity [24].

In this study, nuclear DNA (ncDNA), mitochondrial DNA (mtDNA) and chloroplast DNA (cpDNA) were used to assess the genetic variability and population structure of *P. hartwegii* populations affected by bark beetles and dwarf mistletoes, expecting that genetic variability

will be low in the parasitized populations. We want to contribute to the conservation of mountain pine populations of the PFFANT generating information which helps in the identification of populations genetically valuable.

## 2. Materials and methods

### 2.1. Study zone

Sampling was carried out in the Protection of Flora and Fauna Area Nevado de Toluca, which forms part of the Trans-Mexican Volcanic Belt physiographic region. The geographical coordinates of the study zone are 18° 51′ 31″ and 19° 19′ 03″ N and 99° 38′ 54″ and 100° 09′ 58″ W; it is a priority region for conservation due to its diversity of ecosystems in which pine-fir forests and high mountain prairie dominate [1, 25].

### 2.2. Plant material

We sampled a total of 180 individuals of *P. hartwegii*. A distance of 50 m was between each tree sampled. Samples were classified into four groups (categories): trees with signs of attack by bark beetles (BB), trees with signs of attack by dwarf mistletoe (DM), trees with signs of attack of both parasites (BM), and trees with no signals of any parasite, which were considered as healthy trees (HT). Each sample consisted of young needle tissue. Immediately after collecting the needles, they were placed on ice for transport to a laboratory where they were maintained at −70°C.

### 2.3. DNA isolation

Cetyltrimethylammonium bromide (CTAB) method was implemented with a few modifications [26]. Needle tissue was ground to a fine powder with a chilled mortar and pestle, making two washes, the first with 100% ethanol (v/v) and the second with 75% ethanol (v/v). DNA was re-suspended in 70 μL TE and stored at −20°C until it was used.

### 2.4. PCR amplification reactions

PCRs were performed in a reaction volume of 10 μL, containing: ammonium buffer 15 mM, $MgCl_2$ 25 mM, dNTP mix 10 mM, primer 20 μM, DNA 10 ng/μL and 0.5 U of Taq polymerase (Sigma). To amplify nuclear (ncDNA), we used anchored microsatellites (ASSR) which proved to be genetically stables and heritable: ASSR-15 and ASSR-29 [27] and one operon: UBC 254 [28], one cytochrome oxidase sequence: cox3in, was used to amplify mitochondrial (mtDNA) [30] and one highly polymorphic microsatellite: 10FF/RR, to amplify chloroplast (cpDNA) [31] (**Table 1**).

Amplifications were performed in a Master Cycler Gradient (Eppendorf) Thermal Cycler in all 40 cycles. For ncDNA primers: 1 min of denaturation at 94°C, 1 min of annealing at 48°C and 1 min of extension at 72°C. For mtDNA primers: 1 min of denaturation at 94°C, 1 min of annealing at 48–55°C and 1 min of extension at 72°C. For cpDNA: 1 min of denaturation at

| Primer name | Marker type | Sequence (5′ → 3′) |
|---|---|---|
| UBC-254 | Nuclear operon [28] | 5′-CGCCCCCATT-3′ |
| ASSR-15 | Nuclear anchored microsatellite [29] | 5′-(CT)7GCA-3′ |
| ASSR-29 | Nuclear anchored microsatellite [29] | 5′-(CT)7GTA-3′ |
| ASSR-20 | Nuclear anchored microsatellite [29] | 5′-(CT)7ATG-3′ |
| COX3in | Mitochondrial cytochrome oxidase III gene sequence [30] | 5′-GTA GAT CCA AGT CCA TGG CCT-3′<br>5′-GCA GCT GCT TCA AAG CC-3′ |
| 10 FF/RR | Chloroplast microsatellite [31] | 5′-CAGAAGCCCAAGCTTATGGC,<br>5′-CGGATTGATCCTAACCATAC |

**Table 1.** Description of markers used for the study of genetic variability of *Pinus hartwegii*.

94°C, 1 min of annealing at 58–60°C and 1 min of extension at 72°C; all samples with all primers were given a 7 min of pre-amplification denaturation at 94°C and a 5 min of post-amplification at 72°C.

### 2.5. Electrophoresis in agarose gel

The amplification products were separated by electrophoresis in agarose gel (1.5%) at constant voltage (100 V and 90 mA). Gels were visualized by UV transilluminator (UVP) with ethidium bromide (10 mg/mL).

### 2.6. Scoring of bands and data analysis

DNA patterns were inferred according to dominant nature of markers used, so each amplified product was scored for all genotypes for its presence or absence, we made a binary matrix in which band presence was assigned a value of one (1) and the absence of a band a value of zero (0). Co-migrating bands were assumed as the same locus and the same band when scoring.

