

Chapter 5. Status of Knowledge – Current and Emerging Technologies

5.1 Introduction

Context, information sources

- 5 This is the first synthesis of its kind and constitutes a baseline against which future status assessments can be compared; however there is not a single starting point for this report. Sources of information for this chapter include National Reports prepared by countries and which form the basis of the State of the World's Forest Genetic Resources Report; regional summaries that were prepared in part following regional workshops; background Thematic Studies that were prepared to supplement the information provided by countries, and additional information was synthesized from scientific articles and other published literature. Earlier chapters of the Report describe the status of FGR. This chapter is limited to status of knowledge about the resources. Of necessity the treatment of knowledge of FGR, herein is selective, as volumes would be required to capture all available knowledge. Examples are used in the chapter to illustrate levels, types and applications of genetic knowledge.

Although basic genetic principles are consistent across plant and animal taxa, forest trees differ from agricultural crops in significant ways, and the study, management and conservation of their genetic resources has had to adapt accordingly. Thus the type of knowledge and focus on particular genetic technologies also differ between forest trees and domesticated plants and animals in some regards.

- 20 Even the relatively small proportion of forest tree species that are undergoing incipient domestication, typically also exist as large wild, randomly mating, and unstructured populations (Neale and Kremer 2011). Most forest tree species have narrow regional adaptation so the numbers of species planted commercially are much higher than for food crops (Pautasso, 2009). Trees are keystone species in forest ecosystems, and the diversity of associated species depends on inter- and 25 intraspecific diversity of trees (Whitham *et al.*, 2006). Specific colonising symbiont microbial species are often essential for successful establishment of tree species (Bonfante and Anca, 2009). Tree species are long-lived and generation times are long. Many tree species encompass enormous genetic diversity (Hamrick, *et al.* 1992); even breeding populations in improvement programmes represent relatively large gene pools compared to agricultural crops, and tree genomes may be 30 orders of magnitude larger than those of cultivated crops (Neale and Kremer 2011). Tree species are predominantly out-crossing and in fact, none are known to be predominantly selfing (Petit and Hampe 2006). With a few exceptions, tree breeders do not aim to develop varieties similar to those of agricultural crops. In addition to all of the aforementioned distinctions, the sheer number of tree species that have known or potential value is in the 10's of thousands. This means that forest 35 geneticists and genomicists face very different challenges and require different tools and techniques from those who work with agricultural crops.

- According to Country Reports, approximately 2400 species are actively managed. However, only 40 approximately 700 tree species are subject to some level of selection and improvement globally, and progeny tests have been established for no more than two-thirds of these species. In addition, a number of non-plantation tree species have been studied mainly using molecular markers, but still,

assuming a total global count of at least 80,000 tree species, little more than 1% of the tree species have been subject to genetic study, and less than 1% have been studied with the aim of improving resources for human use. Undoubtedly many species, not yet studied, have untapped potential that could be realised, pending sufficient resources, interest and survival of sufficiently diverse

5 populations.

The tree species that have been most studied using scientific approaches (since about 1950) fall in two categories: those that have been extensively planted in commercial plantations for wood, and species that are valuable in agriculture for fruit and other livelihood benefits. Commercial plantations represent about 7% of the world's forests, but are responsible for more than 50% of 10 world's industrial roundwood production (FRA 2010). Approximately 30 tree species from just 4 genera (*Acacia*, *Eucalyptus*, *Pinus* and *Populus*) account for much of the area planted globally (Carle, et al. 2008). Most of these species have been studied in detail, including quantitative and molecular/genomic analyses. Some of the most studied species include: *Acacia mangium*, *A. nilotica*, *Eucalyptus grandis*, *E. camaldulensis*, *E. globulus*, *Gmelina arborea*, *Tectona grandis*, *Pinus caribaea*, 15 *P. elliottii*, *P. patula*, *P. taeda*, *P. radiata*, *P. nigra*, *P. pinaster*, *P. massoniana*, *P. sylvestris*, *Picea sitchensis*, *P. abies*, *Pseudotsuga menziesii*, *Cunninghamia lanceolata*, *Populus trichocarpa*, *P. deltoids*, *P. nigra*, *P. tremuloides*, and *P. tremula*.

Tree species producing fruit and other food-related crops of global significance have a much longer history of domestication and accumulation of knowledge, and unlike the tree species that have been 20 managed for wood production, well recognised varieties of fruit tree species have been developed over centuries. Yet unlike most agricultural crops, the original species still exist as wild populations capable of sharing genetic material with domesticated varieties. Some of them are under threat, for example several globally significant fruit tree species that originated in Central Asia (Eastwood, et al. 2009).

25 ***What constitutes knowledge of Forest Genetic Resources?***

Forest genetic resources refer to genetic diversity of tree and other woody plant species of present or potential value to humans (FAO 1989). This includes ecological value as well as direct livelihood values. For the purposes of this overview, we assume that any species that has been studied has actual or potential value. Relevant knowledge encompasses quantitative, molecular and genomic 30 information; however recognising that knowledge of a tree species implies more than the existence of a small genetic marker study, it was beyond the scope of this chapter to evaluate level of knowledge on a species by species basis. In addition to "direct" genetic information, knowledge of species distribution and environmental variability within the species range; population size and relative degree of contiguity; and observable morphological variation (in a natural population), can 35 all provide insight into genetic variability of a species. Traditional or indigenous knowledge predates published information and it is less well-documented, but valuable.

Plantation tree species having high commercial value have mostly been subject to some degree of selection, testing and breeding have been undertaken and this implies a particular type of knowledge. If such a species is vegetatively propagated, a different type of knowledge is implied.

40 Other species that are highly valuable are harvested exclusively in the wild and very little may be

known about genetic resources of such species. In some cases the genetic resource of such species may be degraded through dysgenic selection, when the best quality trees are cut leaving only badly shaped trees as contributors to next generation. In the absence of an improvement strategy and planting this may have dramatic effects on the resource sustainability but few conclusive studies 5 have evaluated this, and one of the few that has been published found little evidence (Cornelius, et al. 2005).

Reasons for studying FGR and type of information generated

Uses, management practices, and priorities for research vary by type of tree species, region and socio-economic situation of the people who use them. Most of the scientific genetics research on 10 trees has been devoted to species having commercial value for timber, and most of these are temperate species. About 30 species have been studied intensively, tested, and bred for increased wood production, improved quality (Neale and Kremer 2011) and/or resistance to pests and diseases (Yanchuk and Allard 2009). A body of knowledge has also accumulated over a longer period of time and in a much more fragmented way on tree species that are important for non-timber forest 15 products: fruit and nut-bearing trees, species with valuable medicinal properties, oil or latex producing trees, and species having shade or ornamental value. Traditional knowledge of phenotypic variation extends from informally recorded traditional knowledge to recent scientific studies. Few of these species have been thoroughly studied using modern techniques, with the exception of those having high commercial value, and in such cases, the techniques employed and 20 aims pursued bear resemblance to those of agricultural crop genetic research.

Most tropical timber species are managed in semi-natural forests; in many cases, selectively harvested and their renewal depends on natural regeneration. In general, little is known about the sustainability of the genetic resources comprised by selectively harvested tree species, especially those in the tropics (Wernsdorfer, et al. 2011). However, effective long-term management of these 25 species requires knowledge of population genetic parameters such as gene flow dynamics, and structure of genetic variation in economically and adaptively important traits. Such knowledge is important to ensure that viable populations are maintained in harvested areas and that harvest does not have a dysgenic impact on seed trees. Genetic markers that can be used effectively for identifying species and origin of timber are becoming important as well, to monitor legality of timber 30 harvest.

Conservation of evolutionary potential is important for all use and management categories of trees (Lefèvre et al. 2013). This requires an understanding of the extent and patterns of genetic diversity throughout a species' range. Many studies have been conducted on limited numbers of populations for many species, using various molecular markers to elucidate patterns of diversity. Often studies 35 are limited by insufficient funds, resulting in small sample sizes and thus limited applicability of results; and the use of different markers for subsequent studies in divergent geographic locations of the same species leads to disparate results that may even be contradictory, with little potential for generating a common understanding. Little concrete action has been taken on the basis of most conservation genetic studies, in part because of a gap between the results of studies and applicable 40 conservation knowledge (Knight 2008). The data are still largely insufficient to allow testing if congruent patterns of spatial genetic diversity exist among species (zones of genetic endemism,

richness). This has important implications in terms of landscape management and conservation as forest reserves are ideally localized on the basis of genetic information across all species.

Tree species are much better characterised in some regions than others. In Europe, North America and Australia, at least some genetic knowledge has been generated for most native tree species.

- 5 Species in Latin America have received more attention than those of Asia or Africa, in general, although there are exceptions at the country level. Even in countries where significant resources have been focused on understanding FGR, the evenness of species coverage is variable, with key species studied intensively, but for many, there is little or no information. At the species level, approaches vary widely, with single population studies common for tropical species, up to range-wide surveys for temperate species of broad commercial interest.

Tree improvement

Improvement programmes for wood production have generated knowledge about productivity and quality traits for most tree species that are used extensively in plantations. The earliest genetic studies on forest trees were designed to quantify variation in traits of commercial value for purposes

- 15 of selection and breeding. Most of the effort has been focused on increasing wood volume both for timber and pulp and paper and improving the form of trees for timber production. Many studies have been reported on genetic parameters associated with wood quality, including strength and density, and more recently lignose/cellulose ratio, although these traits have generally been considered to be of secondary importance. Breeding for resistance or tolerance to biotic and abiotic
- 20 factors tends to be restricted to more specialised research programmes (Yanchuk and Allard 2009). Hence much information is available for most commercial plantation species on the complicated growth rate-related traits associated with quantity of wood production, but relatively little genetic information has been generated about other traits.

Agricultural purposes - Domestication of fruit trees and other tree species that are important for

- 25 food, fodder or have cultural or religious significance has been underway for millennia, without the benefit of scientific genetic knowledge for most of that time. The most reliable method for capturing improvements is by cloning trees having desirable properties. Unlike the case of commercial timber species, domesticated varieties of trees important for food and other non-wood amenities have been developed for centuries, simultaneously, in many geographic locations.

- 30 *Conservation* - During the last three decades, since the development and broad use of molecular markers, many studies have been conducted applying these tools to inform conservation strategies and approaches. Neutral genetic markers are used to deduce population-level parameters that are informative about spatial patterns of genetic diversity, reproductive biology (mating system, pollen- and seed-mediated gene flow), species evolutionary history and demography (for example, existence of genetic bottlenecks, localization of refugia sites, founder populations).

Aims of conservation genetic studies are to understand levels and patterns of genetic diversity, impacts of land use changes on intraspecific variation, and vulnerability of populations to threats; and to identify populations having high conservation priority and design approaches for their conservation. A combination of neutral molecular markers and either phenotypic measurements or

genomic markers for adaptive traits is ideal for defining priority populations, but in most cases, resource limitations have resulted in use of neutral markers.

Taxonomy & phylogeny - Patterns of genetic differentiation and speciation have been studied in order to understand evolutionary history of species. Petit and Hampe (2006) reviewed the

- 5 evolutionary consequences of being a tree, noting that the high diversity and very high fecundity of most tree species allows for rapid evolution at the micro scale, but long generation time, large size and other characteristics result in slow macroevolution.

Taxonomic studies rely increasingly on genetic markers to complement or replace conventional morphological methods to determine taxonomic status and understand phylogenetic relationships.

- 10 Many tree genera are incompletely described at the species level, in part because of hybridization and introgression among species; *Quercus* spp in Mexico provide an example where the number of species is 135-150 (González-Rodríguez, et al. 2004) but the exact number is not known. A combination of nuclear markers and morphological traits are employed to differentiate species.

Chloroplast DNA is the genome of choice for phylogenetic studies because of the small size of the genome, and uniparental inheritance, allowing spatial pattern of haplotypes to be interpreted as an estimate of past gene flow. In addition, the chloroplast shows neutral differentiation among divergent populations sooner than nuclear alleles. If population divergence has occurred relatively recently, neutral cpDNA variation will be more likely to show the differentiation than nuclear polymorphisms (Hamilton, et al. 2003). Hamilton, et al. (2003) examined patterns of cpDNA

- 20 haplotype variation and in particular, searched for evidence of selection acting on several insertion/deletion regions of the chloroplast genome of eight species of Lecythidaceae, which is the Brazil nut family. They found that the rate of evolution was highly variable among regions in the genome, but that the variability seemed to be lineage-related rather than region-related. They concluded that the insertion/deletion markers and nucleotide variation in the chloroplast genome

- 25 were selectively neutral and thus should provide unbiased estimates of population parameters.

The Bar Code of Life project attempts to use DNA markers directly to identify species. This is further discussed below, with respect to use of DNA forensics for reducing illegal logging.

5.2 Trait based knowledge of tree genetic resources

Until the advent of biochemical and molecular methods, the only way to estimate genetic values or

- 30 variation was by measuring phenotypes and using statistical tools to separate genetic effects from environmental influences. Although efforts in field studies designed to estimate genetic parameters have declined, such studies are still essential for understanding genetic control of phenotypic traits.

Before the development of genetics as a scientific discipline, trees had been planted for food, wood, shade and religious purposes for thousands of years. Knowledge of trait variation was used in

- 35 traditional farming and subsistence systems to select, save and/or cultivate valuable individual trees on the basis of phenotypic characteristics. Besides food tree species such as *Olea europaea*, *Malus domestica*, *Citrus sinensis*, other cultivated tree species of significant importance include *Cinchona ledgeriana* from Bolivia and transported to Europe to combat malaria, then grown in Asia; and *Hevea*

brasiliensis, the rubber tree in Brazil seeds of which were transported to the UK then Asia, in the late 1800s. All of these species were subject to selection and breeding on the basis of valuable phenotypic characteristics.

Indigenous and traditional knowledge

5 Traditionally living societies generally maintain an intimate relationship with the natural world of their (actual and/or historical) living environments. They are (or until recently were) strongly dependent on the exploitation of natural resources for their livelihoods. Through this intimate relationship with, and dependence on, the natural world, people have developed extensive knowledge about natural resources, often built up over generations. Trees are among the most
 10 important life forms to traditional people in terms of usefulness which they owe to their more complex habit and multipurpose nature compared to herbaceous plants: apart from fulfilling human needs for food and medicine they also contribute considerably as sources of (construction) materials and fuel, and providers of a wide array of ecological services (Thomas and Van Damme 2010). The importance of species with a tree habit for traditional societies to a large extent co-varies with the
 15 floristic composition of their living environments. For example, Clement (1999) calculated that 68% of the 138 species under cultivation or management at the time of European arrival in Amazonia, were trees or woody perennials.

