

Strategies for conservation of germplasm in endemic redwoods in the face of climate change: a review

M. R. Ahuja*

60 Shivertown Road, New Paltz, NY 12561, USA

Received 2 October 2010; Accepted 11 January 2011 – First published online 4 February 2011

Abstract

This study reviews the various conservation strategies applied to the four redwood species, namely coast redwood (*Sequoia sempervirens*), Sierra redwood or giant sequoia (*Sequoiadendron giganteum*), dawn redwood (*Metasequoia glyptostroboides*) and South American redwood or alerce (*Fitzroya cupressoides*), which are endemic in the USA, China and South America, respectively. All four redwood genera belong to the family *Cupressaceae*; they are monospecific, share a number of common phenotypic traits, including red wood, and are threatened in their native ranges due to human activity and a changing climate. Therefore, the management objective should be to conserve representative populations of the native species with as much genetic diversity as possible for their future survival. Those representative populations exhibiting relatively high levels of genetic diversity should be selected for germplasm preservation and monitored during the conservation phase by using molecular markers. *In situ* and *ex situ* strategies for the preservation of germplasm of the redwoods are discussed in this study. A holistic *in situ* gene conservation strategy calls for the regeneration of a large number of diverse redwood genotypes that exhibit adequate levels of neutral and adaptive genetic variability, by generative and vegetative methods for their preservation and maintenance in their endemic locations. At the same time, it would be desirable to conserve the redwoods in new *ex situ* reserves, away from their endemic locations with similar as well as different environmental conditions for testing their growth and survival capacities. In addition, other *ex situ* strategies involving biotechnological approaches for preservation of seeds, tissues, pollen and DNA in genebanks should also be fully exploited in the face of global climate change.

Keywords: climate change; endemics; germplasm conservation; genetic diversity; molecular markers; redwoods; *Sequoia*; *Sequoiadendron*; *Metasequoia*; *Fitzroya*

Introduction

Conservation of germplasm and maintenance of genetic diversity are important considerations for the survival of forest trees, in particular the endemics, in the face of global forest decline and climate change. The four

redwoods discussed in this study are endemics in their native countries: coast redwood (*Sequoia sempervirens* (D. Don) Endl.) and giant sequoia or Sierra redwood (*Sequoiadendron giganteum* (Lindl.) Buchholz), in the USA (Olson *et al.*, 1990; Weatherspoon, 1990); dawn redwood (*Metasequoia glyptostroboides* Hu & Cheng) in China (Chu and Cooper, 1999); and alerce or South American redwood (*Fitzroya cupressoides* (Mol.) Johnst.) in Chile/Argentina (Allnutt *et al.*, 1999). All four relectual genera are long-lived conifers and

*Corresponding author. E-mail: mrahuja@hotmail.com

belong to the family *Cupressaceae*. These redwood genera are monospecific, share a number of common phenotypic traits and have the same basic chromosome number of $x = 11$ (Gadek *et al.*, 2000; Ahuja, 2009). Although polyploidy is rare in conifers, two of the four redwood genera are polyploids (Ahuja, 2005, 2009). *Sequoia* is a hexaploid ($2n = 6x = 66$) and in fact the only hexaploid conifer (Saylor and Simons, 1970; Ahuja and Neale, 2002), while *Fitzroya* is a tetraploid ($2n = 4x = 44$) (Hair, 1968). On the other hand, both *Sequoiadendron* and *Metasequoia* are diploids ($2n = 22$) (Schlarbaum and Tsuchiya, 1984).

The native ranges, local environments and spatial patterns of the four redwoods are shown in Table 1. *Sequoia* extends from south-western corner of Oregon (latitude 42°09'N) to Santa Lucia Mountains of southern Monterey County (latitude 35°41'N) in California, USA. The native redwood forests are confined to a narrow coastal fog belt that is 725 km long and 8–56 km wide. The mean annual temperatures vary between 10 and 16°C, while the mean annual maximum and minimum temperatures range between 12 and –1°C in the *Sequoia* region. The *Sequoia* forests remain frost-free from 6 to 11 months. Most stands of *Sequoia* are found between 30 and 760 m, and this region receives an annual precipitation between 640 and 3100 mm/year (Olson *et al.*, 1990). *Sequoia* has undergone both expansions and contractions in its range in the past 10,000 years, and currently it appears that *Sequoia* is retreating from its southern range and expanding northwards (Sawyer *et al.*, 2000).

The natural range of *Sequoiadendron* consists of 75 groves scattered over 420 km long and 24 km wide belt extending from Tulare to Placer counties on the western slopes, within an altitude range of 1400–2000 m, in the Sierra Nevada region in central California, USA. The annual precipitation varies between 900 and 1400 mm, and the annual mean temperatures vary between 24 and 29°C, while the annual maximum and minimum vary between 1 and –6°C (Hartseveldt *et al.*, 1975; Weatherspoon, 1990). The third redwood *Metasequoia* is now confined to its native range of ~800 km², within an altitude range of 700–1350 m, in western Hubei, eastern Chongqing and northern Hunan provinces (latitudes 29°10'N to 30°10'N) in south-central China. The annual precipitation is ~2360 mm, and the moderate temperature mean is 16.3°C in the *Metasequoia* region (Leng *et al.*, 2007). The fourth redwood *Fitzroya* is also an endemic to the temperate rain forests, within an altitude range of 100–1200 m, in southern South America. It grows in discontinuous populations within an area of ~360 × 200 km in the coastal Cordilleras and central depression in Chile, and on the western slopes of Andes in Chile and

Argentina from latitudes 39°50'S to 42°45'S. The annual mean precipitation varies between 2000 and 4000 mm, and the temperature is between 13 and 16°C in the native habitats of *Fitzroya* (Allnutt *et al.*, 1999; Premoli *et al.*, 2000).

Although the spatial pattern of *Sequoia* is largely continuous in most of its native range (Table 1), it also has fragmented populations in certain parts of its range (Olson *et al.*, 1990; Sawyer *et al.*, 2000). *Sequoiadendron*, by contrast, has mainly scattered groves in its entire range (Weatherspoon, 1990). *Metasequoia* populations are fragmented and discontinuous (Leng *et al.*, 2007), while *Fitzroya* forests are highly fragmented and discontinuous (Table 1) (Premoli *et al.*, 2000). Fragmentation is disadvantageous in outcrossed conifers, as it leads to isolation and inbreeding and a substantial loss of heterozygosity. Common symptoms of inbreeding depression in conifers are abortive embryos, reduced seed set, reduced vigour, growth and survival in the inbreds (Wright, 1976; White *et al.*, 2007). Inbreeding depression has been investigated in *Sequoia* (a hexaploid) and *Metasequoia* (a diploid). Both redwoods exhibited reduced growth rates and survival in the inbred progenies compared with outcrossed trees (Libby *et al.*, 1981; Kuser, 1983). Species with polyploid genomes, particularly allopolyploids, often do not experience severe inbreeding depression (Stebbins, 1957). Even though *Sequoia* is a hexaploid, it did not exhibit any buffering effect against inbreeding depression. It is not clear whether the nature of polyploidy in *Sequoia* (Ahuja and Neale, 2002), which may be either an autoallohexaploid (AAAABB) or a segmental allohexaploid ($A_1A_1A_2A_2A_2A_2$ or $A_1A_1A_2A_2A_3A_3$), but not a strict allohexaploid (AABBCC), might have been responsible for the lack of sheltering effect against inbreeding depression.