We used PopGene 32 [32], Genealex 6.5 [33] and TFPGA [34] to obtain genetic diversity parameters: mean number of alleles per locus (A), mean number of observed alleles (na), effective number of alleles (ne), Nei's genetic diversity indices (*He*); as long as we sampled parasitized trees we used Graphpad Prima 7.0 to perform a chi square test and detect if there were differences in heterozygosis according to the categories considered in sampling. The number of polymorphic alleles (LP), percentage of polymorphic alleles (%LP), population structure fixation indices ($G_{ST}$), indicators of heterozygosis ($H_T$: total genetic diversity of the locus, $H_S$: genetic diversity within populations), gene flow (Nm) and Nei's genetic distance (D) between the four categories we obtained BB, DM, BM, and HT [35].

## 3. Results

We obtained electrophoretic patterns with high reproducibility and clear band resolution. UBC-254: 11 bands, ASSR-15: 7 bands, ASSR-29: 11 bands, Cox3in: 8 bands and 10FF/RR: 7

| | ncDNA | | | | mtDNA | | | | cpDNA | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **BB** | **DM** | **BM** | **HT** | **BB** | **DM** | **BM** | **HT** | **BB** | **DM** | **BM** | **HT** |
| n | 35 | 75 | 35 | 35 | 75 | 35 | 35 | 35 | 75 | 35 | 35 | 34 |
| na | 1.9 ± 0.3620 | 2 | 1.9 ± 0.3620 | 1.7 ± 0.4237 | 1.4 ± 0.5175 | 1.8 ± 0.4629 | 1.6 ± 0.5175 | 1.5 ± 0.5345 | 1.4 ± 0.5345 | 2 | 1.4 ± 0.5345 | 1.6 ± 0.5345 |
| ne | 1.3 ± 0.2964 | 1.3 ± 0.3067 | 1.7 ± 0.2872 | 1.3 ± 0.2666 | 1.0 ± 0.1433 | 1.1 ± 0.1859 | 1.1 ± 0.2395 | 1.0 ± 0.1047 | 1.0 ± 0.0395 | 1.1 ± 0.2033 | 1.0 ± 0.1056 | 1.1 ± 0.1661 |
| *He* | 0.1924 ± 0.1648 | 0.1993 ± 0.1586 | 0.1877 ± 0.1526 | 0.1831 ± 0.1552 | 0.0539 ± 0.1016 | 0.0810 ± 0.1196 | 0.0872 ± 0.1382 | 0.0582 ± 0.0828 | 0.0295 ± 0.0368 | 0.1267 ± 0.1260 | 0.0592 ± 0.0857 | ND |
| % LP | 87.5 | 87.5 | 100 | 75.86 | 23.10 | 75.86 | 86.21 | 33.33 | 55.56 | 33.33 | 55.56 | ND |

**Table 2.** Genetic variability of *Pinus hartwegii* in attacked (by bark beetle and dwarf mistletoe) and nonattacked trees for nuclear DNA (ncDNA), mitochondrial DNA (mtDNA), and chloroplast DNA (cpDNA); bark beetle attacked tree (BB), dwarf mistletoe attacked tree (DM), bark beetle and dwarf mistletoe attacked tree (BM) and healthy tree (HT); sample size (n), number of alleles per locus, effective number of alleles per locus (ne), Nei's genetic diversity, percentage of polymorphic loci (%LP), (ND) no data.


http://dx.doi.org/10.5772/intechopen.79860

bands, combined in 139 band patterns, ranging from 200 to 2000 bp; additionally there were bands only present in HT trees.

### 3.1. Genetic variability

The number of alleles per locus (na) and the number effective alleles (ne) ranged from 1.3 to 2.0 indicating that the number of alleles transferred from one generation to the next is low; these low values of *na* and *ne* consequently act on heterozygosis (Nei's genetic diversity: *He*), which also was low compared with other *Pinus* species (**Table 2**).

Among categories (BB, DM, BM and HT) we observed, in some cases, a tendency of BB, DM and BM to present higher values of *He*, for example with ncDNA BB and DM categories showed *He* values 0.1924 and 0.1993, respectably, higher than in HT trees which was 0.1831, with cpDNA DM trees presented high *He* compared with HT (**Table 2**). According to Chi square tests, the distribution of *He* showed statistically significant differences between the BB, DM, BM and HT categories (**Table 3**).