Ethnobiological research on people-plant interactions has traditionally focused mainly on the
 20 utilitarian (ethnobotanical) dimension of plant species and far less on ecological aspects. However, studies that were dedicated to investigating traditional ecological knowledge (TEK) of plants more broadly have demonstrated the deep knowledge held by people on aspects including species' habitat preference, phenology, pollination systems, seed dispersers, species associations, intraspecific variation, pests and diseases, environmental services provided, and behavior under
 25 different types of management (e.g. Assogbadjo et al 2008; Hmimsa et al 2012; Parra et al 2012; Fraser et al 2012). Most studies that investigated traditional knowledge about plants at subspecies level have focused on ethnolinguistic and ethnotaxonomic aspects, i.e. the ways traditional societies name and classify plants (Berlin 1992). Ethnotaxonomical classifications often coincide to certain extent with corresponding scientific classifications and can provide a first approximation of existing
 30 intraspecific variation in plants. Over-differentiation, the splitting a scientific species or subtaxa into two or more traditional names is primarily encountered with cultivated varieties for which distinctive scientific names are often lacking, although such cultivated varieties may be genetically different (Hunn and Brown 2011), or not (e.g. Assogbadjo et al 2009).

35 TEK can provide very valuable information to inform scientific research on the ecology, management, intraspecific variation and conservation of tree species, considering that such information is still lacking for many tropical tree species. However, TEK about tree species is being lost more rapidly than the respective scientific knowledge is increasing, calling for the urgent need to strengthen efforts in recording remaining knowledge. Participatory tree breeding and domestication
 40 of tree species is a fairly recent approach, which combines TEK about tree use and management with scientific advances in germplasm collection, selection and propagation, and with market development, with the ultimate goal to improve people's livelihoods (Dawson et al 2013). TEK can also be a rich source of inspiration for designing biological approaches to tackling the environmental

problems of our times such as the development of renewable energies and resources (Martin et al. 2010) or ecological restoration (Douterlungne and Thomas 2013).

Tree and landscape management

Traditional societies are typically positioned at the interface between the natural and cultural world.

- 5 They live in close proximity to natural vegetation from which they extract livelihood goods, and at the same time engage in different types of plant management. Plant management in traditional societies covers a continuum, ranging from gathering and protecting plants in wild populations, over deliberately tolerating plants in man-made habitats (also defined as disturbance habitats), to cultivating domesticates as well as non-domesticates. Of all anthropogenic habitats, particularly
- 10 home gardens are the laboratories where people have experimented with plant genetic resources; they contain a combination of strictly wild plants, camp followers (weeds; which can be trees e.g. in Amazonia – Balée 1994), spared and tolerated plants, and cultivated and domesticated plants. Through on-going processes of experimentation and innovation, wild plants with desirable traits are gradually brought into the cultural sphere (from dump heap, through cultivation to domestication)
- 15 (Bennett 1992; Miller and Nair 2006; Clement 1999; Thomas and Van Damme 2010).

Useful wild tree species available in natural vegetation may also enter the cultural sphere when spared during clearing land for human use, as this increases contact intensity between people and such remaining trees in or along the margins of crop fields. Intensity of contact, salience, accessibility

- 20 and availability of plant species are often correlated with their perceived usefulness by people (Adu-Tutu et al. 1979; Byg et al. 2006; Thomas 2009; Thomas et al. 2009a,b; Turner 1988). Tree management is not limited to humanized landscapes such as home gardens and swiddens, but occurs also in natural vegetation where certain species, may receive protection, e.g. by removing competing plants or pests to enhance the target plants' chances of survival. More significant,
- 25 however, is landscape domestication, a process of landscape manipulation which was initiated by early humans all around the world (Chase 1989; Clement 1999; Anderson 2005; Young 2009; Aumeeruddy-Thomas et al 2012; Sheil et al 2012) and reaching its extremes in the highly artificial land use forms of modern society. The impacts of long-standing landscape domestication in forest environments is, for example, evidenced by enrichment in useful species (Wiersum 1997; Shepard
- 30 and Ramirez 2011), anthropogenic forests (Balée 1989), or anthrosols such as black earth soils containing charcoal and cultural waste from prehistoric burning and settlement and carrying distinctive vegetation as a consequence of high nutrient content. In Amazonia, black earth soils are generally associated with forests that are enriched with useful species such as Brazil nut trees (Clement and Junqueira, 2010; Junqueira et al 2011). Long-standing human management often
- 35 leaves a mark in the geographical distribution of the genetic diversity of trees (Vendramin et al 2008; Shepard and Ramirez 2011; Thomas et al 2012). Archaeological knowledge about historical tree use and management can provide powerful entry points to understand contemporary patterns in inter and intra-specific diversity patterns of trees (e.g. Chepstow-Lusty and Jonsson 2000; Goldstein et al 2012).

Risk management

Throughout human history, traditional societies have been positioned in the firing line of environmental and climate change. They are aware of the need to monitor environmental change,

often through the use of indicator plants such as *Barbaceniopsis boliviensis*, a Bolivian Andes plant whose leaves are said to turn yellow as an early warning sign (Thomas 2009). To cope with the adverse impacts of environmental fluctuations traditional societies have developed a plethora of risk management strategies. Most of the strategies are designed to make opportunistic use of space,

- 5 natural resources, social relations, and time. People tend to invest in a diverse portfolio of options because this lowers vulnerability and increases resilience and stability in the availability and supply of livelihood goods and services (Frison et al 2011). A popular strategy is to maximise the accessibility to, and use of different ecological units in the landscape in which people grow a variety of different plants and/or extract floral and faunal elements upon which they can draw for their
- 10 livelihoods (Berkes and Folke, 1994; Ladio and Lozada, 2004; Thomas et al 2009a). This explains why indigenous groups are drawn to environments with high ecological variation, such as ecological edges (Turner et al 2003). Biological and ecological diversification strategies imply the need for diversified knowledge systems, not only about the ecological conditions of different environments, but also about a high number of biotic elements, their useful traits and management. Risk strategies
- 15 based on optimal use of natural resources spread risk in terms of space and resources; crop failure in one ecological zone may be offset by more stable harvest in another and reduced availability of one biotic resource may be compensated by use of (a variety of) alternative resources.

Biological and ecological diversification strategies of traditional societies are often complemented by equally important social risk management strategies (van Oudenoven et al 2011). Smith et al

- 20 (2012) recently suggested that resilience is a dynamic social process determined, in part, by the ability of communities to act collectively and solve common problems. Systems that spread risk and innovation in social space, either consciously or unconsciously, simultaneously stimulate further diversification of available resources and provide individual elements of the network to fall back on
- 25 others in case of unforeseen events (e.g. misharvest). A good example which illustrates this is that in rural communities it is very common to see high variation in germplasm grown in home gardens; different tree species, varieties or genotypes generally occur at low densities and frequencies and what is being cultivated is often very different between gardens, even in the same village (Padoch and De Jong 1991; Ban and Coomes 2004; Perrault-Archambault and Coomes 2008; Jarvis et al 2008;
- 30 Thomas and Van Damme 2010; Wezel and Ohl 2005; Hmimsa et al 2012). A similar pattern is observed at larger scales (across the homegardens of different villages), as well as in predominantly agricultural landscapes (Dawson et al 2013). For example, Van den Eynden (2004) reported that about 60% of all edible plants were only used or known in one of the 42 villages of Southern Ecuador that she investigated. Guarino and Hoogendoijk (2004) postulated that because populations of many,
- 35 if not most, species in homegardens are often very small they are much more prone to genetic drift and rapid genetic divergence or differentiation. This, in combination with the fact that experimentation and selection for particular traits of plant species is often highly dependent on the interests of individual garden owners, may explain the high intraspecific variability found in home gardens. Regardless of the biological and human factors responsible for the high variability of
- 40 germplasm found in different gardens, it is clear that social bonds, relations and interactions are crucial for allowing individual elements operating in the social space uniting different home gardens or villages to benefit from the diversification strengths of the overall system. For example it has been suggested that the number of plant species or varieties found in gardens is positively related to their owners' opportunities for exchange of germplasm, through social and kin networks (Ban and
- 45 Coomes 2004; Perrault-Archambault and Coomes 2008).

The success of some ancient societies can at least partly be related to the same structural components as described above. For example, the rise of the pre-Columbian Inca society has been related to the structural components of *verticality*, *specialization* and *reciprocity*. Verticality is an underlying principle of traditional Andean social, political and economic organization (Murra, 1975).

- 5 It implies that Andeans specialize in extracting and producing resources from multiple ecological zones along steep mountainsides and exchange their complementary resources for those produced by people inhabiting other ecological zones (social reciprocity) (Murra, 1975). These conditions are at the base of Andean people's extraordinary knowledge and use of micro-environments, an incredible range of technological and agricultural innovations, and formalized systems of reciprocity
- 10 (e.g. exchange of labour or agricultural produce) (Alberti and Mayer, 1974).

From the above it is clear that traditional societies are in many cases the creators and keepers of an often remarkably diverse and untapped repository of tree germplasm in varying stages of domestication that is spread out over ecological and social space. There is a large latent potential for modern society to take advantage of traditional diversification strategies for addressing human

- 15 development needs, not only in terms of tangible resources, but also in terms of social and ecological management and organization.

To end this section, it seems important to note that traditional knowledge is not evenly distributed across or within indigenous and local communities, but is known to vary with ethnicity, age, sex, social status, as well as numerous other possible factors (e.g. Thomas 2012), and even within

- 20 knowledge states it is typical to find a high degree of idiosyncrasy, at least in certain plant use categories such as traditional medicine. The primordial knowledge holders about certain tree species also depends on where they grow in the landscape; for example, homen gardens are generally the domain of women, whereas men are often more knowledgeable about trees in the forest. All this of course has important implications when planning research with traditional knowledge holders: it is
- 25 not only important to identify and work with people that hold most knowledge on a topic of interest, but also to include as many people as possible in order to be able to access the full spectrum of often complementary bits of knowledge. Another important aspect of traditional knowledge is that it is highly dynamic and adaptive, depending on the context of use. Indeed practical knowledge is kept alive, at least in part, through its actual use. If the particular use of plants is no longer required,
- 30 accompanying knowledge is likely to disappear eventually. Basically, this is what happens through modernization of traditional lifestyles: people tend to substitute traditional knowledge and plant use with modern knowledge and/or practices. Hence, unless efforts are made to deliberately retain knowledge of plant uses that are no longer applied by or relevant for a society, whether through written or oral transmission it will be lost and/or replaced by "new" knowledge at one time or
- 35 another.

Classical tree improvement

Methods from crop and livestock breeding have been adapted to accommodate the peculiarities of forest trees. Unlike agronomic crops and livestock, most forest tree species have not been domesticated, so the starting point for tree breeders is typically very different from that of crop or

- 40 animal breeders. Tree breeders usually begin working with wild populations instead of varieties or breeds. This means that the type of knowledge, both required and generated through associated

research, differs significantly from that required for agricultural species. Forest trees have other characteristics that are unique, relative to crop and livestock breeding; for example they are long-lived and reproductive maturity typically occurs only after 10 – 40 years of age. As well, most tree species have a highly outcrossing mating system and low tolerance for inbreeding.

- 5 In classical tree breeding, desired phenotypes are selected in the wild and propagules are collected; typically seed for progeny testing and scions or cuttings for establishment of seed orchards. Quantitative genetic data is generated from phenotypic measurements taken under uniform environmental conditions in a series of progeny or clonal trials. Statistical analyses are employed to separate genetic from environmental sources of variation in measured traits. The ratio of genetic to
 10 phenotypic (including environmental) variation provides a measure of the heritability of the trait, and thus the potential for improvement.

Types of knowledge that may be obtained from field trials associated with tree improvement include:

- 15 • Genetic variability and heritability of traits related to growth, product quality and quantity, and resistance or tolerance to insect pests, diseases and adverse environmental conditions
 • Genetic correlations between traits
 • Epigenetic effects
 • Genotype by environment interactions
 • Trait-specific juvenile-mature correlations
 20 • Gene action (additive, dominance) and in some cases, estimates of numbers of genes involved in traits of interest

Tree improvement using genetic theory has been underway for only a little more than a century, and intensive selection and breeding for wood products, only since the 1930s beginning in Sweden, Denmark, the Netherlands, Italy, Germany and Austria (Hitt 1952). Methods for provenance trials to
 25 identify the best seed sources and progeny trials to estimate additive genetic variation and heritability of valuable traits were adopted by mid-century in Europe, North America (McKeand *et al.* 2008), Australia and New Zealand (Burdon, *et al.* 2008). Thousands of trials were established, leading to rapid improvement of growth rate and stem form, and associated broad-scale planting of commercial tree species such as *Pinus radiata*, *Pinus taeda*, *Pinus elliottii*, *Pseudotsuga menziesii*,
 30 *Pinus contorta*, *Picea sitchensis*, and *Picea abies*. Over the course of selection, testing, and breeding for improved traits, a body of quantitative genetic knowledge has been accumulated for the major plantation species.

Most European countries have tree improvement programmes. Northern European countries have the most advanced breeding programmes with a concentration on *Pinus sylvestris* and *Picea abies*,
 35 both of which are important for timber and pulp. According to country reports, at least 25 other native conifer and broadleaf species are subject to improvement, but some of the species are in the beginning stages of selection and breeding. Most of the efforts are on improving productivity, wood quality and environmental adaptation, particularly considering climate change. Breeding for pest resistance was mentioned in by only a few of the countries, such as the Netherlands, which noted
 40 that in addition to selection for adaptation to site conditions, considering survival and bud burst,

trees are selected for resistance to pests and diseases. Genotypes of several species are also being tested there for non-forestry purposes such as their performance as roadside trees. Several European countries have breeding programmes for Christmas trees and other non-wood forest products. In addition to improving trees for timber and pulp, almost half of the countries reported 5 improvement programmes for tree species used for energy. The existence of such breeding programmes focusing on a range of species and traits implies a substantial body of knowledge about genetic variation and heritability.