All four genera are threatened in their native ranges, due to human activity and a changing climate. According to the International Union for Conservation of Nature and Natural Resources (IUCN) categories of threat, *Sequoia* and *Sequoiadendron* are classified as vulnerable, while *Metasequoia* is listed as critically endangered and *Fitzroya* as endangered species (IUCN, 2010). In this study, we review strategies and current practices for conservation of germplasm in the endemic redwoods in the face of climate change.

Conservation of germplasm

Genetic diversity is essential for the survival and conservation of a species in a changing environment. Greater the level of genetic diversity in a species, better are the chances for its survival and deployment over a wide range of environments (Ledig, 1988). Appropriate levels

Table 1. Native ranges, local environments and spatial patterns of redwoods

Range and local environment	<i>Sequoia</i> ^a	<i>Sequoiadendron</i> ^b	<i>Metasequoia</i> ^c	<i>Fitzroya</i> ^d
Native range	725 × 8–56 km Range in northern California and southern Oregon fog belt, USA	420 × 24 km Range in the Sierra Nevada, California, USA	800 km ² In western Hubei, eastern Chogqing and northern Human provinces in south-eastern China	360 × 200 km Range in coastal Cordillera and central depression in Chile, and western and eastern slopes of Andes in Chile and Argentina
Latitude coordinates	35°41'N to 42°09'N	35°51'N to 39°03'N	29°10'N to 30°10'N	39°50'S to 42°45'S
Annual precipitation	640–3100 mm	900–1400 mm	2300 mm	2000–4000 mm
Altitude range	30–760 m	1400–2000 m	700–1350 m	100–1200 m
Mean annual temperature range	10–16°C	24–29°C	16.3°C	13–16°C
Spatial ^e pattern of populations	Mostly continuous, few fragmented	Scattered groves, fragmented	Fragmented and discontinuous	Highly fragmented
Relative ^f levels of genetic diversity	Higher in northern populations, based on microsatellites	Higher in southern populations, based on isozymes	Higher in northern populations, based on RAPDs	Higher in eastern populations, based on isozymes

^a Olson *et al.* (1990); Rogers (1997); Brinegar *et al.* (2007).

^b Hartesveldt *et al.* (1975); Fins and Libby (1982); Weatherspoon (1990).

^c Li *et al.* (2005); Leng *et al.* (2007).

^d Allnutt *et al.* (1999); Premoli *et al.* (2000).

^e Impacted by human activity and climate change.

^f *Sequoia* (Brinegar *et al.*, 2007); *Sequoiadendron* (Fins and Libby, 1982); *Metasequoia* (Li *et al.*, 2005); *Fitzroya* (Premoli *et al.*, 2000).

of genetic variation must be maintained in the species and populations for conservation planning (Ledig, 1986, 1987). This should be based on an understanding of genetic architecture and how genetic variation is organized and distributed within and among populations. Initially, those populations with higher levels of genetic diversity would be worth for germplasm conservation. In addition, selection of genetic variation for traits related to adaptation to climate change, for example time of bud flush, growth rates, drought and cold hardiness, and timing of initiation and cessation of growth, would be more relevant to conservation of genetics resources of redwoods. In other words, the conservation of adaptive gene complexes would be important for the future survival of the redwoods. Molecular markers have been widely applied to characterize patterns of genetic variation within and among populations of a species. These patterns provide baseline information for determining the appropriate levels of genetic diversity within selected populations for gene conservation. However, molecular genetic markers, including isozymes, random amplification of polymorphic DNAs (RAPDs), restriction fragment length polymorphisms (RFLPs), simple sequence repeats (SSRs), and amplified fragment length polymorphisms (AFLPs), which are derived from non-coding DNA sequences, are selectively neutral markers and may not often be predictive of adaptive genetic diversity (Karhu *et al.*, 1996; González-Martínez *et al.*, 2006; Holderegger *et al.*, 2006). Although in recent years, much emphasis has been placed on the utility of adaptive genetic diversity in population genetics and gene conservation (Krutovsky and Neale, 2005; González-Martínez *et al.*, 2006; Hidalgo *et al.*, 2010), very little is known about the molecular genetic control (number of loci involved) involved in the adaptive genetic diversity. Furthermore, if adaptive traits are under a very strong selection pressure and the numbers of gene loci are a few, much of the genetic variance would be lost.

In spite of the controversy regarding the utility of molecular markers in gene conservation, molecular markers have many applications with regard to conservation of genetic resources in forest trees. Molecular markers are useful in the characterization of a number of evolutionary forces that impact the maintenance of genetic diversity, mating systems, gene flow and genetic drift. Molecular markers can also reveal whether a small isolated population is experiencing a bottleneck (Ledig *et al.*, 2002) and thus is particularly susceptible to accelerated decline due to inbreeding depression and genetic drift. Most molecular markers, including isozymes, have provided useful estimates of the levels of genetic variation in a tree species (Hamrick *et al.*, 1992; Millar and Westfall, 1992; Hidalgo *et al.*, 2010). Studies on these markers have revealed that conifers

exhibit greater levels of genetic diversity within populations as compared to between different populations.

Molecular genetic markers (isozymes) have revealed that hexaploid *Sequoia* seems to have a relatively higher level of genetic diversity (Libby *et al.*, 1996; Rogers, 1997, 2000) than *Fitzroya* (a tetraploid) and *Metasequoia* and *Sequoiadendron* (both diploids) (Kuser *et al.*, 1997; Rogers, 2000; Premoli *et al.*, 2000; Chen *et al.*, 2003; Ahuja, 2009). Preliminary studies using molecular genetic markers (microsatellites) have indicated relatively higher levels of genetic diversity (Table 1) in the northern populations of *Sequoia* (Brinegar *et al.*, 2007) and *Metasequoia* (using RAPDs) (Li *et al.*, 2005). Higher levels of genetic diversity were detected in the southern populations of *Sequoiadendron* (employing isozymes) (Fins and Libby, 1982) and eastern populations of *Fitzroya* (using isozymes) (Premoli *et al.*, 2000). Although, these data are not entirely comparable as different population sizes and molecular markers were employed, they provide, at least, preliminary baseline data on genetic diversity for these redwoods. Again, both *Sequoia* and *Fitzroya* are polyploids, and yet *Sequoia*, a complex hexaploid, either an autoallohexaploid (AAAABB) or a segmental allohexaploid ($A_1A_1A_2A_2A_2A_2$ or $A_1A_1A_2A_2A_3A_3$), seemingly has a relatively higher genetic diversity compared with *Fitzroya*, a putative autotetraploid (AAAA), as estimated by isozyme analyses (Rogers, 2000; Premoli *et al.*, 2000). The differences may be due to the type and nature of polyploidy.