### 3.2. Population structure

Estimated population structure based on $G_{ST}$ (fixation index) was very low probably due to high levels of gene flow (**Table 4**). The rates of gene flow (Nm) derived from $G_{ST}$ were very

| Marker | $X^2$ | P |
|---|---|---|
| ASSR-15 | 1.0614 | 0.0001 |
| ASSR-29 | 1.8906 | 0.0001 |
| UBC254 | 0.8028 | 0.0001 |
| 10FF/RR | 0.8732 | 0.0001 |
| COX3IN | 0.5732 | 0.0001 |

**Table 3.** Comparison of genetic variability between groups (BB, DM, BM and HT) in *Pinus hartwegii*.

| | ncDNA | | | | mtDNA | | | | cpDNA | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | MAX | MIN | Mean | SD | MAX | MIN | Mean | SD | MAX | MIN |
| $G_{ST}$ | 0.0321 | 39.8316 | 0.1404 | 0.0006 | 0.0170 | 0.0065 | 0.0230 | 0.0049 | 0.0475 | 0.0312 | 0.0961 | 0.0096 |
| Nm | 15.0849 | 10.8853 | 55.5996 | 3.0604 | 28.9288 | 14.9747 | 59.5707 | 21.2152 | 10.0279 | 17.0230 | 51.3333 | 4.7046 |
| $H_T$ | 0.1946 | 0.0203 | 0.4906 | 0.0302 | 0.0713 | 0.0122 | 0.3294 | 0.0064 | 0.0832 | 0.0064 | 0.2076 | 0.0064 |
| $H_S$ | 0.1884 | 0.0194 | 0.4850 | 0.0120 | 0.0700 | 0.0116 | 0.3218 | 0.0063 | 0.0792 | 0.0058 | 0.1955 | 0.0063 |

**Table 4.** Summary of *Pinus hartwegii* population differentiation ($G_{ST}$), genic flow (Nm), total genetic diversity of the locus ($H_T$), average genetic diversity ($H_S$), for nuclear (ncDNA), mitochondrial (ncDNA) and chloroplast DNA markers (cpDNA).

| | | HT | BB | DM | DM |
|---|---|---|---|---|---|
| ncDNA | HT | — | | | |
| | BB | 0.006 | — | | |
| | DM | 0.014 | 0.007 | — | |
| | BM | 0.012 | 0.004 | 0.007 | — |
| mtDNA | HT | — | | | |
| | BB | 0.002 | — | | |
| | DM | 0.010 | 0.001 | — | |
| | BM | 0.004 | 0.001 | 0.003 | — |
| cpDNA | HT | — | | | |
| | BB | 0.014 | — | | |
| | DM | 0.003 | 0.013 | — | |
| | BM | 0.005 | 0.006 | 0.001 | — |

**Table 5.** Matrix for genetic distance values for bark beetle attacked tree (BB), dwarf mistletoe attacked tree (DM), bark beetle and dwarf mistletoe attacked tree (BM) and healthy tree (HT) in *Pinus hartwegii* samples, evaluated with nuclear (ncDNA), mitochondrial (mtDNA) and chloroplast (cpDNA) markers.

high with all markers, averaging 15.1 migrants per generation (**Table 4**). Based on these results (low-population differentiation/high gene flow), we assume that inbreeding rates are low.

Genetic distances ranged from 0.003 between DM/BM with mtDNA and DNA cp markers to 0.014 between HT/DM and HT/BB with ncDNA and cpDNA markers; for all markers parasitized trees had the highest genetic distances between nonattacked trees (HT) and attacked trees (BB, DM and BM) (**Table 5**).

## 4. Discussion

We found low levels of genetic variability in mountain pine, but also that parasitized trees in some cases had highest levels of heterozygosity; this is not rare if we take into account that plants are subject to various abiotic and biotic stresses, especially those long-lived species like conifers; thus, slow-growing plants will invest heavily in defenses against parasites because of high cost of replacing tissue [9, 36]; substantial variation in susceptibility, damage and resistance are well documented in natural plant populations [21, 37–39].

### 4.1. Genetic variability

Levels of heterozygosis in mountain pine were in general low, but in spite of this with ncDNA, we observed a tendency of parasitized trees (BB and DM) to have more genetic variability and this is relevant because in a population stressed by an increment of parasites, high genetic

diversity individuals will have more chances to adapt themselves to changes in their environment and attack by parasites and pathogens.

Some theories propose that dwarf mistletoes performance is regulated by physiological (genetic) condition of the host and that infections are greater in sites with high stress, also long-term contact and evolutionary history between specific plant and parasites are expected to increase plant defenses, tree species have been co-evolving with mistletoes for 25 million years, so high genetic variability of parasitized organisms is an insight of co-evolution system [16, 40, 41]. The genetics of host resistance due to a co-evolutionary linkage has been reported in some pine species, such as *P. edulis*, *P. lawsonii*, *P. montezumae* and *Fagus sylvatica* with their associated bark beetles [9, 42], and for *P. ponderosa* and Douglas-fir (*Pseudotsuga menziesii*) to dwarf mistletoes [37, 40, 43, 44].