Most of the tree species included in breeding programmes in Europe are native to Europe and to the 10 countries where breeding efforts are underway; however, about one-third of the tree species under improvement are exotic in the reporting countries. In some cases these species originated from North America or other regions in Europe. Knowledge gained about performance of these species growing outside of their native range may be useful within their native range, particularly when matching seed sources to novel environmental conditions.

In North America, tree breeding programmes began generating knowledge about a range of species 15 having timber value in the mid 1900s. Exploration, testing and breeding of *Pseudotsuga menziesii* began in the 1950s in British Columbia, Canada (Orr-Ewing 1962). Beginning in 1958, an arboretum was established with more than 200 *Pseudotsuga menziesii* sources from throughout its range in Canada, the US and Mexico. The objective was to provide as wide a gene pool as possible for a breeding program (Orr-Ewing 1973). Samples of the other five *Pseudotsuga* species were established 20 in the arboretum as well. Subsequent testing and tree improvement projects in British Columbia included, *Tsuga heterophylla*, *Thuja plicata*, *Larix occidentalis*, *Pinus contorta*, and *P. ponderosa*.

As in Canada, early efforts in the northwestern states of the USA focused on *Pseudotsuga menziesii*, 25 with the earliest work initiated by Hoffman in 1912 in Wind River Experimental Forest in southern Oregon. Duffield (1959) set up the first seed source studies, but tree improvement programmes began in the 1960s. Because of the topography and associated high environmental variation over short geographic distances, many small breeding programmes were initiated. By the 1980s there were 125 separate breeding programmes for *Pseudotsuga menziesii* in Oregon, Washington and British Columbia (Johnson 2000). Other species have received much attention in the Pacific Northwest of both countries but exploration, assessment and improvement programmes have been 30 undertaken to varying degrees for *Picea* spp, *Tsuga heterophylla*, *Pinus contorta*, *P. monticola*, *P. lambertiana*, *Thuja plicata*, *Larix occidentalis*, and *Chamaecyparis lawsoniana*.

Elsewhere in the US, the foundation for tree improvement was laid in the 1920s and 30s. The Placerville Institute of Forest Genetics in California was initiated in 1925 by James Eddy, as a private research centre dedicated to improving forest growth through breeding. The Institute was first 35 focused on pines and 49 *Pinus* species from many countries were planted in 1926. The most complete pine arboretum in the world was established there in 1931. In 1935, the Institute was donated to the US Forest Service and it has been an important knowledge base for forest genetics research since that time (USDA Forest Service; accessed 03\02\09).

In the early 1950's Bruce Zobel initiated a tree improvement programme in east Texas, focusing on 40 drought resistance and wood properties (Zobel 2005). Tree improvement co-operatives were established in Texas in 1952, Florida in 1954, and North Carolina in 1956 and programmes developed

rapidly during the rest of the century. Before 1960, there was no genetically improved seed available and all seedlings for planting were produced from woods-run seed, with little control over quality (Dorman 1974). By the mid-1970s, much of the seed used for tree planting in the southern USA was from genetically improved seed orchards.

- 5 In North America, in spite of the high number of species (approximately 85) under some form of tree improvement, the most intensive breeding efforts have focused on just a few species, notably *Pinus taeda* and *Psuedotsuga menziesii*. Tree improvement programmes are underway at various stages for many other species; according to the US country report, more than 70 species in the US are undergoing breeding, selection and testing. However, more than 50% of the improved seedlings are
- 10 just two species of pine: *Pinus taeda* and *P. elliottii* (McKeand, et al. 2008). Some of the most advanced tree improvement programmes are in the USA, where according to Neale and Ingvarsson (2008), more than 11.5 million trees have been tested in progeny tests for just four conifer programmes, more than 41,000 parent trees have been evaluated. Two of the programmes are in the third generation of testing and breeding.
- 15 The US has breeding programmes that are intended for conservation for several species, for example *Castanea dentata* and *Ulmus americana*, both of which have been severely depleted by introduced diseases, and the primary breeding objective is to develop disease resistant genotypes. Through breeding for resistance to these diseases and others, valuable knowledge has been gained about behaviour of introduced diseases and insect pests, and genetic options for control (Schlarbaum
- 20 1998; Loo 2009).

- Mexican forests encompass very high levels of biodiversity with high numbers of species and high intraspecific variability. Tree improvement programmes are underway for nine native species: *Pinus patula*, *P. Douglasiana*, *P. leiophylla*, *P. greggii*, *P. oocarpa*, *P. pseudostrobus*, *Taxus globosa*, *Cedrela odorata*, and *Swetenia macrophylla* (CONAFOR 2012). In addition, improvement programmes are
- 25 underway for several non-indigenous species, including *Eucalyptus urophylla*, *Gmelina arborea* and *Tectona grandis*.

- By the latter decades of the 20th century CAMCORE was a leader in establishing trials and generating data on conifers from Central America, with an initial focus on pine species which were of interest for planting in other continents. Examples include *Pinus tecunumanii*, *P. patula* and *P. caribea*
- 30 (Dvorak, et al. 1996). Many of the trials, which also serve an *ex situ* conservation purpose are located in other tropical or subtropical countries. The knowledge gained from these testing efforts is now also useful in selecting provenances or families for planting in the species' natural range.

- At least 25 North American tree species are used for forestry purposes and have been extensively tested in other countries (Rogers and Ledig 1996). An example of one such species is *Pinus radiata*
- 35 which occurs only as five small populations in its natural range and has not been used for plantation forestry in North America, however it is a very important timber species in other countries (Cope 1993). In its native range, it is used for ornamental purposes, erosion control and fuelwood, but is not considered to have commercial value for timber because of its typical poor crown form. It was first introduced into Australia for ornamental plantings around 1857 (Wu, et al 2007) and its rapid growth led to its use in plantation forestry beginning in the 1920s. Through selection and breeding
- 40

programmes in countries where it has been introduced, the crown form has been significantly improved and it has become one of the most widely planted pine species for timber.

Knowledge gained from early tree improvement trials led to valuable insights about patterns and extent of genetic variation in forest trees. Early studies in New Zealand and Australia on *Pinus*

- 5 *radiata*, for example, demonstrated that although the species' native range was small (Rogers 2002) and the species did not show much variation or promising traits in the wild, there was sufficient heritable variation among the planted trees to make strong and rapid improvement in traits of commercial importance (Burdon, et al. 2008). Wu, et al. 2007 noted that realized genetic gains of as much as 33% were reported for volume at age 15 years from the first generation of selection of
- 10 *Pinus radiata* in New Zealand. They surveyed the literature and reported mean heritabilities from dozens of articles for morphological traits that are important in wood quality such as stem straightness and branch angle, both with an average heritability of about 0.25 and branch size just over 0.50; all of which indicate potential for rapid improvement. Although populations in North America are small, they are genetically isolated from each other, which results in higher variation
- 15 than would likely be the case had the populations been contiguous.

Outside of temperate and boreal forest regions, *Eucalyptus* spp, Teak (*Tectona grandis*), and several other species have been subjected to intensive selection and breeding and are now primarily clonally propagated. *Eucalyptus* breeding programmes often focus on producing high value hybrid clones either between species or between highly selected lines. Most teak field trials are clonal, because of

- 20 the low seed production and difficulty in conducting controlled crosses. Almost 10 years ago a FAO publication stated, "The scale of forest genetics and tree improvement in the tropics is entirely inadequate, both in geographical distribution and species coverage, and bears no relation to its potential value and importance" (FAO 2005). This situation has not substantially changed although native species are being gradually brought into improvement programmes in all regions.

- 25 More than 100 native tree species are listed in the India Country Report as being included in tree improvement programmes. However, most plantations are established with *Eucalyptus*, *Tectona grandis*, *Acacia* spp, *Casuarina*, *Shorea robusta*, *Cedrus deodara*, *Pinus roxburghii*, *Pinus wallichiana*, *Gmelina arborea* and *Grevillea robusta*. Country Reports describe a variety of purposes for tree improvement. In India for example, although the focus for the majority of tree species in
- 30 improvement programmes is wood products, a substantial number of species are bred for fuel production, multi-purpose and non-timber forest products. In Mexico, uses of tree species that are undergoing tree improvement include food, essential oils, forage, gums and resins, Christmas trees, medicines, conservation and restoration.

- 35 In South America genetic improvement of two *Prosopis* species produces seedlings suitable for restoration of degraded land. Two *Nothofagus* species and at least one *Cedrela* species are also subject to selection, breeding and field testing. In addition to these native species, several exotic species from the *Pinus* and *Eucalyptus* genera are undergoing breeding and selection to improve the quantity and quality of their wood. *Salix* and *Populus* species are tested for pulp and paper. Other Latin American countries, such as Chile, do not plant any native tree species for commercial
- 40 production, and all production plantation forestry employs exotic species. In such cases, breeding

programmes increase the adaptedness of selected genotypes of exotic species to local environmental conditions and improve their productivity.

In spite of the great number of tree species (80,000 to 100,000) and numerous large international efforts to generate genetic data, only a tiny fraction of available tree species have been thoroughly

5 studied; mainly temperate conifers and some Eucalyptus species. The efforts have also been limited in the traits studied; mainly growth-related traits, focused on increasing production of wood. Considering what has been learned about trait variation in many tree species, it is clear that a huge untapped potential exists for improving product quantity and quality as well as improving adaptive traits of many tree species.

10 ***Provenance trials***

Provenance trials were originally conceived to identify species-specific sources of planting material that are suitable for different locations (Matyas 1994); the main objective was to evaluate which sources had best performance, usually measured as survival and growth under particular growing conditions. Testing provenances involves collecting seed (or other propagules) from populations of

15 trees covering a range of environments and growing them together in experimental field trials (common garden tests); ideally, a series of trials is established to cover the range of environments where the species occurs or where the species may be planted. Although many of the existing provenance tests were not intended to characterise adaptive traits, survival and growth are basic measures of adaptation of a provenance to the sites where it is planted (Mátyás 1994). Ying and
20 Yanchuk (2006) argued that height growth is generally a valid surrogate for fitness, noting that the trait responds quickly to changing environmental conditions.

An important use of provenance trial results is defining seed zones (or zones of provenance) (Ying and Yanchuk 2006). Seed zones are geographic areas within which seedlings of the tested species, sourced anywhere within the zone, can be planted without loss of local adaptation. For a given

25 species, a provenance trial usually includes trees grown from seed collections from 10 or more trees at each of several, to many, geographic locations; however the planting material may vary from bulk collections from many trees per population, to individual tree collections, which would allow combined progeny/provenance testing. Planting sites may be within or outside of the species' range. The field trials are usually established following a randomised block design at several
30 locations to test performance under different environmental conditions and to allow assessments of genotype x environment interactions (Sáenz-Romero, et al. 2011). Provenance trials provide population level information intended to identify the sensitivity of seed sources to varying environmental conditions.

The types of knowledge obtained from provenance trials include:

- 35
- intraspecific variation in survival and growth across variable environmental conditions
 - the degree to which adaptation tracks environmental gradients
 - existence and type of genotype x environment interactions
 - probable responses of different provenances to climate change

Provenance testing in temperate climates began more than 250 years ago (Langlet 1970), when plantations were established to compare *Pinus sylvestris* seed sources in 1745-55 by Duhamel, the Inspector General of the French navy, using sources from the Baltic region, Russia, Central Europe and Scotland, in the quest for suitable mast material for naval vessels. It was already known that planting material from different sources performed differently at a given planting site.

Unfortunately, the results of this pioneering experiment did not provide lasting knowledge because they were not written up and published. Since then, hundreds of thousands of trees have been planted in provenance trials, starting in Europe then North America and gradually in other regions. Many provenance trials could yield much more information than is often measured or published; nevertheless, the scientific literature is rich with published results from such trials. Examples of very large provenance testing efforts, the results of which have yielded many scientific articles, include the *Picea abies* IUFRO provenance trial established in 1968 which comprised 1100 provenances collected from throughout the range of the species and planted at 20 locations in 13 countries (Krutzsch 1974). *Pinus contorta* provenance trials established by Illingworth in 1974 in the Canadian province of British Columbia and Yukon Territory, included 140 provenances from throughout the species' range in western North America, tested at 62 locations (Wang, et al. 2006 and Wang, et al. 2010).

Phil Wakeley established a test in the southern USA in 1926 that included four provenances of *Pinus taeda* (Rogers and Ledig 1996). He also initiated a large study of geographic variation of four southern pine species (*Pinus taeda, elliottii, palustris, echinata*), which were planted in 66 test plantations across the southeastern USA in 1951 (Wakeley 1954). This set the stage for most of the later genetics work with these species (Zobel 2005).

In Canada, extensive provenance testing has been carried out for 35 native species and some exotics; mostly conifers. In the 1950s, Mark Holst initiated an ambitious program with *Pinus resinosa*, sending seed to other parts of North America and Europe for testing, in addition to establishing many experiments in Ontario. The program was abandoned when it became apparent that genetic variation was lower than for most other conifers (Steill 1994). In Eastern Canada the primary focus for early exploration and testing was *Picea* species with range wide provenance trials established for *P. mariana, rubens* and *glauca*. Provenance trials including native and exotic *Larix* species were also established in eastern Canada.

In Germany, provenance testing is underway for 33 tree species including several North American species.