Although molecular markers have provided reasonable estimates of genetic diversity in the redwood populations, the molecular genetic basis of adaptive genetic diversity still remains to be investigated. Structural and functional genomic information is still lacking in these redwoods. Linkage maps have not been constructed in the redwoods. Because of the large genome sizes in these redwoods, ranging from 10,000 MB in *Sequoiadendron* and *Metasequoia* to 31,500 MB in *Sequoia* (Ahuja and Neale, 2005), genome sequencing would be a very difficult and a challenging problem in the redwoods. Initially, gene discovery based on identifying expressed sequence tags would be an alternative to genome sequencing in the redwoods. Therefore, presently, the conservation of germplasm would rely on the availability of molecular marker-based genetic information that would be relevant to the genetic diversity in the redwood populations.

In order to evaluate the plans for conservation of germplasm, it is important to recognize threats to genetic diversity (human activity and climate change) and to have adequate knowledge on geographic variation of adaptive traits, so that, selected populations may

be prioritized relative to the threat. In this context, a better understanding of geographical variation in adaptive traits and its relationship to the endemic environment would be helpful in determining which populations of redwoods are most threatened (for example, northern or southern, coastal or inland, low or high altitude, fragmented or continuous, sensitive to frost or drought) and should be given priority for germplasm conservation. However, for global conservation of redwood germplasm, selected genotypes with high levels of diversity (both neutral and adaptive) from the entire species range should be included in the face of uncertainty regarding the climate change.

Conservation of germplasm can be accomplished by *in situ* (on site, within the original ecosystem) and *ex situ* (outside the natural habitat) methods, which take into account the maintenance of genetic diversity (Fig. 1). The conservation strategy has to be holistic, which is based on the entire gene pool of a species. One of the objectives of gene conservation is to ensure that functionally adaptive alleles will be available in the future for the breeding programmes and evolution of the species (Ledig, 1986; Hattemer, 1995). In order to hedge against uncertainty regarding the level of climate change in the future, it would be desirable to maximize genetic diversity by selecting individuals heterozygous for a number of genes that exhibit hybrid vigour (Ledig and Kitzmiller, 1992; Hamrick, 2004; Geburek and Konrad, 2007). Since climate change is unlikely to

stabilize for a long time in the foreseeable future, the maintenance of both neutral and adaptive genetic diversity (Volis and Blecher, 2010) will be absolutely essential for the survival of the forest trees. One of the best strategies might be to deploy intimate mixture of seed from the selected genotypes from disparate regions and environments for future *in situ* and *ex situ* forest tree plantations in the face of uncertain climate change (Ledig and Kitzmiller, 1992). Finally, it would be desirable to use molecular markers (Tikader *et al.*, 2009; Hidalgo *et al.*, 2010) to monitor the genetic fidelity of populations/clones during the conservation and management of redwood germplasm in the *in situ* and *ex situ* programmes.

In situ conservation

In situ preservation of a forest tree includes conservation of stands and populations via regeneration by generative and vegetative methods within the area of natural occurrence (Fig. 1). Although this type of conservation has been in practice in the national parks and nature reserves, it is not necessarily based on genetic criteria. However, a better approach to conservation of genetic resources in the redwoods in the USA, China, Chile and Argentina should be based on gene-ecological approaches that ensure not only the conservation of the redwoods but also the associated plant and animal species in the ecosystem (Parker and Donoso, 1993; Li, 1999; Noss *et al.*, 2000; Everts and Popper, 2001; Premoli *et al.*, 2003). The regeneration of populations is essential, and new generation of trees should originate from the controlled hybridizations within the conserved but diverse populations to enhance performance (hybrid vigour) that would be advantageous for an overall conservation of genetic diversity in the face of climate change. However, the potential consequences of genetic swamping should be curtailed in these gene conservation management programmes. Furthermore, the number of genotypes, both for vegetative and for generative progeny, should be large enough to preserve the common alleles and the adaptive gene complexes. Thus, a gene conservation strategy seeks not only to preserve germplasm in the old growths and secondary growth populations, but also to maintain sufficient genetic variability to allow adaptation in the new environment.

In the context of *in situ* conservation of redwoods, it is important to recognize the species' composition, their abundant pattern, genetic architecture, ecological dynamics, genetic diversity and ecosystem protection and maintenance (Libby *et al.*, 1996; Noss *et al.*, 2000). Each redwood species has a unique endemic environmental niche in its native country (Table 1). An additional understanding of the soil and nutritional requirements,

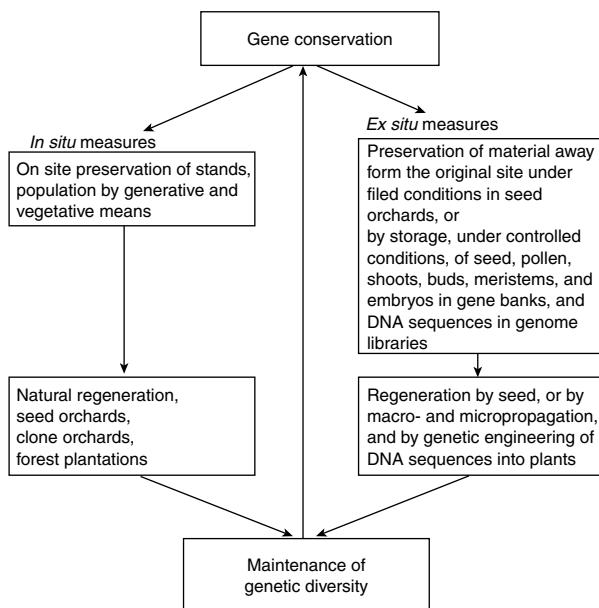


Fig. 1. Conservation of forest tree genetic resources by *in situ* and *ex situ* strategies. Gene conservation approaches should aim at maintaining genetic diversity in the forest tree species.

and companion forest and other vegetation can also provide the direction for the maintenance and conservation of the redwoods in the present and future ecosystems in the face of climate change. The contingency planning for the conservation of genetic resources of future redwood forests would be to move their genetically diverse populations in protected areas and reserves outside of their native habitats before the anticipated rapid climate changes impact them. That implies that *ex situ* strategies for the conservation of redwood germplasm should be fully explored at this stage.

Ex situ conservation

The *ex situ* measures conserve the genetic resources outside the natural habitat of a species (Fig. 1). These include (1) seed and clonal orchards under nursery and field conditions, and (2) biotechnological approaches for preservation of germplasm. We examine in the following sections the status of these *ex situ* preservation methods in redwoods. Implementing *ex situ* programmes can be time consuming and expensive. Among other things, *ex situ* conservation has a drawback in that the plant material is subjected to a selection pressure that may be quite alien to that in nature under which the original populations evolved (Ledig, 1986). However, these problems can be partially circumvented by regularly monitoring genetic fidelity and variation in the genotypes by employing molecular markers.