In PFFANT, pine forests are under high abiotic stress like the presence of human settlements and logging which in turn could be causing biotic stress situations like incrementing incidence of parasites (e.g., because of the loss of trees, bark beetles move to sites where there were no infections and the infection spreads in trees which are predicted to have low defenses). The location of populations parasitized and with high genetic variability is a valuable data to be taken into account for conservation science.

*P. hartwegii* form dense forest stands in the PFFANT, and their associated parasites are also present with a patchy distribution. They are not present in all populations, indeed there are healthy trees in populations highly parasitized (also called "scape trees") it could suggest that the parasites of mountain pine select their host based on genetic cues. There is a hypothesis called "gene by gene coevolution" which pose that host-parasite relationship had been kept polymorphisms in plants, these has been recognized in the existence of two cases: host-specificity, and variation in host preference [40, 45], we suggest that in view of the levels of *He* in parasitized trees, it is possible that a gene-by-gene coevolution case is present in the interaction of mountain pine with its parasites, although there is a need for more studies to probe it. It appears that we are facing a coevolution dynamics, where trees with higher genetic diversity are attacked due to its genes; parasites track specific host genotypes under natural conditions [46–48] not only to phenotypic characteristics. In other words, plant genetic diversity affects enemies and mutualists [49].

### 4.2. Population structure

Organelle genomes provide information about the relative capacity of dispersal of males and females; mtDNA is maternally inherited, and cpDNA is paternally inherited in pines, this shapes gene flow and genetic diversity within and among populations in a particular way [22, 50, 51], we found low levels of $G_{ST,}$ in comparison with some other pine species at organelle genomes [24] and registered high levels of gene flow, these data could suggest that mountain pine subpopulations studied at PFFANT are poorly differentiated which in turn could mean that the population as a whole must be managed and conserved as genetically unique; also we registered the highest with mtDNA on this we can infer that *P. hartwegii*'s most important means of gene flow seems to be the seed (**Table 4**), but it is also probably due to the

reforestation plans performed at PFFANT by government institutions, which in most of the cases is performed with pines from off-site seed carrying foreign DNA.

High levels of deforestation have been reported at PFFANT, mainly due to illegal logging leading to forest fragmentation, which in turn has devastating effects on forest trees, taking off the best conformed trees, which reduce levels of gene flow and allele diversity, promoting inbreeding and genetic drift [52]. Since conifers are wind pollinated and long lived organisms, effects of inbreeding and or genetic drift are not drastic yet (in view of our results) and the levels of genetic variability reported here may only reflect *standing genetic variation*, when a population passes through selective pressures it adapts mainly from standing genetic variation [53, 54].

Genetic distances differed according to marker type; there has been reported high polymorphism in *Dendroctonus ponderosa* related to geographic region [55]. Our results may be explained by differences in land use in which trees were sampled, which could impose different selection pressures in parasites and hosts in response to biogeographic discontinuities [42, 56]. We observed, for example that dwarf mistletoe infections response to altitude, at least *Arceuthobium vaginatum* and *A. globossum* (parasites of *P. hartwegii*) disappear above 3600 masl (personal observation at FFPANT). The lowest genetic distances we found were between DM and BB trees with the tree markers (**Table 4**), and these trees are genetically similar and have high levels of polymorphism, the differences between HT trees and DM, BB trees may consist in those which parasitized trees possess and make parasites choose them [40, 45], while markers we used are not specifically related with defense and resistance pathways in plants, we report a reliable difference which can serve as a first insight to conduce more studies on genetic resistance of conifers to bark beetles and dwarf mistletoes.

### 4.3. Conservation implications

The presence of probably immune trees (HT) and resistant trees (BM) must be taken into account to conserve mountain pine forests of the FFPANT, specially confronting an increase of infected areas by bark beetles and dwarf mistletoes; BM are trees whose seeds could be used in reforestation programs, especially in areas where infections are growing, and HT seeds could be used to create barriers which impede the spread of outbreaks.

*Pinus* forests of the FFPANT are home and a potential distribution area of many species of plants and animals some of which also have high levels of genetic variability which in turn makes FFPANT an important place to conservation of species [4, 57–60]. These forests provide water to urban areas, hence the importance of preserving this natural area should not be dismissed; there is an urgent need to protect these forests, as long as forest fragmentation is increasing.