Table ??: An example of thorough provenance testing: provenance tests established by the Länder or BLAG-FGR institutions (2010) in Germany
BLAG-FGR 2011 [National Report Germany]

Scientific Name	Common Name	Provenance tests carried out by
<i>Abies alba</i>	European Silver Fir	BY, BW, SN, RP, NW-FVA
<i>Abies grandis</i>	Giant Fir, Grand Fir	BB, BY, NW-FVA, vTI
<i>Abies nordmanniana</i>	Caucasian Fir	RP
<i>Abies procera</i>	Noble Fir	BY, NW-FVA
<i>Acer pseudoplatanus</i>	Sycamore	BB, BY, SN
<i>Alnus glutinosa</i>	Common Alder, European Alder	vTI
<i>Betula maximowicziana</i>	Monarch Birch	vTI

<i>Betula pendula</i>	European Birch, Silver Birch	BY, vTI
<i>Betula pubescens</i>	Downy Birch	BY
<i>Castanea sativa</i>	Spanish Chestnut, Sweet Chestnut	RP
<i>Fagus sylvatica</i>	Common Beech, European Beech	BB, BW, BY, SN, RP, MV, NW-FVA, NW, vTI
<i>Fraxinus excelsior</i>	Common Ash, European Ash	BB, BW, BY, NW-FVA
<i>Larix decidua</i>	European Larch	BB, BY, MV, SN, vTI
<i>Larix kaempferi</i>	Japanese Larch	vTI
<i>Malus sylvestris</i>	Apple, Wild Crab	RP
<i>Picea abies</i>	Common Spruce, Norway Spruce	BW, BY, SN, MV, NW-FVA, TH, vTI
<i>Picea sitchensis</i>	Sitka Spruce	NW-FVA, vTI
<i>Pinus contorta</i>	Lodgepole Pine, Shore Pine	BY, vTI
<i>Pinus nigra</i>	Austrian Pine, Black Pine	BB, BY, vTI
<i>Pinus ponderosa</i>	Ponderosa Pine	BY
<i>Pinus strobus</i>	Eastern White Pine, Weymouth Pine	BB
<i>Pinus sylvestris</i>	Scots Pine	BB, BY, RP, MV, NW-FVA, vTI
<i>Populus tremula</i>	Aspen	RP
<i>Prunus avium</i>	Gean, Mazzard, Wild Cherry	BW, BY, SN, RP
<i>Pseudotsuga menziesii</i>	Douglas Fir	BB, BW, BY, SN, RP, MV, NW-FVA, NW, TH, vTI
<i>Pyrus pyraster</i>	Wild Pear	RP
<i>Quercus petraea</i>	Sessile Oak	BB, BY, NW-FVA, RP, vTI
<i>Quercus robur</i>	English Oak, Oak, Pedunculate Oak	BW, NW-FVA, RP, NW
<i>Quercus rubra</i>	American Red Oak	vTI
<i>Robinia pseudoacacia</i>	Acacia, Black Locust, Robinia	NW-FVA, vTI
<i>Sequoiadendron giganteum</i>	Giant Sequoia, Wellingtonia	RP, vTI
<i>Sorbus domestica</i>	Service Tree	BY, RP, NW-FVA
<i>Taxus baccata</i>	Common Yew, English Yew	BW, RP
<i>Tilia cordata</i>	Little Leaf Linden	BY, SN, RP, NW-FVA

Provenances of many tree species that are planted for wood production in Europe, North America, Australia and some countries in other regions have been well studied, in many cases both within and outside of their natural ranges. Although fewer species native to the tropics have been studied, and it is often not easy to find information gained from existing trials, there are exceptions. In West

- 5 Africa for example, 25 provenances from throughout the range of the multi-purpose *Parkia biglobosa* were established in 1995 at two test locations in Burkina Faso that receive contrasting mean rainfall amounts (Ouedraogo, et al. 2012). Results are now available to guide selection of planting material for Burkina Faso locations. According to an overview based on country reports, at least eight other West African species (*Acacia senegalensis*, *Adansonia digitata*, *Allanblackia parviflora*, *Tamarinda indica*, *Vitellaria paradoxa*, *Khaya senegalensis*, *Terminalia ivorensis*, *Triplochiton* spp.) have been included in provenance trials in the region.
- 10

Other regions of Africa report lower numbers of tested native species; Southern Africa has expended substantial effort on testing provenances and developing tree improvement programmes, but most of the plantation species are exotics.

- 15 Country reports indicate that about 12 native species have been evaluated at the provenance and or progeny level in SE Asia, and 17 in South Asia; 12 native species have been evaluated in China, Japan

and Korea. In Central Asia there is little information available about provenances but *Haloxylon aphyllum* sources have been tested and drought and pest resistant variants have been identified. *Abies sibirica* has been tested in Kazakhstan.

Globally provenance testing is often done outside of species' native ranges to identify sources of

- 5 planting material for plantation forestry. Understanding growth parameters of the exotic provenances performing under particular environmental conditions can be useful as well, in matching provenances of those species to novel conditions resulting from climate change, within the natural range of the species. Thus applying the information from provenance trials, where a species is exotic, to restoration of harsh sites within the species' natural range is an unplanned potential
- 10 value of the trials.

During the past two decades, the establishment of new provenance tests has declined because large field trials were generally considered to be too expensive, slow to produce results and difficult to maintain. The days of collecting large quantities from seed from scores or hundreds of provenances and planting them in dozens of long-term field trials are likely finished but new approaches have

- 15 emerged that provide useful information without such high cost. In the Northwest of the USA, for example, most provenance testing is done using short- term genecology studies to map genetic variation across the landscape. These common-garden experiments are carried out in nursery environments where site conditions are uniform so the observed variation of adaptive traits such as growth rate, phenology, form, cold and drought tolerance, across the landscape, is due to their
- 20 genetic composition or epigenetic effects. If the pattern of genetic variation tracks physiographic or climatic characteristics of the seed-source locations, it provides evidence for selection and may be important for adaptation. (from US country report).

Genetic field trials have given way to the sometimes unfulfilled promise of lab-based molecular analyses. In recent years it has been increasingly recognised that the knowledge gleaned from

- 25 provenance trials cannot be substituted by molecular data, and the recognition of a gap between molecular and quantitative knowledge, especially important in the understanding adaptive traits. The resurgence of interest in field trials, in large part because of the recognition of their value for predicting responses and designing strategies to counter impacts of climate change, has led to several attempts to capture data before it is lost as scientists retire. The Centre for Forest

- 30 Provenance Data in Oregon, USA, led by Brad St Clair at the US Forest Service and Oregon State University, is one such effort (<http://cenforgen.forestry.oregonstate.edu/index.php>). Researchers are invited to submit and use provenance trial data. Although it is intended to have global reach, only western North America is taking advantage of the opportunity to house data there, to date. Data has been uploaded from 25 studies on 8 North American tree species.

- 35 As noted above, multi-location provenance trials that have been established in the past to determine appropriate seed sources for production in specific sites can provide information that is highly valuable for predicting responses to climate change. Results of these experiments have been reported extensively (e.g. <http://curis.ku.dk/portal-life/en/publications/search.html>). These results can be coupled to precipitation and temperature data and time series to observe adaptive genetic
- 40 variation across provenances under specific climate conditions (Sáenz-Romero et al. 2006; Schueler et al. 2012). Such analyses are useful for assessment of climate change impact of tree species

distributions and productivity in planted forests and agroforestry systems under future climate projections according to Global Climate Models (GCMs) (Sáenz-Romero et al. 2006). Collection of existing provenance trial data of tree species will help to carry out systematic analyses of climate change vulnerability of the selected species in specific geographic areas.

- 5 Provenance trial survival and height growth data of subtropical pine species native to Mexico and Central America, have been used to validate climate change impact predictions for 2050 of natural species distributions with environmental envelop modelling (EEM) (van Zonneveld et al. 2009). Survival data have been used to calibrate EEM to make more realistic predictions (Benito Garzón et al. 2011). EEM can over-predict climate change impact on species' distributions because species can
- 10 also survive and perfom well outside their existing climate niche (van Zonneveld et al. 2009). The estimations of EEM of tree species improve considerably when provenance performance data are included in the analyses (Benito Grazón et al. 2011). Trait data can be coupled with climate data to develop empirical productivity models to identify the seed sources that perform well for desired traits under expected climate change (Leibing et al. 2013).
- 15 Provenance trial results have value beyond production forestry; they can be useful in restoration of degraded sites because often the conditions in an area to be restored are substantially different from those of surrounding forest; including more prone to drought, depleted soil, and absence of other species that would normally be part of a functioning forest ecosystem. Often early successional or pioneer species are needed as a first stage to reclaim a forest site. Provenance trials
- 20 have been conducted mainly for early to mid-successional species, which tend to be the fastest growing and the most easily cultivated, hence these results are useful if data have been generated on the response of different provenances to diverse environmental conditions. Species with well-defined spatial patterns for which adaptive traits track environmental conditions closely, will have different results in a provenance trial from species that show little variation across large distances.
- 25 In the first case, applying the results is a simple matter of matching planting sites to trial sites and choosing the provenances having best performance. In the second case, planting material can be chosen from a wider amplitude of sources. Mátyás (1996) noted that although seldom recognized as such, provenance research may be among the most important contributions of forestry to biological sciences. Of course, this is only true in regions where there have been serious efforts to test
- 30 provenances.

Changing climate in combination with other causes of site degradation will likely render much of the area that could benefit from forest ecosystem restoration substantially different from the environment in which local remnant trees became established. Several approaches have been recommended, tested and are now being employed, in some cases, to plan planting projects that

- 35 may involve rescue of rare and threatened species (Ledig et al. 2010), or that simply aim to increase the likelihood that planted material will be adapted to future climatic conditions (Wang, et al. 2010; Hamann, et al. 2011; Rehfelt, et al. 1999; Beaulieu, et al. 2004; Saenz-Romero, et al. 2006). Using an approach called "universal response function" which integrates environmental and genetic variables, described by Wang, et al. (2010) is claimed to increase precision compared to the other approaches,
- 40 in situations in which number of provenances or test sites is less than desirable. Each of the methods described has potential value in the planning phases of restoration projects, for identifying suitable sources of planting material, but only in cases where provenance testing has been carried out.

Perhaps the most valuable knowledge gained from provenance testing is that every species is different in their responses to environmental variation. The northwestern US represents one of the areas with most intense provenance testing over the past half century and more. The US country report notes that all conifers in the region exhibit clinal variation in all or part of their ranges, but the amount and patterns of variation differs for each species. The geographic distance on an elevational gradient as well as climatic distance (number of frost-free days) needed to detect seed source differences also differs among conifer species sampled in the same region. For example, Rehfeldt (1994) described Douglas-fir (*Pseudotsuga menziesii*) and lodgepole pine (*Pinus contorta*) as specialists because their populations appear to be adapted to relatively narrow niches. The opposite is true for two generalist species, western redcedar (*Thuja plicata*) and western white pine (*Pinus monticola*). Similarly, in the Southeast, geographic variation is more complex for loblolly pine (*Pinus taeda*) than for the other southern pines (Schmidling 2001).

Table xx. From US country report: *Species differences in amount of environmental difference needed to show a genetic difference (from Rehfeldt., 1994)*.

Species	Elev. difference to find genetic difference	Frost-free days to find genetic difference	Evolutionary mode
Douglas-fir	200 m	18	Specialist
Lodgepole pine (<i>Pinus contorta</i> Dougl. ex Loud. (Pinaceae))	220 m	20	Specialist
Engelmann spruce (<i>Picea engelmannii</i> Parry ex Engelm. (Pinaceae))	370 m	33	Intermediate
Ponderosa pine (<i>Pinus ponderosa</i> P.& C. Lawson (Pinaceae))	420 m	38	Intermediate
Western larch (<i>Larix occidentalis</i> Nutt. (Pinaceae))	450 m	40	Intermediate
Western redcedar (<i>Thuja plicata</i> Donn ex D. Don (Pinaceae))	600 m	54	Generalist
Western white pine (<i>Pinus monticola</i> Dougl. ex D. Don (Pinaceae))	none	90	Generalist

15

The fact that evidence has been documented for clinal and/or ecotypic variation in all forest tree species examined indicates the importance of matching seed sources to environmental conditions. The evidence that patterns and amounts of adaptive variation also differ among all species evaluated implies that there are no model species and no shortcuts. Some form of provenance or genecological testing is needed for all planted tree species.

20

Table xx. From US country report: *Evidence from reciprocal transplant studies showing local sources as optimal or near-optimal (from Johnson et al. 2010). Additional studies are summarized in Hereford (2009).*

FAMILY	GENUS (common name)	SPECIES (reference)
Betulaceae	<i>Alnus</i> (alder)	<i>A. rubra</i> (Hamann and others, 2000)
Cupressaceae	<i>Chamaecyparis</i> (false-cedar)	<i>C. thyoides</i> (Mylecraine and others, 2005)
Fagaceae	<i>Quercus</i> (oak)	<i>Q. rubra</i> (Sork and others 1993)
Pinaceae	<i>Abies</i> (fir)	<i>A. grandis</i> (Xie and Ying 1993);
Pinaceae	<i>Pinus</i> (pine)	<i>P. contorta</i> (Ying & Hunt 1987; Ying & Liang 1994; X & Ying 1995; Wu & Ying 2004); <i>P. lambertiana</i> (Harr and others, 1983); <i>P. ponderosa</i> (Squillace & Silen 19 Wright, 2007); <i>P. taeda</i> (Frank 1951; Wakely 1944)

- 5 Globally, more than 500 tree species are subject to tree improvement, most of which include provenance and/or progeny testing (from country reports). The results of such tests provide valuable information to determine sources of planting material that are adapted for a particular site and the range within which reproductive material of these species can be moved without loss of adaptation. In many cases the full potential of this knowledge is not realised because:
- 10 • provenance trials have not been maintained and measurements have stopped after an initial assessment
- measurement data that have been collected have not been analysed to their full potential
- results are not readily available, often not entered in an computerised database and not published
- 15 • data are lost as scientists retire.

Results of many provenance trials have not been published, nor do they exist in electronic form, in many cases. However a concerted effort must be made to locate and use existing information about the 500 or more species that have been tested.

Participatory tree domestication

- 20 People began selecting and planting trees for their own purposes thousands of years ago, similarly to other useful species, but impeded by long generation times and the highly out-crossing mating system of many tree species, little progress was made toward domestication. More recently scientists began working with local people in what is known as participatory domestication. This collaborative approach was initially developed in Central Africa focusing on domesticating fruit and
- 25 nut tree species valuable to local people (Leakey, et al. 2005). The objective is to bring together local knowledge and objectives with scientific knowledge and theory to speed the process of improvement of specific traits for particular uses (Tchoundjeu, et al. 2012). Principles and methods for agroforestry domestication, much of which involves local participation, have been compiled by the World Agroforestry Centre (ICRAF) (Dawson et al 2012).
- 30 The central Africa participatory domestication approach differs from the tree improvement methods described above in that multiple species are typically targeted for improvement, at the same time.

The multispecies approach is less risky for the small producer participants than banking on a single species (Tchoundjeu, et al. 2012) Progress for a given species is likely to be slower than for the commercial plantation species because of less concentrated effort and in some cases, a greater range of traits of interest. The typical production method for improved stock is by cloning, either 5 grafting or rooting cuttings, which allows multiple traits to be selected at the same time (Leakey 2004).