Seed and clonal orchards

Seed and clonal orchards under nursery and field conditions are a routine for the *ex situ* conservation of forest tree species (Melchior *et al.*, 1986; Ledig, 1986, 1988; Millar, 1993; Hattermer, 1995; Behm *et al.*, 1997). *Ex situ* plantations of redwoods have also been tested for their performance within the USA and in a number of countries. *Sequoia* has been successfully grown out of its fog belt for more than 100 years in Placerville and in the foothills of Sierra Nevada in California, Seattle, Washington, Hawkinsville, Georgia, USA; and Victoria, British Columbia, Canada (Kuser, 1981). It is now known that *Sequoia* can be grown in many parts of the world: Western Europe, Turkey, Crimea, New Zealand, Chile, South Africa and Tasmania (Kuser *et al.*, 1995). A range-wide international provenance trial, which included 180 clones from 90 locations throughout the natural range of *Sequoia*, was carried out in four plantation sites in the USA, two in France and one each in Spain, England and New Zealand (Kuser *et al.*, 1995). Early results from this study suggest that provenances

from the north end of the *Sequoia* range survive well in South Carolina and suffer less frost damage in northern France. Provenances from Humboldt County (middle of the *Sequoia* range) do survive well in Lafayette, California, and Entacon, France (Kuser *et al.*, 1995). This implies that the survival of redwoods outside their native range depended on the comparable climate/soil conditions in the distant locations. Therefore, differences in the origin of the material are important consideration for *ex situ* plantations of *Sequoia*.

Single trees or stands of *Sequoiadendron* were also planted more than 100 years ago in a number of countries of Europe (Hartseveldt, 1969). In several countries, solitary trees in Arboretums/Parks or stands of *Sequoiadendron* have survived (France, Hungary, Greece, Belgium, Netherlands, Denmark, Norway and Germany), while in other countries (Yugoslavia and Romania), *Sequoiadendron* has not performed well (Libby, 1981). Although healthy *Sequoiadendron* trees/stands still exist in Europe, the species is sensitive to frost and disease damage. In Germany, *Sequoiadendron* has performed well in southwest Germany, but not in northern Germany. One study tested frost tolerance in *Sequoiadendron* at the seedling stage (Guinon *et al.*, 1982), and the second investigated the survival of 14-year-old trees under field conditions in Germany (Melchior and Hermann, 1987). Significant differences were observed in the degree of frost tolerance, as measured by the freezing test conditions (temperatures ranging from -5 to -14°C), in the 2-year-old seedlings from 22 provenances (representing the entire natural range of *Sequoiadendron* in California) in Germany (Guinon *et al.*, 1982). Significant differences were also observed in the survival and growth performance of 14-year-old *Sequoiadendron* from four provinces at three different locations in Germany (Melchior and Hermann, 1987). In the northern location at Grossshansdorf, Germany, *Sequoiadendron* was badly damaged by frost and infection by *Armillaria mellea*. The authors recommended the use of frost-tolerant genotypes of *Sequoiadendron* for suitable locations in Germany (Melchior and Hermann, 1987).

Metasequoia has also been planted outside China and in different countries of the world as single trees in Arboretums/Parks or stands (Sato, 1999). *Metasequoia* seems to be more resilient to environmental extremes, for example, it has survived the heavy snows in the botanical gardens of Hamilton and Montreal, Canada, sizzling summer temperatures of Adelaide, Australia (Sato, 1999), and perhaps in other regions of the world with similar climates. In the USA, *Metasequoia* was introduced more than 50 years ago and showed good growth in the eastern and western USA (Kuser, 1999). Even though *Metasequoia* is an endemic, it has

a moderate reservoir of genetic (Kuser *et al.*, 1997) and phenotypic and ecotypic diversity (Li, 1999) for *ex situ* plantations (Li *et al.*, 2005) worldwide.

Although single trees may have been planted in the botanical gardens/parks in some countries, only small experimental stands of *Fitzroya* have been mainly restricted to Chile and Argentina (C. Donoso, pers. commun.). Of all the endemic redwoods, perhaps *Fitzroya* is the most endangered species and must be conserved in *ex situ* plantations and by other *ex situ* approaches for the conservation of its genetic resources. Genetic diversity in *Fitzroya* is lower than other conifers (Premoli *et al.*, 2000), but still has a moderate level of diversity, similar to *Metasequoia* and *Sequoiadendron* (Fins and Libby, 1982; Kuser *et al.*, 1997), and should be able to adapt to different environments. Therefore, it is high time that, like other three redwoods, *ex situ* plantations of *Fitzroya* are also established in suitable locations in Chile/Argentina and other countries for testing their adaptability and survival.

Biotechnological approaches for preservation of germplasm

Biotechnological approaches for preservation of germplasm include (1) *in vitro* storage of tissues at non-frozen temperatures and (2) storage of germplasm (seed, tissues, pollen and DNA) at sub-zero and ultra-low temperatures (cryopreservation).

In vitro storage of tissues

Tissues (meristems and shoots) can be used as a resource for clonal propagation and conservation of germplasm, since these can be maintained in culture at 4–25°C over a long period of time (Aitkin-Christie and Singh, 1987; Ahuja, 1994, 1999). The potential of tissue culture for differentiation and organogenesis has been investigated in *Sequoia* for more than 50 years. We have come a long way from the early studies with callus cultures (Ball, 1950) to differentiation of plantlets from shoot cultures of juvenile and mature trees, up to 90 years of age, in *Sequoia* (Boulay, 1997; Arnaud *et al.*, 1993; Bon *et al.*, 1994; Liu *et al.*, 2006). In general, stump shoots from the base of mature trees are more responsive than shoots from the crown of the same mature tree to *in vitro* differentiation and organogenesis. We had employed tissue culture using bud meristems from four frost-tolerant *Sequoia* trees (23 years old) from a plantation near Cologne, Germany, to clonally propagate them in 1986 at the Institute of Forest Genetics, Grosshansdorf, Germany (Ahuja, 1996). More than 1000 clones were

produced by tissue culture, and several hundred clones were tested under field conditions for their overwintering capacity. Frost tolerance capacity of *Sequoia* clones from the frost-tolerant donor trees was tested for many years in Grosshansdorf and Trenthorst, in northern Germany. The *Sequoia* clones grown in the Grosshansdorf nursery seemed to be frost-tolerant and have survived in the winters in Germany. However, only those frost-tolerant *Sequoia* clones survived in the field trial in Trenthorst were sheltered by the tree canopy during early growth (Ahuja, 1996). Therefore, it would be necessary to shelter the putative frost-tolerant *Sequoia* clones for several years of early growth for their survival in climates with harsh winters.

Tissues (bud meristems) from juvenile and mature trees (up to 100 years old) of *Sequoiadendron* have been cultured *in vitro*, and plants have been regenerated from such cultures (Monteuuis, 1987, 1991; Bon and Monteuuis, 1991; Monteuuis *et al.*, 2008). Tissue culture offers opportunities for clonal propagation from selected genotypes of redwoods, including frost-tolerant genotypes in *Sequoia*, for *ex situ* plantations. To my knowledge, tissue culture studies for clonal propagation have not been reported in *Metasequoia* and *Fitzroya*.

Storage of germplasm under sub-zero and ultra-low temperatures

Storage of germplasm (seeds, dormant buds, meristems, embryos, cells, pollen and DNA) under sub-zero temperatures (0 to –80°C) and cryopreservation (–196°C) offers opportunities for conservation of germplasm (Table 1) in forest trees (Sakai, 1986; Ahuja, 1989, 1994; Engelmann, 2004; Suszka *et al.*, 2005). Seeds of redwoods have been stored at low temperatures for various lengths of time. *Sequoia* seeds containing 6–10% moisture in airtight sealed bottles stored at 5°C for 3 years retained 14% viability, but seed viability dropped to 0% after 16 years of storage (Schubert, 1952). *Sequoia* seeds stored at –2 to –4°C retained viability for 1 year but lost viability rapidly after removal from cold storage (Metcalf, 1924). On the other hand, after storage at –16°C for 7 years, *Sequoia* seeds retained 12–15% viability (Boe, 1974). *Sequoiadendron* seeds, which had an 18% germination capacity, dropped their viability to 8% after storage at 5°C for 14 years (Schubert, 1952). Dry seeds of *Metasequoia* have been satisfactorily stored in airtight bottles at 2–4°C (Johnson, 1974).