It has been reported that there are differences in susceptibility to parasites attack among tree species and even among individuals within a species; when different species interact, selective changes may occur as a result of the interaction; models of host–parasite interactions support the idea that variation in host resistance is, at least partially, genetic and assume the presence of genetic variation [23, 46, 48]. In conifers, some theories have been proposed to explain this

variability, including variations in terpene or phenolic content [61], differences in constitutive defenses [62, 63] and differences in growing conditions [64, 65]. Population genetics theory predicts that under many selective regimes, fitness will increase the number of heterozygous loci [23]; many authors have reported that genetic variability of trees affected by parasites, in terms of heterozygosity, they report that heterozygous individuals were resistant and suggest that heterozygosity and plant resistance are positively correlated [61, 66–69].

More studies are needed in order to reaffirm the results reported here, may be with other kinds of molecular markers, and to continue the improvement of management and preservation of the FFPANT. Forests worldwide need to be protected in face of an imminent climate change.

## Acknowledgements

The authors wish to thank Consejo Nacional de Ciencia y Tecnología (CONACyT) and the Universidad Autónoma del Estado de México (UAEMéx) for financial support. Also, they thank the CIEAF students who helped in the laboratory and fieldwork.

## Author details

Rosa Laura Heredia-Bobadilla[1], Guadalupe Gutiérrez-González[1], Amaury-M. Arzate-Fernández[1]* and Sergio Franco-Maass[2]

*Address all correspondence to: amaury1963@yahoo.com.mx

1 Centro de Investigación y Estudios Avanzados en Fitomejoramiento, Universidad Autónoma del Estado de México, Toluca, Mexico

2 Instituto de Ciencias Agropecuarias y Rurales, Universidad Autónoma del Estado de México, Toluca, Mexico

## References

[1] Candeau-Dufat R, Franco-Maass S. Aplicación de un sistema de información geográfica para determinar la relación entre la dinámica y condiciones de vida de la población de las localidades del parque nacional nevado de Toluca y la generación de impactos ambientales en sus ecosistemas. Investigaciones Geográficas Boletín del Instituto de Geografía UNAM. 2000;**62**:44-68

[2] Heredia-Bobadilla RL, Gutiérrez-González G, Franco-Maass SF, Arzate-Fernández AM. Genetic variability of sacred fir (*Abies religiosa*) in the Nevado de Toluca National Park. International Journal of Biodiversity and Conservation. 2012;**4**(3):130-136. DOI: 10.5897/IJBC11.187

[3] Arriola-Padilla V, Estrada-Martínez E, Ortega-Rubio A, Pérez-Miranda R, Gijón-Hernández AR. Deterioro en Áreas Naturales Protegidas del centro de México y del Eje Neovolcánico Transversal. Investigación y Ciencia. 2014;**22**(60):37-49

[4] Villers-Ruiz L, García del Valle L, López-Blanco J. Evaluación de los bosques templados en México: una aplicación en el Parque Nacional Nevado de Toluca. Investigaciones Geográficas Boletín del Instituto de Geografía UNAM. 1998;**36**:7-19

[5] CONANP. Área de Protección de Flora y Fauna Nevado de Toluca [Internet]. 2013. Available from: http://nevadodetoluca.conanp.gob.mx [Accessed: 01-06-2017]

[6] SEMARNAT. Acuerdo por el que se da a conocer el Resumen del Programa de Manejo del Área Natural Protegida con categoría de Área de Protección de Flora y Fauna Nevado de Toluca. Available from: http://www.conanp.gob.mx/que_hacemos/pdf/programas_manejo/RESUMEN_NEVADO_TOLUCA.pdf, [Accessed: 22-11-2016]

[7] Raffa KF, Aukema BH, Bentz BJ, Carroll AL, Hicke JA, Turner MG, Romme WH. Cross-scale drivers of natural disturbances prone to anthropogenic amplification: The dynamics of bark beetle eruptions. Bioscience. 2008;**58**(6):501-517. DOI: 10.1641/B580607

[8] Ramírez-Dávila JF, Porcayo-Camargo E. Estudio de la distribución espacial del muérdago enano (*Arceuthobium* sp.) en el Nevado de Toluca, México, utilizando el método del SADIE. Madera y Bosques. 2009;**15**(2):93-111

[9] Bentz BJ, Hood SMM, Hansen EM, Vandygriff JC, Mock KE. Defense traits in the long – lived Great Basin bristlecone pine and resistance to the native herbivore mountain pine beetle. New Phytologist. 2017;**213**:611-624. DOI: 10.1111/nph.14191

[10] Keane PJ, Kile GA, Podger FD, Brown BN. Diseases and Pathogens of Eucalypts. Australia: La Trobe University Bundoora; 2000. 565 pp

[11] Sáenz C, Snively AE, Linding R. Conservation and restoration of pine forest genetic resources in Mexico. Silvae Genetica. 2003;**52**:233-237