Most examples of participatory domestication are in the tropics. In the past two decades the number of tree species that are discussed in the literature on agroforestry domestication has increased from about 10 to 50 (Leakey, et al, 2012). Much of the recent progress in domestication 10 has combined local with scientific knowledge. Small-scale village nurseries have proliferated in West and Central Africa, in particular, and have become on-farm enterprises and de facto applied experimental sites, where knowledge about cultivation practices for a range of tree species is acquired.

5.3 Knowledge of Population Genetics based on Molecular Markers

15 Application of molecular marker approaches to forest trees

The use of allozymes opened new avenues for understanding population genetics of forest trees, beginning in the late 1970s (Mitton, et al. 1979; Guries and Ledig 1978). Allozyme analyses were instrumental in accumulating knowledge on relative amounts and patterns of genetic diversity, gene flow, inbreeding levels and mating systems for many tree species (see for example, Hamrick et al., 20 1991, 1992). Isozyme analyses have been carried out on for most temperate and many (although far from most) tropical species, allowing researchers to test Hardy-Weinberg based theory and predictions with real data. Millar and Westfall (1992) reviewed the usefulness of allozyme markers in conservation of genetic diversity of forest trees, for example, in designing sampling for conservation, quantifying distribution and amount of genetic variability among and within 25 populations and monitoring changes.

Ledig and others (1983, 1997, 2000, 2002) published extensively on population and conservation genetics of temperate conifers in Mexico and southern USA, on the basis of isozymes. They found, for example that relict *Picea* populations in temperate Mexico were genetically depauperate as theory would predict on the basis of their isolation and small size. Ledig and Conkle (1983) 30 demonstrated that Torrey pine which occurs in two small populations in western US, had the lowest genetic diversity of any tree species that had been studied; with no difference detected within populations, but several alleles found only in one of the two populations. They identified the likely cause to be genetic drift. Hamrick and others (1991, 1992) demonstrated relationships between life history traits and amount and distribution of genetic diversity helping to move application of 35 population genetics for forest trees from pure theory to evidence-based hypotheses. Although Duminil, et al (2007) have since demonstrated that positive correlations among life history traits and genetic parameters have to be interpreted with caution when phylogenetic relationships are not taken into account, Hamrick's work is useful, particularly for formulating hypotheses about patterns of genetic diversity.

Allozymes are usually considered to be neutral in population genetic analyses but many of the enzymes are crucial for metabolic processes and the variants are not neutral in all circumstances. Mitton (1997) described situations in which variants of particular “housekeeping” enzymes apparently resulted in selective advantages, particularly for heterozygous individuals of a number of species. Such effects were found to be small but significant in a number of studies (Bush and Smouse 1992). DNA markers having a stronger claim to neutrality, gradually replaced allozymes because they provide direct information on genetic variation, the number of markers available can be orders of magnitude greater than for allozymes, results may be more repeatable, and samples are more easily handled because DNA is more durable than protein. Although initially the use of DNA markers was expensive, prone to error and time consuming, during the past 25 years, their use has dominated population genetic analyses.

Studies using DNA markers proliferated in North America, Australia and Europe, and gradually spread to other regions. Neale and Williams (1991) and Neale, et al. (1992) reviewed use and applications of early DNA molecular markers (RFLPs, RAPDs) in forest trees, especially their value in tree breeding. Although these markers had distinct advantages over allozymes, for example in the vastly greater number of potential markers, problems such as low repeatability of results, led to adoption of other approaches. As more informative but also more expensive approaches were developed, microsatellites (SSRs) became the most frequent markers used in population genetic studies.

Chloroplast DNA markers have been particularly useful in elucidating genetic structure of populations. Petit et al (2005) reviewed the use of organelle compared with nuclear markers, mainly SSRs, in evaluating population structure of plants based on 138 species, including 37 conifers. They determined that pollen is generally more important than seed in gene flow and that although nuclear marker variability is lower in gymnosperms than angiosperms; the opposite is true for maternally inherited organelle markers. For the proportion of total diversity among vs within populations, there was no correlation found between nuclear and maternally inherited markers.

Temperate species are generally better studied and much more is known about their population genetic structure and diversity than for tropical tree species. Within the tropics, some regions have had much more attention than others. Many studies have been carried out in Latin America (Table xx), where at least 239 studies have focused on about 70 tree species since 1990. Latin America is comparatively better covered than other tropical regions, followed by Asia with at least 172 studies on about 60 species. In Africa, 114 studies were found which reported on genetic diversity of about 40 tree species including a few exotics. Several major European projects during the 1990s contributed significantly toward the high number of studies in Latin America.

The number and scope of the studies per species vary greatly; some relatively important species have been subject to many studies, for example at least 21 molecular studies have been conducted on *Araucaria angustifolia* in Latin America; and at least 15 studies on *Acacia mangium* in Asia. However for most species, even those that have been the subject of at least one molecular study, there is little information.

Approaches and techniques vary widely, with sample sizes ranging from single population studies to distribution-wide surveys and markers varying from a few allozymes to genome-scale genetics

Table xx. Indicative numbers of tropical tree species studied using molecular markers by region since 1990, and approximate number of studies.

Region	DNA analyses -number of species	Isozyme studies – number of species	Number of studies
Asia	51	22	172
Africa	39 (Incl. exotics)	3	114
Australia	24	7	87
Latin America	63	19	239
Total	177	51	612

Marker-based understanding of population genetic processes

- 5 Molecular markers been used to quantify genetic diversity and its partitioning between and within populations. They have also been used to understand population genetic processes that influence or determine levels and patterns of genetic diversity, particularly gene flow, genetic drift and mating systems. Some general results regarding genetic processes derived from marker studies include:
- 10 • Gene flow more influenced by pollen than seed (Petit, et al. 2005)
 • Long distance gene flow in northern and temperate forests where most species are wind pollinated (Petit and Hampe 2006)
 • Fragmentation can lead to greater gene flow among fragments and breakdown of population structure (Young, et al. 1996)
 • Gene flow varies with pollinator energy in tropical species but is frequently several
 kilometres (Petit and Hampe 2006)
 • Most forest trees are strongly out-crossed (Petit and Hampe 2006)
 • Mating system can adapt to accommodate pollen availability (Lowe, et al. 2005)
 • Many tree species naturally have large effective population sizes (Petit and Hampe 2006)
 • Genetic drift detected in small populations that have been isolated for many generations
 (Ledig, et al. 1997)

This general knowledge and related species specific information that has only been possible through marker analyses, has been valuable for testing hypotheses based on population genetic theory, and for informing various types of management and conservation decisions. However, because of the relative ease and availability of these markers, their interpretation has often been over-extended
 25 (Holderegger, et al. 2006) beyond the capacity of neutral measures. In general they do not provide reliable information about genetic variation that is subject to selection and they have contributed little to the understanding of natural selection and adaptation (Gonzalez-Martinez, et al. 2006). The need to extend their use beyond selectively neutral processes led to development of QTL approaches.

30 5.4 Genomic advances

Gene discovery begins with construction of maps and this has been more challenging for forest trees than for agricultural crops because of the large size of the genome, especially for gymnosperms; the high heterozygosity of most species, longevity, and intolerance to inbreeding which prevents creating of highly homozygous lines. Neale and Kremer (2011) briefly described the history of

genetic mapping for forest trees, noting that it began by mapping restricted fragment length polymorphism (RFLP), then random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers, all of which were less than ideal, in part because of their poor reproducibility. The discovery and development of simple sequence repeat (SSR) markers improved

5 the reproducibility problem but their development was too expensive for broad application for mapping purposes. Single nucleotide polymorphism (SNP) markers combined with high-throughput technology have made highly saturated genetic maps possible, however, and rapid progress has been made during the past decade. SNP frequency is very high in forest trees, providing unlimited numbers of markers, with no reproducibility problems at ever decreasing cost, so this marker is

10 increasingly the first choice for many applications.

Several recent comprehensive reviews describe the state of the art of forest tree genomics (see, for example, Neale and Kremer 2011; Gailing, et al. 2009; Grattipaglia, et al. 2009). Similarly to other genetic technologies, trees lag behind key agronomic crops and tropical tree species have tended to receive less attention than temperate ones. However, the number of scientists taking on the

15 challenge of applying the latest technologies to trees is growing. Neale and Kremer (2011) noted that despite drawbacks associated with long generation times, large genomes, lack of well-characterized mutants for reverse genetic methods, and low funding, forest biology is now well - positioned to make rapid strides. Gailing, et al. (2009) argued that forest trees have some distinct advantages over other plant species with respect to using genomic approaches to understanding the

20 genetic basis of adaptation because forest trees generally have high diversity within populations that are still wild; and forest tree populations are subject to natural selection.

Genomic advances have opened doors to understanding molecular biology of trees and, in particular, next-generation high throughput sequencing technologies have made the possibility of sequencing and understanding the large and complex genomes of tree species an affordable reality.

25 There have been significant accomplishments; for example draft sequences have been produced for several tree species, most recently *Picea abies* (Nystedt, et al. 2013) and *Picea glauca* (Birol, et al. 2013) the first conifers for which draft sequences were published. *Populus trichocarpa* was the first tree to have the entire genome sequence published (Tuskan, et al 2006). The *Eucalyptus grandis* draft sequence was published in 2011 by Myburg, et al.

30 As a result of the combination of rapid advances and rapidly decreasing costs, genetic maps, markers and candidate gene sequences have become available for a range of species, allowing for investigation of complex questions and the application of these tools to practical problems in breeding programmes (Haminishi 2011).

The first forest tree species for which the entire genome was sequenced, *Populus trichocarpa* (Tuskan, et al 2006), provides an important reference genetic map, which has contributed to its model species status. Besides those mentioned above, other tree species for which entire genome sequencing is underway or nearly completed, with completed drafts, include other *Populus* spp, *Malus domestica* (Velasco, et al. 2010), *Prunus persica*, *Citrus sinensis*, *Carica papaya*, *Amborella trichopoda*, *Coffea canephora*, *Castanea mollissima*, *Theobroma cacao*,

35 (<http://www.nature.com/ng/journal/v43/n2/full/ng.736.html>), *Salix purpurea*, *Quercus robur*, *Azadirachta indica*, *Pinus taeda* (Neale and Kremer 2011), *Pinus lambertiana*, *Pseudotsuga menziesii*, *Larix sibirica*, *Pinus pinaster*, and *Pinus sylvestris*. Despite the complexity and size of

conifer genomes, their high economic value and the investment in such species for plantation forestry has led to significant sequencing efforts. Important commodity tree crops, including fruit trees have also received significant attention.

Although the main driver influencing the choice of species for sequencing is undoubtedly their economic value and the importance of identifying genes for important traits for marker facilitated selection which will reduce time and cost in tree improvement programmes, there are many knowledge spin-offs. Pavy et al (2013) noted that the availability of large SNP databases allow investigation of polymorphism patterns in genetically distant species to examine evolutionary pathways. For example, Nystedt, et al (2013) were able to confirm that the large genome of conifers is probably not due to a relatively recent whole genome duplication event, but instead is the result of gradual and continuing genome expansion over time via the steady accumulation of long-terminal repeat transposable elements that are not eliminated, as they are in angiosperms. They found that whole genome duplication probably predated the divergence between angiosperms and gymnosperms. These are examples of the type of knowledge gained through sequencing of whole genomes.

Knowledge of genetic basis of adaptive traits, productive traits and resistance to pests and diseases.

Much of what is known about genes that are important for adaptive, productive and resistance traits was discovered prior to full genome sequencing, based on genome-wide analysis techniques such as microarray analysis (Hamanishi and Campbell 2011). Genome-wide techniques can reveal global gene expression patterns and thus are important for identifying groups of genes that respond to specific stimuli, such as drought. Early microarray experiments used sets of Expressed Sequence Tags (ESTs) from specific tissues from *Populus* or *Pinus* species to understand gene expression patterns (Sterky, et al. 1998; Hertzberg, et al. 2001; Heath, et al. 2002) and identify candidate genes involved in wood formation and drought tolerance.

Gonzalez-Martinez, et al. (2006) described methods used for identifying candidate genes in trees, noting that for most trees candidate genes are identified by transferring information from model species for which gene function has been elucidated or in gene expression studies. Another approach is to carry out neutrality tests on population nucleotide sequence data for individual genes or groups of genes. Deviation from neutrality may indicate selection. This approach has been used to identify genes associated with stress tolerance and disease resistance.

Next generation high throughput sequencing can shed light on epigenetic effects which have profound multi-generational impacts on plant responses to environmental stimuli, such as drought or cold (Hamanishi and Campbell 2011). *Populus* trees vary in their epigenetic effects, caused by methylation of genes under stress conditions, turning them off, or on. Using combinations of EST and SNP markers will help to elucidate the mechanisms by which DNA methylation influences gene expression under stress conditions.

Grattapaglia, et al. (2009) reviewed the status of genomic knowledge about growth traits in forest trees and noted that although recent advances have greatly increased the understanding of complex, interacting mechanisms, applying genomic tools to increase productivity and growth was not yet practical because of the number of component traits, each of which is genetically variable.

5.5 Combining molecular tools with tree improvement – marker assisted selection

The first applications of molecular genetic information in tree improvement were for other purposes than identifying genes responsible for traits of interest. Early uses of genetic fingerprinting with molecular markers used in tree improvement programmes included (FAO 2011):

- 5 • measuring genetic diversity of breeding population accessions between indigenous provenances and naturalized landrace origins;
 • testing paternity contributions to offspring grown in field tests;
 • verifying genetic identity during vegetative propagation.

As genetic mapping became possible with molecular markers, there was a level of interest in the 10 linking quantitative traits with markers, launching numerous QTL projects. For a variety of reasons, including the rapid decay of linkage disequilibrium, requiring very close association between markers and genes for traits of interest (Neale and Kremer 2011), QTLs did not provide the expected insights as quickly as hoped and attention turned to other approaches including association genetics and DArT. As genomic tools have become increasing accessible, both in terms of ease of application and 15 cost, a current bottleneck in linking traits to the growing knowledge of forest tree genomes has shifted to the cost and time required for sampling in the field and phenotyping. There is still a need to measure the range of phenotypic variation in traits of interest in order to link the genomic markers to phenotypic expression.