Seeds, buds, meristems and cells have been successfully cryopreserved in a number of forest tree species (Stanwood, 1985; Ahuja, 1986, 1989, 1999; Bonner, 1990; Rynänen, 1996). In addition, conservation of DNA at –20 to –80°C in genebanks offers prospects

for the application of genomics to germplasm conservation (Adams, 1997; Rice *et al.*, 2006). Cryopreservation of germplasm has not been researched in the redwoods so far, but this avenue offers new options for storage of germplasm of the redwoods for future exploitation.

Future of redwoods

The redwoods are threatened in their native endemic locations in the face of climate change, and, therefore, the conservation of their germplasm is essential. Although these redwoods are protected in the national parks, reserves and in privately owned forests in their habitats, we have to consider their conservation in view of global warming and climate change. It would be desirable to conserve them (1) *in situ* on the model conservation sites (Noss *et al.*, 2000) that have comparable environmental conditions to their endemic climate regions (Table 1) and (2) in *ex situ* locations, similar to endemic conditions and also different environments to challenge their genotypes, and by other biotechnological *ex situ* strategies for conservation of their germplasm in genebanks.

Although there is an uncertainty about the actual amount of global warming, recent estimates predict an increase in global mean temperature, as a result of human activity, by 2.4–6.4°C (IPCC, 2007), and significant changes in the rainfall cycles (Trenberth *et al.*, 2003) by the end of the current century. Global climate change is impacting species distributions and functioning and their terrestrial ecosystems (Parmesan, 2006; Thuiller *et al.*, 2008). There is substantial evidence to suggest that the species ranges are shifting (Parmesan and Yohe, 2003; Root *et al.*, 2005; McKenney *et al.*, 2007; Kelly and Goulden, 2008), and some species are facing extinction risks, whereas others have become extinct (Thomas *et al.*, 2004; Schwartz *et al.*, 2006). Different models have been used to predict plant migration patterns to potentially suitable habitats under the future climate change scenarios. Many North American tree species will likely shift their ranges at a rate of 10–100 km/100 years to keep pace with the predicted climate changes in this century (Davis and Zabinski, 1992; Iverson and Prasad, 2002; Iverson *et al.*, 2004, 2005). In a recent study, Iverson *et al.* (2008) have examined the potential response of 134 tree species in the USA under different climate change scenario in this century. Depending on the climate change scenario, they predicted that more than a quarter of the species could shift their ranges more than 400 km northwards, and in the hottest climate change scenario, most of the species would advance up to 800 km northwards (Iverson *et al.*, 2008).

However, the use of molecular markers as indicators of potential migration capacity based on two North American tree species (*Fagus grandifolia* and *Acer rubrum*) under rapid climate change scenario has provided lower estimates of less than 10 km/century, which seem to be consistent with their life history and dispersal capacity (McLachlan *et al.*, 2005). It would appear that the potential migration rates of forest tree species in the face of rapid climate change, based on different models and methodologies, have resulted in widely different estimates of range shift (Pearson, 2006).

Based on the dispersal capacity, we speculate that if the climate changes slowly raising the global temperature by 1–2°C and the southern areas in the northern hemisphere start becoming hot and dry, the tree species from such climatic zones may have to move only a few km north to colonize areas that are suitable habitats for their survival. The reverse would be the case in species in the southern hemisphere, where the ranges would move further south. However, if the earth warms 2–6°C in this century, the climate niches of the tree species may have to move, depending upon the species, 10–100 km or even more in this century. Tree species whose seeds are dispersed by birds may be able to colonize new areas in that range. But, species whose seeds are dispersed by wind (unless strong winds) may not be able to spread more than a few hundred metres from their stands. Though redwoods have winged seeds, their dispersal is usually very close to their plantations. Therefore, rapid climate change would pose a challenging problem for colonization of the redwoods into new habitats. And then there are human-created impediments (industry and new settlements) that would become barriers to dispersal and migration. What will be the fate of redwoods in a rapid future climate change scenario? Although the extinction of endemic redwoods seems unlikely in the foreseeable future because of human conservation intervention (parks and reserves), they are still vulnerable to climate change in their native ranges.

A recent study has shown that there is a 33% reduction in the summer fog in the coast redwood (*Sequoia*) region along the California coast during the past century, and this climate change may impact recruitment of new coast redwood trees in the forest (Johnstone and Dawson, 2010). Anticipated changes in temperature and precipitation cycles in the 'climate envelopes' of other redwoods (*Sequoiadendron*, *Metasequoia* and *Fitzroya*) may also affect their growth and survival. If the retraction of *Sequoia* in its southern range and expansion in the northern range is any indication of the threat from climate change or other causes to the species (Sawyer *et al.*, 2000), then a similar phenomenon might be happening to the other three redwoods in their native ranges.

That would imply that *Sequoiadendron* and *Metasequoia* may also be contracting in the southern ranges and expanding northwards, while *Fitzroya* may be retracting in the northern range and expanding southwards. Since California has varied terrains, anticipated migration of endemics, depending on the magnitude of emissions, may be more complex and, as a result, species may migrate in different (altitude and latitude) directions, thus disrupting the present endemic floras (Loarie *et al.*, 2008). And *Sequoiadendron*, which is already vulnerable endemic in California, may have an unpredictable fate in the face of climate change. The redwoods ranges have temperate temperatures (10–29°C zones) and mild winters (Table 1), but the redwoods are sensitive to frost. Therefore, development/isolation of frost-tolerant genotypes in redwoods may offer excellent opportunities for future *ex situ* reserves in colder climates.

Faced with a threat of climate change, it might be useful to deploy an intimate mixture of seeds from widely divergent populations from different environments (Ledig and Kitzmiller, 1992) as a resource for seed orchards and planting material for potential future climatic conditions. In addition, conservation planning in a changing climate calls for strategies that locate, configure and maintains areas that are managed to promote biodiversity and ecosystem stability (Hannah *et al.*, 2007; Pressey *et al.*, 2007; Thuiller *et al.*, 2008). In the face of uncertainty regarding climate change, it would be prudent to pursue flexible approaches that include adaptive strategies (actions that promote and maintain genetic diversity) and mitigation approaches (actions that sequester carbon and reduce overall greenhouse gas emissions) for forests of the future (Millar *et al.*, 2007). In any event, it is imperative that germplasm conservation of redwoods must proceed unabatedly, with more emphasis on *ex situ* strategies to 'Save the Redwoods'.

Acknowledgements

I am thankful to the Institute of Forest Genetics, USDA, Placerville, and Davis, California, for research and library facilities for this review. I thank David Neale for interesting discussions, and anonymous reviewers for comments on the manuscript.