[12] Shaw DC, Chen J, Freeman EA, Braun DM. Spatial and population characteristics of dwarf mistletoe infected trees in an old-growth Douglas-fir-western hemlock forest. Canadian Journal of Forest Research. 2005;**35**:990-1001. DOI: 10.1139/x05-022

[13] FAO. Global Forest Resources Assessment 2015 [Internet]. 2015. Available from: http://www.fao.org/3/a-i4793e.pdf [Accessed: 05-01-2017]

[14] Cibrián-Tovar D, Tulio MJ, Campos BR, Yates HO, Flores L. Insectos Forestales de México. México: Universidad Autónoma Chapingo; 1995. 453 p

[15] Cibrián-Tovar D, Alvarado-Rosales D, García-Díaz SE. Enfermedades Forestales en México. México: Universidad Autónoma Chapingo; 2007. 587 p

[16] Scharpf R, Parmeter JR Jr. Population Dynamics of Dwarf Mistletoe on Young True Firs in the Central Sierra Nevada. California: United States Department of Agriculture; 1982. 9 p. DOI: 10.2737/PSW-RP-161

[17] Way DA. Parasitic plants and forests: A climate change perspective. Tree Physiology. 2011; **31**(1):1-2. DOI: 10.1093/treephys/tpq113

[18] Klutsch JG, Beam RD, Jacobi WR, Negrón JF. Bark beetles and dwarf mistletoe interact to alter downed woody material, canopy structure, and stand characteristics in northern Colorado ponderosa pine. Forest Ecology and Management. 2014;**315**:63-71. DOI: 10.1016/j.foreco.2013.12.024

[19] González-Martínez S, Krutovsky KV, Neale DB. Forest-tree population genomics and adaptive evolution. New Phytologist. 2006;**170**:227-238. DOI: 10.1111/j.1469-8137.2006.01686.x

[20] Neale DB, Kremer A. Forest tree genomics, growing resources and applications. Nature Reviews Genetics. 2011;**12**:111-122. DOI: 10.1038/nrg2931

[21] Burdon RD. Genetic diversity and disease resistance: Some considerations for research, breeding, and deployment. Canadian Journal of Forest Research. 2001;**31**:596-606. DOI: 10.1139/x00-136

[22] Petit RJ, Duminil J, Fineschi S, Hampe A, Salvini D, Vendramin GG. Comparative organization of chloroplast, mitochondrial and nuclear diversity in plant populations. Molecular Ecology. 2005;**14**:689-701. DOI: 10.1111/j.1365-294X.2004.02410.x

[23] Hedrick PW. Genetics of Populations. Canada: Jones and Barlett Learning; 2011. 675 p

[24] Floran V, Sestras RE, García-Gil MR. Organelle genetic diversity and phylogeography of scots pine (*Pinus sylvestris L.*). Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2011; **39**(1):317-322. DOI: 10.15835/nbha3916103

[25] Arriaga L, Espinoza JM, Aguilar C, Martínez E, Gómez L, Loa E. Regiones terrestres prioritarias de México. México: Comisión Nacional para el Conocimiento y uso de la Biodiversidad; 2000

[26] Zhou Z, Miwa M, Hogetsu T. Analysis of genetic structure of a *Suillus grevillei* population in a *Larix kaempferi* stand by polymorphism of inter-simple sequence repeat ISSR. New Phytologist. 1999;**144**:55-63. DOI: 10.1046/j.1469-8137.1999.00504.x

[27] Yamagishi M, Abe H, Nakano M, Nakatsuka A. PCR-based molecular markers in asiatic hybrid lily. Scientia Horticulturae. 2001;**96**:225-234. DOI: 10.1016/S0304-4238(02)00095-X

[28] Wu J, Krutovskii KV, Strauss SH. Nuclear DNA diversity, population differentiation, and phylogenetic relationships in the California closed-cone pines based on RAPD and allozyme markers. Genome. 1999;**42**:893-908. DOI: 10.1139/g98-171

[29] Yamagishi M, Abe H, Nakano M, Nakatsuka A. PCR-Based Molecular Markers in Asiatic Hybrid Lily. Scientia Horticulturae. 2002;**96**:225-234

[30] Wen-Qing Q, Yang HJ, Xue YB, Hu SY. Inheritance of chloroplast and mitochondrial DNA in Chinese fir *Cunninghamia lanceolata*. Acta Botánica Sinica. 1999;**41**(7):695-699

[31] Marshall HD, Newton C, Rotland K. Chloroplast phylogeography and evolution of highly polymorphic microsatellites in lodgepole pine (*Pinus contorta*). Theoretical Applied Genetics. 2002;**104**:367-378. DOI: 10.1007/s001220100687