Quantitative trait loci

20 Quantitatively inherited traits are controlled by many genes and most phenotypic traits of interest in forest trees fall into this category. Each gene controls a relatively small amount of variance in a quantitatively inherited trait (Brown, et al. 2003) and such genes are known as quantitative trait loci (QTL). Much effort has gone into identifying linkages between various molecular markers and QTLs over the past two decades, by determining the number and location of chromosomal regions 25 affecting variation in a trait and finding statistically significant associations between markers closely associated with the chromosomal regions and quantitative phenotypic traits in a segregating population (Brown, et al. 2003). Mapping QTLs allows elucidation of the genetic structure of complex traits and for application to marker-assisted selection in well-studied breeding populations of tree species that are undergoing genetic improvement. The main focus of QTL analysis has been on 30 growth traits which are the main target of breeding programmes but have relatively low heritability and are controlled by multiple genes (Grattipaglia et al 2009).

35 QTL mapping requires highly marker-saturated linkage maps and phenotypic measurements of all pedigree individuals in large segregating populations (Gonzalez-Martinez, et al. 2006). Such maps have been successful in showing existence of loci accounting for between 5-15% of observed variance (Guevara, et al. 2005) in spite of significant challenges such as the instability of associations across different environments. In long-lived organisms the expression of QTL is likely to change on a seasonal or yearly basis. Factors influencing ability to detect QTLs include sample size, genetic background, environment and interactions among quantitative gene loci (Brown, et al. 2003).

Hamanishi and Campbell (2011) described use of QTL mapping for finding linkages with specific traits, noting that genetic maps have been generated for many forest trees and many QTL have been identified for drought-related traits. They pointed out that although knowledge gained from QTL analysis in tree species is useful for tree breeding, progress is slow because of time required for identifying genes in species with limited genomic sequence availability.

Echt, et al (2011) described advances in mapping the loblolly pine genome. They have combined SSR markers with previously mapped ESTP and RFLP markers to produce a map that is useful for a variety of population genetic and germplasm management applications. In addition the mapped markers can facilitate understanding of the evolution of candidate adaptive trait genes that require unambiguous identification of parental and clonal genotypes.

10

Association genetics approaches

Association mapping was developed to overcome problems with QTL mapping experiments. It uses linkage disequilibrium (LD) mapping to understand the genetics basis of complex traits, relying on the association between complex traits and chromosome regions containing genetic markers.

15 Unlike QTL mapping, searching for loci using association genetic approaches does not depend on pedigreed families. Association genetics uses linkage disequilibrium between a phenotypic traits and markers and can be applied for any large sample of a natural segregating population (Ingvarsson and Sweet 2011). Many more markers are needed than have typically been used in QTL mapping and this has been made possible because of the rapid development of sequencing. The hundreds of

20 thousands of SNP markers needed for association mapping can be generated rapidly and relatively inexpensively from any species. The bottlenecks now are field-related, first collecting enough samples to ensure that associations are robust and second, phenotyping the sampled populations.

Ideally, replicated field trials will provide the most precise phenotype information, but time and cost constitute serious impediments.

25 Association genetics may start with candidate genes or a genome-wide approach. Using candidate genes as markers, individual alleles can be found that are involved in controlling traits. Neale and Kremer (2011) summarised association genetic studies using candidate genes for stem growth, wood quality, pest resistance, bud phenology, cold hardiness and drought tolerance in six genera of forest trees including two conifers and four angiosperms. Gonzalez-Martinez et al. (2006) reported testing

30 18 candidate genes for association with drought tolerance in *Pinus taeda* using this approach. All but two were found to be selectively neutral. A drawback of using the candidate gene approach is that as with QTLs, individual associations between markers and traits account for only very small proportions of the genetic variation.

An association mapping approach has the potential to be very useful for tree breeding strategies in
35 the future is a whole-genome scan (Hamanishi and Campbell 2011). For example, in *Picea glauca*, Namroud et al. (2008) identified SNPs in expressed genes and used them as genetic markers for mapping purposes to identify potential associations between local adaptation of candidate genes and phenotypic attributes of populations. This approach can be used for identifying genes under

potential selection for drought tolerance and other adaptive traits in non-model tree species through association mapping.

DArT methods

Some of the most rapid advances in application of genomic tools to breeding are focused on Eucalyptus species. Faria, et al. (2010) reported development of 20 microsatellite markers from ESTs, which are fully transferable across six Eucalyptus species. They predict that the usefulness of the markers will extend to all 300+ species in the sub-genus. In addition, according to the authors, the markers provide excellent resolution and potential for use in breeding. Intra-genus species transferability of markers is highly desirable and new methodology known as Diversity Arrays Technology (DArT) offers exciting prospects for very rapid genome-wide screening of thousands of polymorphisms (Petroli, et al. 2012) across related species. Because the markers are gene-based, they are useful for genomic selection and can dramatically enhanced breeding and improvement of forest trees that have been subject to intensive genomic study (Grattapaglia and Kirst 2008). Several Eucalyptus species were used to create more than 8000 DArT markers which were used for high resolution population genetic and phylogenetic studies (Steane, et al. 2011). Among uses that Steane et al (2011) noted for DArT markers, are species differentiation, identification of interspecific hybrids, and resolution of biogeographic disjunctions within species.

Resende, et al. 2012a used more than 3000 DArT markers to evaluate the efficiency and accuracy of genomic selection in two unrelated Eucalyptus breeding populations and they reported that for growth and wood quality traits they were able to match accuracies attained by conventional phenotypic selection. However they cautioned that, in spite of the potential for the approach to revolutionize tree improvement, experimental support is required and in the short term, it is likely that predictive models will population specific. Resende, et al. (2012b) applied the same approach to Pinus taeda populations across multiple ages and found high accuracies across environments within a given breeding zone, but not when models generated at early ages were used to predict phenotype at age six.

5.6 Genetic modification

Early efforts and challenges in modification of forest trees, particularly conifers were reviewed by Sederoff (1995), who was a pioneer in the application of these technologies to trees.

Genetic modification of forest trees poses challenges both biologically and in terms of public acceptance. Walter and Menzies (2010) reported that activities related to genetic modification of forest trees is taking place in at least 35 countries and 16 of them have field trials which are generally small and of short duration. The first genetically modified tree, a poplar, was produced more than 25 years ago (Fillati et al 1987). The number of tree species that have been successfully transformed remains low, especially among conifers, and within the proportion of attempted genotypes from which transgenic plants have been recovered, remains low ((Meilan, et al 2010).

Traits targeted by transgenic experiments include pest, herbicide and abiotic stress resistance, hormone regulation, lignin and cell wall biosynthesis and growth (McDonnell 2010). Among forest

tree species, *Populus* species and hybrids by far the greatest number of transgenic experiments have been conducted using compared to any other forest tree species or genus.

Except for a few hundred hectares of genetically modified *Populus* planted in China, no commercial planting has been reported. However genetic modification protocols have been developed and

- 5 tested for many commercially important plantation tree species for traits such as stem shape, herbicide resistance, flowering characteristics, lignin content, insect and fungal resistance (FAO 2011). McDonnell, et al. (2010) summarised the progress on genetic modification of 10 species and hybrids for many of these traits (Table xx).

- 10 Transgenic trees are likely to be planted within crossing distance of wild populations of the same species. Robledo-Arnuncio, et al. (2010) concluded that it is highly probable that transgenes from genetically modified trees would move into conventional forest because of the efficiency of dispersal systems over the long lifetime of tree species. Concerns about genetically modified forest trees dispersing pollen or seed, which will spread transgenic, selectively advantageous propagules into
15 natural populations, has led to a strong focus on sterility mechanisms. Attaining a stable form of both male and female sterility in transgenic trees before releasing them is not a trivial problem. Researchers have encountered significant obstacles in the search for sterility, but it has led to expanded knowledge of genetic control of reproductive functions and floral genomics (reviewed by Brunner, et al 2010).

- 20 Although most genetic modification is done with the aim of increasing or improving wood production, it can also be a tool for conservation, for example in the case of American chestnut. Barbakat, et al. (2011) compared canker transcriptomes from American (*Castanea dentata*) and Chinese chestnut (*Castanea mollissima*) to identify candidate genes that may be involved in
25 resistance to chestnut blight (*Cryphonectria parasitica*). They identified several candidate genes for resistance and gained a better understanding of the resistance pathway in Chinese chestnut.

- 30 Table xx. Number of published successful transgenic experiments achieving gene expression or over-expression in transgenic cells (number of genes used in the various experiments in parentheses, where relevant), by tree species or genera and modification objective (summarised from McDonnell et al. 2010).

Species/genus	Pest resistance	Herbicide resistance	Abiotic stress resistance	Hormone regulation	Lignin	Cell wall biosynthesis	Growth
<i>Populus</i> spp and hybrids	8 (>5)	11 (9)	11 (10)	9 (7)	26(10)	6 (6)	8 (7)
<i>Pinus taeda</i>	1		1		1		
<i>Pinus radiata</i>	1	1			1		
<i>Picea glauca</i>	2 (2)						
<i>Eucalyptus camaldulensis</i>		1	1		1		
<i>Eucalyptus grandis x urophylla</i>			1				
<i>Picea abies</i>		1			1		
<i>Larix decidua</i>		1					
<i>Larix leptoleuropaea</i>			1				
<i>Pinus strobus</i>			1				

5.7 FGR Knowledge related to conservation and restoration

Types of knowledge and its application

Many studies have been conducted to understand mating systems and gene flow patterns of forest tree species with an initial focus on Europe, North America, and Australia. More recently,

5 number of studies targeting tropical species has steadily increased; among many examples, the study by Fuchs and Hamrick (2011) on continuous and remnant populations of the endangered tropical tree *Guaiacum sanctum* (Zygophyllaceae). They found that isolated populations had maintained high genetic diversity because of long distance gene flow indicating that the species has potential to adapt and expand populations if suitable habitat is available. This adds to the growing
 10 body of knowledge gene flow among populations of neotropical species. There is still considerable disequilibrium among continents however; tropical American species have been more studied (Ward et al. 2005) than African and Asian ones. In any case, the percentage of studied species is still very low in relation to the high levels of tree species endemism in tropical regions.

15 Spielman et al. (2004) conducted a meta-analysis to compare heterozygosity of threatened species and that of their nearest non-threatened relative. They found that for paired groups of 15 gymnosperm species and 6 angiosperms, heterozygosity was lower in 67 and 81% of the threatened species, respectively. Overall the difference in heterozygosity level between the threatened and non-threatened species was on average, 35% for both gymnosperms and angiosperms.

20 Conservation actions when species are threatened by invasive insects or disease require a different type of genetic information. For example Potter, et al. (2012) studied *Tsuga canadensis*, a North American conifer that is threatened throughout much of its range by an introduced adelgid to inform a ex situ conservation strategy. Information required included range-wide population structure including distribution of genetic diversity within and among populations, occurrence of rare alleles and levels of inbreeding. They used microsatellite markers to identify locations of glacial refuges
 25 which are of interest because they typically have high genetic diversity. They confirmed a negative relationship between population isolation and diversity; and a positive relationship between diversity and population size. The information that they generated through the range wide study will be used to refine seed collection areas to ensure that the patterns of genetic diversity on the landscape are represented in the collections, and that areas with high genetic diversity and unique
 30 or rare alleles will be included.

35 Research by Fady, et al (2008) on the impact of natural and anthropomorphic factors on populations of *Cedrus libani*, a species of the eastern Mediterranean mountains that has long been influenced by human activities, determined that there are two genetically isolated groups in Lebanon and Turkey. Using a combination of cpDNA markers and allozymes, they identified priority populations for conservation and proposed how to identify appropriate source populations for assisted gene flow.

Azevedo, et al. (2007) reported on the population genetic structure of an Amazonian tree species, *Manilkara huberi* that is endangered due to over-exploitation for its high value wood. Based on seven microsatellite loci, they examined mating system, and patterns and structure of genetic diversity, to guide conservation and management of the species. The species has limited pollen flow

and a highly structured spatial genetic pattern. The researchers reported evidence for genetic isolation of populations, indicating that further fragmentation of the species' distribution may result in loss of subpopulations and their associated genetic variability. This means that at least several large populations should be maintained to conserve evolutionary potential of the species. They
 5 were able to estimate on the basis of population genetic parameters, that in order to maintain an effective population size of 500, seed should be collected from more than 175 maternal trees, in order to keep.

The above examples are but a few of the many studies carried out over the past three decades using molecular markers to identify conservation priorities. Such thorough studies cannot be carried out
 10 for all species that are at risk due to increasing land pressures, over-exploitation, climate change and other causes, but lessons learned can be applied more broadly.

Genomic approaches will be relevant for conservation and sustainable management of natural populations of trees in the near future. For example, the emerging potential for conducting
 15 association studies in a well-defined ecological and evolutionary context, where correlations can be estimated between phenotypes and genotypes at a fine scale (Neale and Kremer 2011) will facilitate identification of populations having high conservation value.

Combining spatial analysis with genetic markers to prioritize conservation

The formulation of effective *in situ* conservation strategies can be maximized by an understanding of
 20 spatial patterns of tree species genetic diversity (Petit et al. 1998). Areas of high genetic diversity should be targets for *in situ* conservation as they are considered more likely to contain interesting materials for use and genetic improvement. The recent development of new powerful molecular tools that reveal many genome-wide polymorphisms has created novel opportunities for assessing genetic diversity. This is especially the case when these markers can be linked to key adaptive traits
 25 and are employed in combination with new geospatial methods of geographic and environmental analysis (e.g. Escudero et al. 2003; Manel et al. 2003; Holderegger et al. 2010; Chan et al. 2011). New methods to prioritize populations and geographic areas for *in situ* conservation, and to enable monitoring of genetic diversity over time and space, are now available and can and should be exploited to improve *in situ* conservation.

30 Geospatial analysis of genetic diversity has been undertaken for a wide range of tree species because the maintenance of genetic resources of most of these species depends largely on *in situ* conservation. Among recent examples, a geographic grid-based gap analysis for Norway spruce (*Picea abies* (L.) H.Karst) in Austria was used to identify new conservation units that complement the coverage of mitochondrial and nuclear molecular marker variation, and adaptive genetic
 35 diversity by the current species' conservation unit network (Schueler et al. 2012). Another recent case study is the prioritization of *Prunus africana* populations at continental scale on the basis of nuclear and chloroplast microsatellites, combined with climate clustering as a proxy for adaptive variation (Vinceti et al. 2013).