References

- Adams RP (1997) Conservation of DNA: DNA banking. In: Callow JA, Ford-Lloyd BV and Newbury HJ (eds) *Biotechnology and Plant Genetic Resources, Conservation and Use*. London: CABI Publishing, pp. 163–174.
- Ahuja MR (1986) Storage of forest tree germplasm in liquid nitrogen (–196°C). *Silvae Genetica* 35: 249–251.
- Ahuja MR (1989) Storage of forest tree germplasm at sub-zero temperatures. In: Dhawan V (ed.) *Application of Biotechnology in Forestry and Horticulture*. New York: Plenum Press, pp. 215–228.
- Ahuja MR (1994) Reflections on germplasm preservation of trees. In: Pardos JA, Ahuja MR and Rosello RE (eds) *Biotechnology of Trees*. Madrid: Investigacion Agraria Sistemas y Recursos Forestales, pp. 227–233.
- Ahuja MR (1996) Micropropagation and field testing of frost-tolerant *Sequoia sempervirens* genotypes. In: LeBlanc J (ed.) *Proceeding of the Conference on Coast Redwood Forest Ecology and Management*. Arcata: Humboldt State University, pp. 153–155.
- Ahuja MR (1999) Biotechnology in forest tree gene banks. In: Edwards DGW and Naithani SC (eds) *Seed and Nursery Technology of Forest Trees*. New Delhi: New Age International (P) Limited Publishers, pp. 23–36.
- Ahuja MR (2005) Polyploidy in gymnosperms: revisited. *Silvae Genetica* 54: 59–69.
- Ahuja MR (2009) Genetic constitution and diversity in four narrow endemic redwoods from the family Cupressaceae. *Euphytica* 165: 5–19.
- Ahuja MR and Neale (2002) Origins of polyploidy in coast redwood (*Sequoia sempervirens* (D. Don) Endl.) and relationship of coast redwood to other genera of Taxodiaceae. *Silvae Genetica* 51: 93–100.
- Ahuja MR and Neale DB (2005) Evolution of genome size in conifers. *Silvae Genetica* 54: 126–137.
- Aitkin-Christie J and Singh AP (1987) Cold storage of tissue cultures. In: Bonga JM and Durzan DJ (eds) *Cell and Tissue Culture in Forestry*, vol. 1. Dordrecht: Martinus Nijhoff Publishers, pp. 285–304.
- Allnutt TR, Newton AC, Lara A, Premoli A, Armesto JJ, Vergara R and Gardner M (1999) Genetic variation in *Fitzroya cupressoides* (alerce), a threatened South American conifer. *Molecular Ecology* 8: 975–987.
- Arnaud Y, Franclet A, Tranvan H and Jacques M (1993) Micropropagation and rejuvenation of *Sequoia sempervirens* (Lamb) Endl.): a review. *Annales des Sciences Forestieres* 50: 273–295.
- Ball EA (1950) Differentiation in a callus culture of *Sequoia sempervirens*. *Growth* 14: 295–325.
- Behm A, Becker A, Dorflinger H, *et al.*, (1997) Concept for the conservation of forest genetic resources in the Federal Republic of Germany. *Silvae Genetica* 46: 24–34.
- Boe KN (1974) *Sequoia sempervirens* (D. Don) Endl. – Redwood. In: Schopmeyer CS (ed.) *Seeds of Woody Plants in the United States*. Washington, DC: Forest Service, USDA, pp. 764–766.
- Bon MC and Monteuis O (1991) Rejuvenation of a 100-year-old *Sequoiadendron giganteum* through *in vitro* meristem culture. I. Organogenic and morphological arguments. *Physiologia Plantarum* 81: 111–115.
- Bon M-C, Riccari F and Monteuis O (1994) Influence of phase change within a 90-year old *Sequoia sempervirens* on its *in vitro* organogenic capacity and protein patterns. *Trees* 8: 283–287.
- Bonner FT (1990) Storage of seeds: potential and limitations for germplasm conservation. *Forest Ecology and Management* 35: 35–43.
- Boulay M (1997) Multiplication rapide du *Sequoia sempervirens* en culture *in vitro*. *Annales AFOCEL* 1977: 37–66.