[32] Yeh FC, Rongcai R, Boyle T, Ye Z, Mao-Xiyan J. Pop Gene Version 1.3. Canada: University of Alberta; 1999

[33] Peakall R, Smouse PE. GENALEX 6: Genetic analysis in Excel Population genetic software for teaching and research. Molecular Ecology Notes. 2006;**6**:288-295

[34] Miller MP. Tools for Population Genetic Analyses (TFPGA). USA: Northern Arizona University; 1997

[35] Nei M. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics. 1978;**89**(3):583-590

[36] Polyakova GG, Pashenova NV, Polyakov VI, Zrazhevskaya GK. Induction of conifer immune responses by phytopathogenic fungus metabolites. Russian Journal of Plant Physiology. 2008;**55**(4):496-502. DOI: 10.1134/S1021443708040109

[37] Roth LF. Resistance of ponderosa pine to dwarf mistletoe. Silvae Genetica. 1974;**23**:116-119. DOI: 10.2737/PSW-RP-208

[38] Roth LF. Genetic control of dwarf mistletoe. In: Scharpf RF, Parmeter JR Jr, editors. Symposium Proceedings: Dwarf Mistletoe Control through Forest Management; 11–13 April 1978; Berkeley, CA. Gen. Tech. Rep. PSW-31. Berkeley, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Forest Range and Experiment Station; 1978. pp. 69-72

[39] Yanchuk AD, Murphy JC, Wallin KF. Evaluation of genetic variation of attack and resistance in lodgepole pine in the early stages of a mountain pine beetle outbreak. Tree Genetics and Genomes. 2008;**4**:171-180. DOI: 10.1007/s11295-007-0098-9

[40] Shamoun SF, DeWald LE. Management strategies for dwarf mistletoes: Biological, chemical, and genetic approaches. USDA Forest Service General Technical Report; 2002. pp. 75-82

[41] Bickford CP, Kolb TE, Geils BW. Host physiological condition regulates parasitic plant performance: *Arceuthobium vaginatum* subsp. *cryptopodum* on *Pinus ponderosa*. Oecologia. 2005;**146**:179-189. DOI: 10.1007/s00442-005-0215-0

[42] Krabel D, Petercord R. Genetic diversity and bark physiology of the europan beech (*Fagus sylvatica*): A coevolutionary relationship with the beach scale (*Cryptococcus fagisuga*). Tree Physiology. 2000;**20**:485-491. DOI: 10.1093/treephys/20.7.485

[43] Scharpf RF. Host resistance to dwarf mistletoes. In: Hawksworth FG; Scharpf RF, editors. Biology of Dwarf Mistletoes, Symposium Proceedings; 8 August 1984; Fort Collins, CO. Gen. Tech. Rep. RM-111. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station. pp. 70-76

[44] Scharpf RF. Resistance of Jeffrey pine to dwarf mistletoe. In: Weber HC, Forstreuter W, editors. Proceedings of the 4th International Symposium on Parasitic Flowering Plants; 2–7 August 1987. pp. 745-753

[45] Thompson JN, Burdon JJ. Gene-for-gene coevolution between plants and parasites. Nature. 1992;**360**:121-125. DOI: 10.1038/360121a0

[46] Sorci G, Møller AP, Boulinier T. Genetics of host-parasite interactions. Science. 1997;**12**(5): 196-200

[47] Carius HJ, Little TJ, Ebert D. Genetic variation in a host-parasite association: Potential for coevolution and frequency-dependent selection. Evolution. 2001;**55**(6):1136-1145. DOI: 10.1111/j.0014-3820.2001.tb00633.x

[48] Koskela T, Salonen V, Mutikainen P. Interaction of a host plant and its holoparasite: Effects of previous selection by the parasite. Journal of Evolutionary Biology. 2001;**14**:910-917. DOI: 10.1046/j.1420-9101.2001.00352.x

[49] Moreira X, Mooney KA. Influence of plant genetic diversity on interactions between higher trophic levels. Biology Letters. 2013;**9**:20130133. DOI: 10.1098/rsbl.2013.0133

[50] Neale DB, Sederoff RR. Inheritance and evolution of conifer organelle genomes. In: Hanover JW, Keathley DE, Wilson CM, Kuny G, editors. Genetic Manipulation of Woody Plants. Basic Life Sciences. Vol. 44. USA: Springer; 1998

[51] Hipkins VD, Krutovskii KV, Strauss SH. Organelle genomes in conifers: Structure, evolution, and diversity. Forest Genetics. 1995;**1**(4):179-189

[52] Jump AS, Peñuelas J. Genetic effects of chronic habitat fragmentation in a wind-pollinated tree. Proceedings of the National Academy of Sciences. 2006;**103**(21):8096-8100. DOI: 10.1073/pnas.0510127103