40 One effective method to describe genetic diversity in geographic space is to use circular neighbourhood-type analyses. This approach is especially effective when working with

georeferenced individuals rather than with populations (van Zonneveld et al. 2012a). Such an approach has been used to identify genetic diversity hotspots for the *in situ* conservation of a number of important tree species, including a high-value timber species *Cedrela balansae* C.DC. in northern Argentina (Soldati et al. 2013), cacao (*Theobroma cacao* L) in its Latin-American centres of origin and domestication (Thomas et al. 2012), in the fruit tree cherimoya (*Annona cherimola* Mill.) in the Andes (van Zonneveld et al. 2012a), and in the bush mango (*Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill. and *I. tenuinucleata* Tiegh) in Central Africa (Lowe et al. 2000).

In addition to these large scale studies to map genetic diversity, there are many other studies that have assessed geographic patterns of genetic diversity at a smaller scale than those mentioned above. Most of these studies have been carried out in temperate and boreal zones and more studies are required in the biodiversity-rich tropical regions (Pautasso 2009). However the number of molecular studies is increasing, including in the tropics. The results can be used in meta-analysis to detect overall geographic patterns of genetic diversity for species with similar life history traits or other analogies. The results from such studies can be extrapolated to provide conservation recommendations for other tree species as well for which no genetic studies have been carried out but which share common ecological features with those species that have been subjected to molecular studies in a part of their observed distribution.

One approach to extrapolate patterns observed from these analyses and prioritize areas to maximize capture of tree genetic resources is to identify Pleistocene refugia and converging post-glacial migration routes. These areas harbour high inter-specific and intra-specific diversity (Petit et al. 2003). Georeferenced observation points of such species from herbaria and genebanks can be used to predict Pleistocene species distributions on the basis of past climate data (Waltari et al. 2007). Such data are freely available from the PMIP2 website (<http://www.pmip2.cnrs-gif.fr>) although it still needs to be down-scaled. Georeferenced plant data and climate models are increasingly available through online platforms such as the Global Biodiversity Information Facility (www.gbif.org) and WorldClim (www.worldclim.org), respectively. These data, where available and when they are of reasonable quality, can be fed into Environmental Envelope Modelling (EEM) to predict past species distributions and reconstruct potential Pleistocene refugia (Waltari et al. 2007; Thomas et al. 2012).

Climate change and FGR

Much research has been galvanised by predictions of impacts of climate change on forest populations and species. The impact of specific climatic changes on tree species will vary with biological, genetic and distributional properties of the species and populations within the species. When confronted with significant climatic changes, there are three possible outcomes for populations of native tree species (Aitken 2008). They may be extirpated resulting in loss of unique genes or gene combinations; they may survive in place, as a result of phenotypic plasticity or adaptation, or a combination of the two; or they may migrate following the changing climate to establish in new locations having climatic conditions for which they are adapted. However migration by seed is likely to be too slow for many tree species if climate change is rapid. Trees, given their long generation time are of particular concern.

- Interestingly, past climate-driven demographic events have left some signatures in the genomes of species. Such signatures can be traced back using molecular markers. Phylogeographic methods have thus allow retracing the impact of past-climate changes on the evolutionary demographic history of plants (Hewitt, 2004; Heuertz et al., 2006; Lowe, Harris, Dormontt, & Dawson, 2010; Petit, 5 Brewer, et al., 2002). Modelling the impact of past climate changes on species diversity provides useful information to predict the future evolution of species.

- Spatial modelling using GIS mapping tools to examine vulnerability of genetic resources to impacts of climate change is used increasingly. Van Zonneveld (pers. comm.) demonstrated that the highest 10 diversity in populations of *Annona cherimola*, a species of the Andean foothills in Latin America (van Zonneveld, et al. 2012), fall in areas with highest expected climate-related impacts to forest ecosystems based on predictions of global climate models. Vinceti, et al. (2013) carried out a similar analysis with *Prunus africana*, a widely distributed, but ecologically restricted species found in all of the Afromontane regions. On the basis of climate models, they predicted that the number of populations will be reduced by about half in the area having highest genetic diversity.
- 15 The major challenge facing conservation genetics is to link traits that are important for adaptation to changing climates with molecular markers. Technologies that are being developed for breeding and improvement have relevance, particularly the whole genome association genetic mapping approaches that do not require pedigrees but they still require a higher level of ecological and phenotypic variation knowledge than is available currently for most species of conservation concern,
- 20 especially in the tropics.

- Examples of impacts of already changing climate on tree species and their genetic resources are adding up, but still not readily available in the published literature. Based on available data and deduction, climate change is likely to have impacts on FGR through several processes which may include: loss of populations and their unique genetic variation as a result of extreme climatic events 25 and regeneration failure, especially at the receding end of distributions; more severe pest and disease attack in some areas; altered fecundity of some tree species; pollination failure because of asynchronicity between flowers and pollinators or loss of pollinators; decline or loss of fire-sensitive species because of increased fire frequency; changes in competitive relationships resulting in new species invasions and potential hybridization (Loo, et al. 2011).
- 30 Changes in fecundity are expected because of sensitivity to spring temperatures (e.g. as observed in the southeastern United States of America; Clark et al., 2011) and other factors (Restoux, 2009). For example, in central Spain, a decline in cone production in *Pinus pinea* over the last 40 years has been correlated with warming, especially with hotter summers (Mutke et al., 2005).

Genetic technologies for reducing illegal logging

- 35 Unsustainable and illegal logging is a driver of deforestation and forest degradation worldwide. Commercial timber extraction and logging activities account for more than 70% of forest degradation in Latin America and (sub)tropical Asia (Kissinger et al, 2012). New policy instruments in Europe, the USA and Australia prohibit the sale of illegally harvested wood and wood products in by requiring operators to provide proof of the identity of the species traded and the origin of their

products. Accurate species identification and the tracking of the geographic origin of timber along the chain-of-custody are therefore necessary to control the flow of illegal woods and wood products. However, there is a mismatch between the legislated requirements and the capacity of importers to fully comply because existing methods to document species identification (wood anatomy and

- 5 chemistry) and origin (mostly paper-based documentation, tagging) are insufficient, ambiguous and easily falsifiable. Significant efforts have been focused on extracting DNA from wood samples during the past 10 years and it is now feasible to use DNA markers to complement existing tools, both to identify species and track the origin of timber along the supply chain. Species identification using DNA sequences draws upon the Barcode of Life project (Stockle and Hebert 2004), whereby DNA
10 sequences that vary among but not within species are used to differentiate species. The concept has been more useful for fauna than flora; plants require two or more sequences for confident identification.

15 Lowe and Cross (2011) reviewed progress toward use of DNA as a forensic tool for identifying species and origin of wood. Barcodes using the two core sequences (matK + rbcL), are expected to distinguish at least 50% of plant species. For a broad selection of plant species, the percentage is increased to about 80% with the addition of the nrDNA ITS sequence (Hollingsworth 2011). If needed, additional specific sequences may be used to increase the resolution and likelihood of correct identification for timber species. Extraction of high quality DNA from wood samples is an
20 ongoing challenge.

For purposes of controlling illegal logging, ability to determine the origin of wood may be as important as identifying species. If spatial genetic structuring is strong, the geographic origin of a log
25 may be determined with a high degree of precision. Information obtained from the studies that have been carried out during development of methods for tracking timber feed into the growing body of molecular knowledge of forest trees.

An alternative approach is to obtain wood samples from the standing trees prior to harvest which allows tracking individual logs by matching fingerprints, if necessary at any point on the supply chain.

Table xx: Examples of the use of DNA and markers to control illegal logging

Level of verification	Species	Range	References
Species identity	Multiple species from the Meliaceae family	Worldwide	Holtken, et al. 2011
Declared region/ country of origin	<i>Cedrela odorata</i>	Neotropics	Cavers, et al. 2013
	<i>Neobalanocarpus heimii</i>	Peninsular Malaysia	Tnah, et al. 2009; Tnah, et al. 2010
	<i>Swietenia macrophylla</i>	Americas	Degen, et al. 2013; Lemes, et al. 2003; Novick, et al. 2003; Lemes, et al. 2010
	<i>Brosimum alicastrum</i>	Central America	Poelchau & Hamrick 2013
	<i>Carapa guianensis, C. surinamensis</i>	Amazonia	Scotti-Saintagne, et al. 2013
	<i>Shorea spp.</i>	Southeast Asia	Tsumara, et al. 2011
	<i>Milicia excelsa</i>	Central Africa	Dainou, et al. 2010; Bizoux, et al. 2009

	<i>Pterocarpus officinalis</i>	Caribbean	Muller, et al. 2009
Declared concession of origin	<i>Entandrophragma cylindricum</i>	Cameroun	Jolivet & Degen 2012
Individual log tracking	<i>Intsia palembanica</i>	Papua-Indonesia	Lowe, et al. 2010

The [Global Timber Tracking Network \(GTTN\)](#) led by [Bioversity International](#) is working with scientists and other stakeholders to define the rules and agreed upon international standards for genetic labs that will conduct forensic testing. The network is creating a reference database of DNA fingerprints

- 5 for traded species to help identify species and track the origin of wood and wood products along the supply chain.

“Core-barcodes” have been developed (Fig. 1) for only about half of the 800 commercial timber species. Some species with the conventional barcodes are represented in the Barcode of Life database by few individuals sampled from just a part of the distribution range. For such species, the 10 intraspecific variation and the effect of geographical scale of sampling on DNA barcoding are still problematic (Bergsten, et al. 2012; Lou and Golding 2012). Additional sequences are used [e.g. Mahogany family, (Muellner, et al. 2011)], when the core-barcodes are not universal, have bad sequence quality and lacked discriminatory power (Hollingsworth 2011).

15 The practical application of forensic DNA analysis in the timber trade requires adaptation of the methods:

- to work for samples (wood and wood products) with degraded and low quantity DNA,
- simplification of the laboratory procedures for use in small labs, without the need for sequencing or capillary electrophoresis techniques. This is relevant in many timber producing countries where the risk of illegal logging is high, but law enforcement agencies 20 and national labs are poorly equipped to enforce forest laws before wood and wood products are shipped overseas.

This simplification is possible with the use of PCR-RFLPs after SNP detection and has been successfully developed for CITES-protected species (Holtken, et al. 2011) but it remains to be done for many commercial species.

25 Genetic reference maps to trace the origin of timber are, or soon will be available for about 50 species, for at least a portion of their natural range. With the new generation sequencing techniques, progress in molecular marker development for many species is advancing quickly and costs are decreasing. The most immediate need is widespread sampling to cover the range of as many timber species as possible to have a complete and robust tracking system.

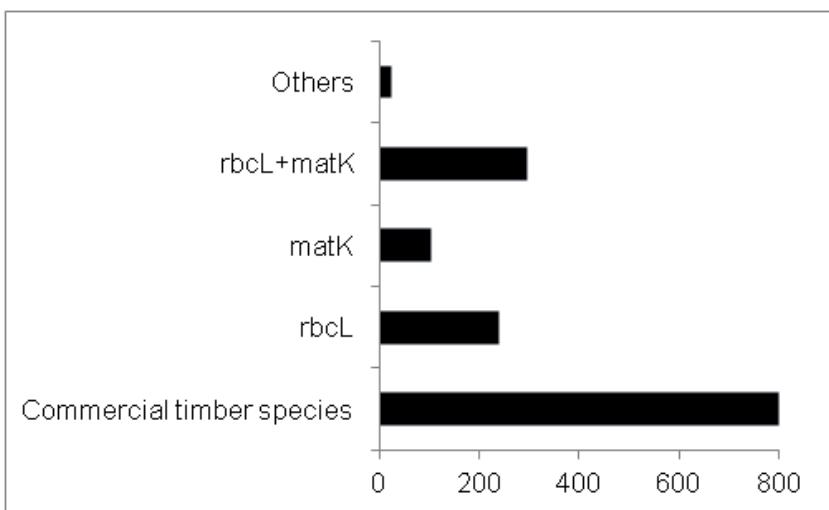


Fig. xx: Commercial timber species having barcodes recorded in the Barcode of Life database

Existing data are available mainly for timber species traded in the global market. But timber from illegal logging activities is used also within producing countries. For example, 85% of the timber
5 production in Brazil is for domestic consumption. Therefore it is important to invest also in developing national capacity for regional and local species (those that are not traded internationally) to reduce illegal logging within countries. In general, the level of knowledge is lower for such species and data needed to track wood origin is lacking.

Finally, capacity building in timber producing countries including training in molecular techniques
10 and ensuring availability of basic lab equipment is essential to foster routine use of DNA as a forensic tool.

In situ conservation

In situ conservation of forest genetic resources was not considered uniformly by different country reports, which complicates interpretation. The strict definition of *in situ* conservation of genetic
15 resources implies reserves established specifically for conserving genetic diversity of targeted tree species. Many European countries have such reserves and reported on this basis. Other countries such as Canada do not have such reserves, and reported no *in situ* conservation of FGR. In Canada, genetic resource gap analyses have established that in British Columbia, for example, all tree species are represented with adequate population size in existing protected areas but across the species'
20 ranges, gaps remain in some of the biogeoclimatic zones. In this case, secure *in situ* protect is in place for most species across much of their natural distribution (Krakowski, et al. 2009; Chourmouzis, et al. 2009). Other countries that do not have forest genetic reserves designated strictly for that purpose reported *in situ* conservation on the basis of protected areas designated for a variety of purposes, but without the benefit of genetic gap analyses to understand the degree to
25 which genetic resources are protected.

Large areas have been designated “protected” with legal protection but often little or no enforcement in many countries, and no information about populations of tree species within the areas, so tallying the extent of protected areas does not provide much useful information regarding *in situ* conservation of genetic resources. In many cases, even if inventories of tree species and

populations were carried out, they were done at least a decade ago and many protected areas have been subject to a variety of human activities. Consequently it is commonly not known whether the species are still found within the protected areas.

In Europe, EUFORGEN (<http://www.euforgen.org/>) has encouraged and provided expertise to 5 facilitate the development of gene conservation reserves in many countries. Technical guidelines have been developed for conservation and use of genetic resources of 33 tree species. A set of standards have been developed and accepted for effective in situ conservation, which includes periodic monitoring of the FGR contained within the reserves. The information collected through 10 monitoring will be extremely useful for understanding factors which influence the effectiveness of conservation. The accomplishments of EUFORGEN in understanding, conserving and monitoring 15 genetic resources of forest trees provides a very useful model for other regions.