- Brinegar C, Bruno D, Kirkbride R, Glavas S and Udransky U (2007) Applications of redwood genotyping by using microsatellite markers. *USDA Forest Service Technical Report PSW-GTR 194*, pp. 47–55.
- Chen XY, Li YY, Wu TY, Zhang X and Lu HP (2003) Size-class differences in genetic structure of *Metasequoia glyptostroboides* Hu et Cheng (Taxodiaceae) plantations in Shanghai. *Silvae Genetica* 52: 107–109.
- Chu K and Cooper SC (1999) An ecological reconnaissance in the native home of *Metasequoia glyptostroboides*. *Arnoldia* 59: 40–46.
- Davis MB and Zabinski C (1992) Changes in geographical range resulting from greenhouse warming effects on biodiversity in forests. In: Peters RL and Lovejoy TE (eds) *Global Warming and Biological Diversity*. New Haven: Yale University Press, pp. 297–308.
- Engelmann F (2004) Plant cryopreservation: progress and prospects. *In Vitro Cellular and Developmental Biology – Plants* 40: 427–433.
- Evarts J and Popper M (2001) Conservation and management of redwood forests. In: Evarts J and Popper M (eds) *Coast Redwood A Natural and Cultural History*. Los Olivos: Cachuma Press, pp. 165–205.
- Fins L and Libby WJ (1982) Population variation in *Sequoiadendron*: seed and seedling studies, vegetative propagation and isozyme variation. *Silvae Genetica* 31: 102–110.
- Gadek PA, Alpers DL, Heslewood MM and Quinn CJ (2000) Relationship within Cupressaceae sensu lato: a combined morphological and molecular approach. *American Journal of Botany* 87: 1044–1057.
- Geburek T and Konrad H (2007) Why the conservation of forest genetic resources has not worked. *Conservation Biology* 22: 267–274.
- González-Martínez SC, Krutovsky KV and Neale DB (2006) Forest-tree population genomics and adaptive evolution. *New Phytologist* 170: 227–238.
- Guinon M, Larson JB and Spethmann W (1982) Frost resistance and early growth of *Sequoiadendron giganteum* seedlings of different origin. *Silvae Genetica* 31: 173–178.
- Hair JB (1968) The chromosomes of the Cupressaceae. I. Tetraclineae and Actinostrobaeae (Callitroideae). *New Zealand Journal of Botany* 6: 277–284.
- Hamrick JL (2004) Response of forest trees to global environmental changes. *Forest Ecology and Management* 197: 323–335.
- Hamrick JL, Godt MJW and Sherman-Boyles SL (1992) Factors influencing levels of genetic diversity in woody plant species. *New Forests* 6: 95–124.
- Hannah L, Midgley G, Andelman S, Araújo M, Hughes G, Martínez-Meyer E, Pearson R and Williams P (2007) Protected areas needs in a changing climate. *Frontiers of Ecological Environment* 5: 131–138.
- Hartesveldt RJ (1969) *Sequoia* in Europe with a review of their discovery and their resultant importation into Europe. *Final Contract Report to the National Park Service. Contract no. 14-10-0434*, pp. 22.
- Hartesveldt RJ, Harry HT, Schellhammer HS and Stecker RR (1975) *The Giant Sequoia of the Sierra Nevada*. Washington, DC: US Department of Interior, National Park Service, p. 180.
- Hattemer HH (1995) Concepts and requirements in the conservation of forest genetic resources. *Forest Genetics* 2: 125–134.
- Hidalgo E, González-Martínez SC, Lexer C and Heinze B (2010) Conservation genomics. In: Jansson S, Bhalerao R and Groover A (eds) *Genetics and Genomics of Populus*. Heidelberg: Springer Verlag, pp. 349–368.
- Holderegger R, Kamm U and Gugerli F (2006) Adaptive vs neutral genetic diversity: implications for landscape genetics. *Landscape Ecology* 21: 797–807.
- IPCC (2007) Climate Change 2007. *The physical science basis. Summary for policymakers*. Available at www.ipcc.ch
- IUCN (2010) *IUCN red list of threatened species*. Version 2010.2. Available at www.iucnredlist.org
- Iverson LR and Prasad AM (2002) Potential redistribution of tree species habitat under five climate change scenarios in the eastern US. *Forest Ecology and Management* 155: 205–222.
- Iverson LR, Schwartz MW and Prasad AM (2004) How fast and far might tree species migrate in the eastern United States due to climate change. *Global Ecology and Biogeography* 13: 209–219.
- Iverson LR, Prasad AM and Schwartz MW (2005) Predicting potential changes in suitable habitat and distribution by 2100 for tree species in the eastern United States. *Journal of Agricultural Meteorology* 61: 29–37.
- Iverson LR, Prasad AM, Matthews SN and Peters M (2008) Estimating potential habitat for 134 eastern US tree species under six climate scenarios. *Forest Ecology and Management* 254: 390–406.
- Johnson LC (1974) *Metasequoia glyptostroboides* Hu and Cheng – Dawn Redwood. In: Schopmeyer CS (ed.) *Seeds of Woody Plants in the United States*. Washington, DC: Forest Service, USDA, pp. 540–542.
- Johnstone JA and Dawson TE (2010) Climate context and ecological implications of summer fog decline in the coast redwood region. *Proceedings of the National Academy of Sciences USA* 107: 4533–4538.
- Karhu AP, Hurme P, Karjalainen M, Karvonen P, Kärkkäinen K and Neale DB (1996) Do molecular markers reflect patterns of differentiation in adaptive traits in conifers? *Theoretical and Applied Genetics* 93: 215–221.
- Kelly AE and Goulden ML (2008) Rapid shifts in plant distribution with recent climate change. *Proceedings of the National Academy of Sciences USA* 105: 11823–11826.
- Krutovsky KV and Neale DB (2005) Forest genomics and new molecular genetic approaches to measuring and conserving adaptive genetic diversity in forest trees. In: Geburek T and Turok J (eds) *Conservation and Management of Forest Genetic Resources in Europe*. Zvolen: Arbora Publishers, pp. 369–390.
- Kuser JE (1981) Redwoods around the world. *American Forests* 87: 30–32.
- Kuser J (1983) Inbreeding depression in *Metasequoia*. *Journal of Arnold Arboretum* 64: 475–581.
- Kuser JE (1999) *Metasequoia glyptostroboides*: fifty years of growth in North America. *Arnoldia* 59: 76–79.
- Kuser JE, Bailly A, Francllet A, Libby WJ, Martin J, Reydelius J, Schoenike R and Vagle N (1995) Early results of a range-wide provenance test of *Sequoia sempervirens*. *Forest Genetics Resources (Rome: FAO)* 23: 21–25.
- Kuser JE, Sheely DL and Hendricks DR (1997) Genetic variation in two *ex situ* collections of the rare *Metasequoia glyptostroboides* (Cupressaceae). *Silvae Genetica* 46: 258–264.
- Ledig FT (1986) Conservation strategies for forest gene resources. *Forest Ecology and Management* 14: 77–90.
- Ledig FT (1987) Genetic structure and conservation of California's endemic and near-endemic conifers. In: Elliot