[53] Hermisson J, Pennings PS. Soft sweeps: Molecular population genetics of adaptation from standing genetic variation. Genetics. 2005;**169**:2335-2352. DOI: 10.1534/genetics.104.036947

[54] Barret RDH, Schluter D. Adaptation from standing genetic variation. Trends in Ecology and Evolution. 2007;**23**(1):38-44. DOI: 10.1016/j.tree.2007.09.008

[55] Stock MW, Amman GD, Bentz JB. Isozyme studies of barck beetle population genetics and systematics. USDA Forest Service General Technical Report. 1992;**138**:7-9

[56] Leonardi S, Piovani P, Scalfi M, Piotti A, Giannini R, Menozi P. Effect of habitat fragmentation on the genetic diversity and structure of peripheral populations of beech in central Italy. Journal of Heredity. 2012;**103**(3):408-417. DOI: 10.1093/jhered/ess004

[57] Sánchez-Jasso J, Aguilar-Miguel MX, Medina-Castro JP, Georgina M. Riqueza específica de vertebrados en un bosque reforestado del Parque Nacional Nevado de Toluca. Revista Mexicana de Biodiversidad. 2013;**84**:360-373

[58] Medina JP, Salgado-Miranda C, García-Cornejo M, Soriano-Vargas E. Coccidia in passerines from the Nevado de Toluca National Park, México. Acta Parasitologica. 2015;**60**(1): 173-174. DOI: 10.1515/ap-2015-0024

[59] Heredia-Bobadilla RL, Monroy-Vilchis O, Zarco-González MM, Martínez-Gómez D, Mendoza-Martínez G, Sunny A. Genetic structure and diversity in an isolated population of an endemic mole salamander (*Ambystoma rivulare* Taylor, 1940) of Central México. Genetica. 2016;**144**(6):689-698. DOI: 10.1007/s10709-016-9935-9

[60] Sunny A, González-Fernández A, D'Addario M. Potential distribution of the endemic imbricate alligator lizard (*Barisia imbricate imbricata*) in highlands of central México. Amphibia-Reptilia. 2017;**38**:225-231. DOI: 10.1163/15685381-00003092

[61] Liu JJ, Ekramoddoullah AKM, Hunt RS, Zamani A. Identification and characterization of random amplified polymorphic DNA markers linked to a major gene ($Cr_2$) for resistance to *Cronartium ribicola* in *Pinus monticola*. Genetics and Resistance. 2006;**96**(4):395-399. DOI: 10.1094/PHYTO-96-0395

[62] Eyles A, Bonello P, Ganley R, Mohammed C. Induced resistance to pests and pathogens in trees. New Phytologist. 2009;**185**:893-908. DOI: 10.1111/j.1469-8137.2009.03127.x

[63] Pastorino RM, Corley J. Bases genéticas de la resistencia de los árboles a las plagas, Serie Técnica: Manejo Integrado de Plagas Forestales; 2009. 15 p

[64] Beaulieu J, Plourde A, Daoust G, Lamontagne L. Genetic variation in juvenile growth of *Pinus strobus* in replicated Quebec provenance-progeny tests. Forest Genetics. 1996;**3**(2): 103-112

[65] King JN, Yanchuk AD, Kiss GK, Alfaro RI. Genetic and phenotypic relationships between weevil (*Pissodes strobi*) resistance and height growth in spruce populations of British Columbia. Canadian Journal of Forest Research. 1997;**27**(5):732-739. DOI: 10.1139/x97-009

[66] Benet H, Guries RP, Boury S, Smalley EB. Identification of RAPD markers linked to a black leaf spot resistance gene in Chinese elm. Theoretical and Applied Genetics. 1995;**90**:1068-1073. DOI: 10.1007/BF00222923

[67] Devey ME, Delfino-Mix A, Kinloch BB Jr, Neale DB. Random amplified polymorphic DNA markers tightly linked to a gene for resistance to White pine blister rust in sugar pine. Proceedings of the National Academy of Sciences. 1995;**92**(6):2066-2070

[68] Kondo T, Terada K, Hayashi E, Kuramoto N, Okamura M, Kawasaki H. RAPD markers linked to a gene for resistance to pine needle gall midge in Japanese black pine (*Pinus thunbergii*). Theoretical and Applied Genetics. 2000;**100**(3):391-395. DOI: 10.1007/s001220050051

[69] Mopper S, Mitton JB, Whitham TB, Cobb NS, Christensen KM. Genetic differentiation and heterozygosity in pinyon pine associated with resistance to herbivory and environmental stress. Evolution. 1991;**45**(4):898-999. DOI: 10.1111/j.1558-5646.1991.tb04365.x

IntechOpen