- TS (ed.) *Conservation and Management of Rare and Endangered Plants*. Sacramento: California Native Plant Society, pp. 587–594.
- Ledig FT (1988) The conservation of diversity in forest trees. *Bioscience* 38: 471–479.
- Ledig FT and Kitzmiller JH (1992) Genetic strategies for reforestation in the face of global climate change. *Forest Ecology and Management* 50: 153–169.
- Ledig FT, Hodgekiss PD and Jacob-Cervantes V (2002) Genetic diversity, mating system, and conservation of Mexican subalpine relict, *Picea mexicana* Martinez. *Conservation Genetics* 3: 113–122.
- Leng Q, Fan S-H, Wang L, Yang H, *et al.*, (2007) Database of native *Metasequoia glyptostroboides* trees in China based on new consensus surveys and expeditions. *Bulletin of Peabody Museum Natural History* 48: 185–233.
- Li J (1999) *Metasequoia*: an overview of its phylogeny, reproductive biology, and ecotypic variation. *Arnoldia* 59: 54–59.
- Li YY, Chen XY, Zhang X, Wu TY, Lu HP and Cai YW (2005) Genetic differences between wild and artificial populations of *Metasequoia glyptostroboides*: implications of species recovery. *Conservation Biology* 19: 224–231.
- Libby WJ (1981) Some observations on *Sequoiadendron* and *Calocedrus* in Europe. *California Forestry and Forest Products* 49: 1–12.
- Libby WJ, McCutchan BG and Millar CI (1981) Inbreeding in selfs of redwood. *Silvae Genetica* 30: 15–25.
- Libby WJ, Anekonda TS and Kuser JE (1996) The genetic architecture of coast redwood. In: Leblanc J (ed.) *Proceeding of the Conference on coast Redwood Forest Ecology and Management*. Arcata: Humboldt State University, pp. 147–149.
- Liu C, Xia X, Yin W, Huang L and Zhou J (2006) Shoot regeneration and somatic embryogenesis from needles of redwood (*Sequoia sempervirens* (D. Don) Endl.). *Plant Cell Reports* 25: 621–628.
- Loarie SR, Carter BE, Hayhoe K, McMahon S, Moe R, Knight CA and Ackerly DD (2008) Climate change and the future of California's endemic flora. *Public Library of Science ONE* 3: e2502.
- McKenney DW, Pedlar J, Lawrence K, Campbell K and Hutchinson MF (2007) Potential impacts of climate change on the distribution of North American trees. *Bioscience* 57: 939–948.
- McLachlan JS, Clark JS and Manos PS (2005) Molecular indicators of tree migration capacity under rapid climate change. *Ecology* 86: 2088–2098.
- Melchior GH and Hermann S (1987) Differences in growth performance of four provenances of giant sequoia (*Sequoiadendron giganteum* (Lindl) Buchh.). *Silvae Genetica* 38: 65–68.
- Melchior GH, Muhs HJ and Stephan BR (1986) Tactics for forest tree resources in the Federal Republic of Germany. *Forest Ecology and Management* 17: 73–81.
- Metcalf W (1924) Artificial reproduction of redwood (*Sequoia sempervirens*). *Journal of Forestry* 22: 873–893.
- Millar CI (1993) Conservation of germplasm of forest trees. In: Ahuja MR and Libby WJ (eds) *Clonal Forestry II. Conservation and Application*. Heidelberg: Springer Verlag, pp. 42–65.
- Millar CI and Westfall RD (1992) Allozyme markers in forest genetic conservation. *New Forests* 6: 347–371.
- Millar CI, Stephenson NL and Stephens SL (2007) Climate change and forests of the future: managing in the face of uncertainty. *Ecological Applications* 17: 2145–2151.
- Monteuuis O (1987) *In vitro* meristem culture of juvenile and mature *Sequoiadendron giganteum*. *Tree Physiology* 3: 265–272.
- Monteuuis O (1991) Rejuvenation of a 100-year-old *Sequoiadendron giganteum* through *in vitro* meristem culture. I. Organogenic and morphological arguments. *Physiologia Plantarum* 81: 111–115.
- Monteuuis O, Doubeau S and Verdeil JL (2008) DNA methylation in different origin clonal offspring from a mature *Sequoiadendron giganteum* genotype. *Trees* 22: 779–784.
- Noss RF, Strittholt JR, Heilman GE, Frost PA and Sorensen M (2000) Conservation planning in the redwoods region. In: Noss RF (ed.) *The Redwood Forest. History, Ecology, and Conservation of Redwoods*. Washington, DC: Save-the-Redwoods League Island Press, pp. 201–2228.
- Olson DF, Roy DF and Walters GA (1990) *Sequoia sempervirens* (D. Don) Endl. Redwood. In: Burns RM and Honkala BH (eds) *Silvics of North America, vol. 1. Conifers*. Agriculture Handbook 654. Washington, DC: US Department of Agriculture, Forest Service, pp. 541–551.
- Parker T and Donoso C (1993) Natural regeneration of *Fitzroya cupressoides* in Chile and Argentina. *Forest Ecology and Management* 59: 63–85.
- Parmesan C (2006) Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution and Systematics* 37: 637–669.
- Parmesan C and Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421: 37–42.
- Pearson RG (2006) Climate change and the migration capacity of species. *Trends in Ecology and Evolution* 21: 111–113.
- Premoli AC, Kitzberger T and Veblen TT (2000) Conservation genetics of the endangered conifer *Fitzroya cupressoides* in Chile and Argentina. *Conservation Genetics* 1: 57–66.
- Premoli AC, Vergara R, Souto CP, Lara A and Newton AC (2003) Lowland valleys shelter the ancient conifer *Fitzroya cupressoides* in the Central Depression of southern Chile. *Journal of the Royal Society of New Zealand* 33: 623–631.
- Pressey RL, Cabeza M, Watts ME, Cowling RM and Wilson KA (2007) Conservation planning in a changing world. *Trends in Ecology and Evolution* 22: 583–592.
- Rice N, Cordeiro G, Shepherd M, Bundock P, Bradbury L, Pacey-Miller T, Futado A and Harry R (2006) DNA banks and their role in facilitating the application of genomics to plant germplasm. *Plant Genetic Resources* 4: 64–70.
- Rogers DL (1997) Inheritance of allozymes from seed tissues of the hexaploid gymnosperm, *Sequoia sempervirens* (D. Don) Endl. (Coast redwood). *Heredity* 78: 166–175.
- Rogers DL (2000) Genotypic diversity and clones size in old-growth populations of coast redwood (*Sequoia sempervirens*). *Canadian Journal of Botany* 78: 1408–1419.
- Root TL, MacMynowski DP, Mastrandrea MD and Schneider SH (2005) Human modified temperature induce species changes: joint attribution. *Proceedings of the National Academy of Sciences USA* 102: 7465–7469.
- Ryynänen L (1996) Survival and regeneration of dormant silver birch buds stored at super-low temperatures. *Canadian Journal of Forest Research* 26: 617–623.
- Sakai A (1986) Cryopreservation of germplasm of woody plants. In: Bajaj YPS (ed.) *Biotechnology in Agriculture and Forestry, vol. 1. Trees I*. Heidelberg: Springer Verlag, pp. 113–129.
- Satoh K (1999) *Metasequoia* travels the globe. *Arnoldia* 59: 72–75.

- Sawyer JO, Gray J, West J, Thorburgh DA, Noss RF, Engbeck JH, Marcot BG and Raymond R (2000) History of redwoods and redwood forests. In: Noss RF (ed.) *The Redwood Forest. History, Ecology, and Conservation of Redwoods*. Washington, DC: Save-the-Redwoods League Island Press, pp. 81–118.
- Saylor LC and Simons HA (1970) Karyology of *Sequoia sempervirens*: karyotype and accessory chromosomes. *Cytologia* 35: 294–303.
- Schlarbaum SE and Tsuchiya T (1984) Cytotaxonomy and phylogeny in certain species of *Taxodiaceae*. *Plant Systematics and Evolution* 147: 29–54.
- Schubert GH (1952) Germination of various coniferous seeds after cold storage. *USDA Forest Service Research Note* PSW-83, pp. 7.
- Schwartz MW, Iverson LR, Prasad AM, Matthews SN and O'Connor RJ (2006) Predicting extinctions as a result of climate change. *Ecology* 87: 1611–1615.
- Stanwood PC (1985) Cryopreservation of seed germplasm for genetic conservation. In: Kartha KK (ed.) *Cryopreservation of Plant Cell and Organs*. Boca Raton: CRC Press, pp. 199–226.
- Stebbins GL (1957) Self-fertilization and population variability in the higher plants. *American Naturalist* 91: 337–354.
- Suszka B, Chmielarz P and Walkenhorst R (2005) How long can seeds of Norway spruce (*Picea abies* (L.) Karst.) be stored? *Annales of Forest Sciences* 62: 73–78.
- Thomas CD, Cameron A, Green RE, *et al.*, (2004) Extinction risk from climate change. *Nature* 427: 145–148.
- Thuiller W, Albert C, Araújo MB, *et al.*, (2008) Predicting global change impacts on plant species distributions: future challenges. *Perspectives in Plant Ecology, Evolution and Systematics* 9: 137–152.
- Tikader A, Vijayan K and Kamble CK (2009) Conservation and management of mulberry germplasm through biomolecular approaches – a review. *Biotechnology and Molecular Biology Reviews* 3: 92–104.
- Trenberth KE, Dai A, Rasmussen RM and Pearson RB (2003) The changing character of precipitation. *Bulletin of the American Meteorological Society* 84: 1205–1217.
- Volis S and Blecher M (2010) Quasi *in situ*: a bridge between *ex situ* and *in situ* conservation of plants. *Biodiversity and Conservation* 19: 2441–2454.
- Weatherspoon CP (1990) *Sequoiadendron giganteum* (Lindl.) Buchholz, Giant Sequoia. In: Burns RM and Honkala BH (eds) *Silvics of North America. vol 1. Conifers*. Agriculture Handbook 654. Washington, DC: US Department of Agriculture, Forest Service, pp. 552–562.
- White TL, Adams WT and Neale DB (2007) *Forest Genetics*. Cambridge, MA: CABI Publishing..
- Wright JW (1976) *Introduction to Forest Genetics*. New York: Academic Press.