It is important that conservation of genetic resources of tree species be an explicit objective of 15 conservation areas. In cases of well protected areas with effective conservation enforcement there may be a need for management actions to maintain genetic resources that are often not allowed by protected area legislation.

Technologies for ex situ conservation

Ex situ conservation includes field gene banks such as clone banks and seed orchards, arboreta and botanical gardens, seed banks, in vitro collections and cryo-banks. Field gene banks are often 20 associated with tree improvement programmes, consisting of clone banks, provenance, progeny and other field trials, or seed orchards. Different types of knowledge are required for each method. Increased emphasis in recent years on expanding collections in botanical gardens, especially in China and other highly diverse countries, has contributed significantly to knowledge of cultivation of 25 tropical tree species, particularly endemics (<http://www.bgci.org/global/>).

A total of 626 species, listed as priorities in Country Reports, are also represented in some form of *ex situ* 25 conservation and 135 of them are conserved in more than one country. According to Country Reports, 1800 tree species are conserved *ex situ*. Many of these are likely in botanical gardens. Although there is a perception that tree seed, especially from tropical trees cannot be stored under conventional seed storage conditions (cold and dry), many valuable tree species for human use in all 30 regions are orthodox in their seed storage behaviour and seed banks to ensure longterm availability of valuable genetic resources are generally under-rated and under-used in this respect. Scientific collaboration in plant conservation has led to substantial innovation in seed storage in recent years, particularly diagnosing tree seed storage behaviour, increasing tree seed longevity in the dry state and improving storage biotechnology.

In all regions and environmental conditions, seeds of some tree species are “recalcitrant” meaning 35 that they cannot be stored in conventional seed banks due to sensitivity of the seeds to desiccation. For example, it is estimated that approximately 60% of tree species in the Amazon Basin produce recalcitrant seeds. This reduces the effectiveness of seed banks in areas where threats to tree species and their populations are greatest. However, during the last 20 years, considerable progress 40 has been made in: 1) understanding the mechanisms of desiccation-induced viability loss on drying, including the role of reactive oxygen species and programmed cell death (Kraner, et al. 2006); 2)

estimating the proportion of the world's flora that produce such seeds (i.e., diagnosis); and 3) developing methods that can help conserve such species in *ex situ* cryo-banks (i.e., storage biotechnology).

Danida Forest Seed Centre (now part of Forest Landscape Denmark) and Bioversity International

- 5 (formerly International Plant Genetic Resources Institute; IPGRI) led a global initiative (1996-2002) involving about 20 countries to screen recalcitrant and intermediate (partial desiccation tolerance but with sensitivity to storage at -20°C and 0°C) seeds of 52 tropical forest trees belonging to 27 families. The project assessed seed responses to multiple desiccation states and subsequent storage 10 at a range of temperatures (Hong and Ellis 1996) as a reliable approach to understand seed storage behaviour. For a summary of the findings of the project, see Sacandé et al. (2005).

An alternative screening approach that deals only with aspects of the effects of drying and short term storage at the initial moisture content of the seed sample (at receipt or harvest), gives a good indication tolerance of rapid artificial drying similar to that used in seed banks; additionally, the moist-stored control can show reduced germination (rapid loss of viability and thus short-lifespans),

- 15 level germination (no effect of short-term storage) or increased germination (evidence of seed maturity during the storage period). Called the 100-seed test, as that is the target number of seeds to use (many less than the previous protocol), the method has been adopted by tree seed experts at INPA in Brazil, University of Queensland, Australia and the University of KwaZulu Natal, Durban, Republic of South Africa.

- 20 It is important to develop predictive biological models to indicate risks associated with handling seed having particular characteristics because the large number of tree and other higher plant species, estimated to be as many as 353000 (Scotland and Wortley 2003; Chapman 2009), renders the physiological screening of all species unlikely in the foreseeable future. Early studies revealed broad associations between heavier seed in the Araucariaceae (Tompsett 1984) and Dipterocarpaceae with

- 25 seed desiccation sensitivity. Further development using multiple criteria (Hong and Ellis 1998) indicated associations between habitat and desiccation intolerance across a broad range of vegetation types; with low frequency (c. 10% or less) in the driest regions of the world, and high frequency (close to 50%) for tropical moist evergreen forests (Tweddle, et al. 2003). For about 70 African tree species that have been studied (Pritchard, et al. 2004b), predictions have been correct,

- 30 in that those that produce recalcitrant seeds disperse their seed in the rainy season in seasonally dry environments, as they must maintain water status or else they die. Such an association is more likely to be apparent in seasonally dry environments than in areas that are moist year-around. Another ecological prediction that has proven useful is that recalcitrant seeds should not need proportionally as great a defence mechanism against consumption, e.g., thick seed coats, as germination will be

- 35 relatively quick (Hong and Ellis 1998). For 104 trees of Panama (Daws, et al. 2006) it was possible to develop a predictive model for the probability of a seed being recalcitrant using this relationship.

In comparative studies of seed morphology in relation to desiccation tolerance and other physiological responses in nearly 200 rainforest species in three continents, from 42 to 62 % of species were found to have non-orthodox behaviour, with an overall average of 51% (Hamilton, et

- 40 al. 2012; Ferraz, et al. 2004; Ellis, et al. 2007). Generally the relationship between seed size and tolerance for desiccation held (Hamilton, et al. 2012). As Daws and Pritchard (2008) discovered, working with *Acer pseudoplatanus*, it is important to pay attention to phenotypic plasticity in the

seed storage response between plants growing inside and outside the native distribution range as this can be responsible for species 'jumping' storage categories or being miss-classified.

The half-life (time for viability to fall from 97.9 to 50%) of seed in storage varies greatly, in part depending on the oil content of the seed in relation to mass. Estimates range, for example, from

- 5 0.95 for *Dipterocarpus alatus*, to 342 years for *Liquidambar styraciflua* (Daws, et al. 2006). The information required to make such predictions is not available for most tree species, but recent advances in understanding mean that predictions can be fairly accurate. The shortest life-spans are driven by the need to store the seeds partially hydrated at 17 - 10% moisture content. In contrast, the longest lived seeds can be stored much drier at around 3% moisture content. There are
- 10 examples of very long-lived seed; some seeds of three woody species of the Cape Flora of South Africa were germinated after about 200 years storage under museum or cellar conditions.

Besides improving protocols through application of new seed physiology knowledge, which has resulted in some species that had previously been considered recalcitrant, being reclassified to intermediate, for example, and development of techniques for extending the viability of non-

- 15 orthodox seed, the main innovation for recalcitrant seed has been improvement in cryopreservation techniques. Cryopreservation is necessary for species that produce fully hydrated recalcitrant seeds. The lifespan of these seeds is limited mainly by the extent to which they can be safely cooled. For temperate and tropical species cooling is limited by the risk of ice formation and chilling stress, respectively. Thus in practice, storage can be close to 0°C for temperate recalcitrant seeds and 15°C
- 20 for tropical representatives on this functional trait. Usually under such conditions, lifespan is limited to a few months and rarely more than one year. Consequently, alternative conservation solutions are needed if the seeds of these species are to be conserved longer-term *ex situ*.

The main development of the last 25 years in plant cryopreservation has been the improvement in methods for vitrification, particularly encapsulation-dehydration (Fabre and Dereuddre 1990) and

- 25 the use of complex solutions of cryoprotectants that reduce the risk of ice formation of partially hydrated tissues during cooling and rewarming. Most of the research has been on species that are of interest to commercial forestry or are valuable fruit trees. There are many examples of enablement of vitrification of the intracellular constituents during cooling following partial drying in air; for example, the embryos or embryonic axes of five species of citrus cryopreserved after
- 30 desiccation to c. 15% moisture content (Malik, et al. 2012). In this example, longevity of the embryos at -20°C was limited to a few months only, whilst the cryopreserved samples retained high levels of viability after 6 – 8 years.

Having evolved over millennia to ensure the dispersal of the species genomes, pollen and seed are ideal starting materials for innovative conservation programmes. *Ex situ* approaches of significance

- 35 include enhancing seed production through artificial pollination and treatments ensuring efficient seed germination to generate the next cohort of plants (beyond the scope of this review). But such work is underpinned by knowledge of the species distribution and the application of horticultural skills to seedling growth.

Knowledge generation and management needs: knowledge of seed biology of forest tree species is

- 40 limited and the resources that are available are scattered. An on-going series of tree seed leaflets including 150 species has been published since 2000 by the University of Copenhagen, in collaboration with the World Agroforestry Centre (ICRAF) is available online. Other initiatives such

as the study of seed from 100 Panamanian tree species, has generated important information (Sautu, et al. 2006). The Tropical Tree Seed Manual (Vozzo 2002) which includes 175 tree species is another highly useful publication. It would be beneficial to bring all of the information together on one portal so that *ex situ* conservation actions can be supported. Sources of information should 5 include compendia of national and regional forest seed programmes.

Morphological characterization of tree species field collections

Ex situ field genebanks maintain sources of variation of functional traits for direct use in production through propagation and breeding programs. Morphological and biochemical characterization of 10 conserved accessions provide a wealth of trait-based knowledge. Such collections are established for tree species that are considered important for agroforestry or arboriculture production and include many fruit tree species. Most of these collections are field genebanks because in some cases, the 15 species are recalcitrant, meaning that seeds cannot be stored using conventional methods. More generally, field genebanks enable characterization. However, field genebanks are also costly to maintain (Dawson et al. 2013). Their existence can only be justified when genebank material can be accessed by users and meets their needs. The National Genetic Resources Program of the United 20 States Department of Agriculture, for example, holds collections of a list of tree species that are characterized (<http://www.ars-grin.gov/cgi-bin/npgs/html/croplist.pl>). They include many temperate fruit tree species, but also tropical ones such as peach palm (*Bactris gasipaes* Kunth), the pili-nut (*Canarium ovatum* Engl.) and carambola (*Averrhoa carambola* L.). Data on traits can be consulted freely online and germplasm can be requested accordingly.

There is no centralised information system for tree species collections, thus characterization 25 information, if collected, is generally not accessible, especially not of those in developing countries. Germplasm maintained in those collections that have been characterized, are often not accessible for potential users, and in this way genebanks lose their connection with users and their needs. An exercise with national research agricultural institutions from the Amazon, on the status of *ex situ* 30 conservation, characterization and evaluation demonstrated a high number of collections and promising advances in morphological characterization of prioritized local fruit tree species including acai (*Euterpe spp.*), peach palm, Cupuaçu (*Theobroma grandiflorum*), camu camu (*Myrciaria dubia*), bacuri (*Platonia insignis*), Buriti (*Mauritia flexuosa*), and abiu (*Pouteria caimito*) (Scheldeman et al. 2006).

Thus even though for only a limited number of tree species field *ex situ* collections exist, still a considerable number of these collections is already morphologically characterized. Unfortunately, 35 many of these collections are not known by a general public, and existing characterization data is difficult to access or not available for general public. To enhance the use of genebank characterization, such information should be systemized and be made accessible at a central point.

Application of Genetic principles in forest ecosystem restoration

Forest ecosystem restoration is of growing interest as a means to combat negative impacts and mitigate climate change associated with continued deforestation and degradation of forest 40 ecosystems worldwide. Very commonly however, little attention is paid in restoration initiatives to ensuring the use of appropriate sources of forest reproductive material. Bozzano et al (2013)

conducted a survey and analysis of the degree to which genetic considerations are recognized and applied in restoration projects, globally. They found that geographical origin of planting material is often considered, in that there is a common understanding that local sources are preferable, but there is little awareness of other genetic factors. Even when guidelines exist for numbers of source trees for provision of reproductive material, the guidelines are often inadequate.

Examples of knowledge gaps include: a quantification of the risks associated with genetic mismatching of source of planting material to site conditions or narrow genetic base, particularly considering climate change; thresholds for optimum level genetic diversity in restoration material; and genotype by environment interactions. It would also be valuable to understand the potential for combining species rescue with ecosystem restoration, i.e. the potential of individual restoration projects to contribute to species conservation and serve as future seed sources, especially for rare, endemic and endangered tree species.

Species selection and availability of reproductive material is another issue of concern. Exotic species or seed sources are very commonly used for restoration and in some cases it is clearly justified. In other cases however, this can not only result in wasted efforts, but can threaten native species if the exotic ones become invasive. Often the reason for using exotic species is simply local availability in nurseries and level of knowledge about nursery production requirements. There is a need to expand knowledge of native tree species to understand their potential to achieve diverse restoration objectives in different states of site degradation and ecological and socio-economic contexts including understanding the ecological and socio-economic trade-offs related to the use of exotic versus native species, and the factors that currently constrain wider use of native species.

5.8 Conclusions and recommendations

All countries reported an inadequate public awareness of FGR – thus an impediment to generating new knowledge to alleviate the woeful information gaps, is lack of general understanding of its importance.

1. Knowledge of FGR is reported to be inadequate for well-informed policy or management, in most countries.
2. Among the 80 – 100,000 tree species, a small proportion has been studied to describe genetic parameters (less than 1%) although both the number of studies and the number of species studied has increased significantly in the last decade.
3. Most studies conducted during the past two decades are at the molecular level, either using DNA markers or genomic technologies to characterise genetic resources; the quantity of information at the molecular level is accumulating much faster than whole-organism information, with the consequence that little of the accumulating knowledge has direct application in management, improvement or conservation.
4. A few species are very well studied and genetically characterised, based on both molecular and quantitative studies. These are mainly temperate conifers, Eucalyptus, several Acacias, teak, and a few other broadly adapted, widely planted and rapidly growing species.
5. Quantitative genetic knowledge has led to significant productivity gains in a small number of tree species that have high value as plantation timber.

- 5 6. Genomic knowledge of forest trees lags behind that of model herbaceous species, including the important agricultural crops, but the entire genome of several tree species has been or is in the process of being sequenced and novel approaches have been developed to link markers to important traits. Genomic or marker assisted selection is close to being realised but phenotyping and data management are the biggest bottlenecks.
- 10 7. Progeny and provenance trials established for many species in many countries were abandoned as funding and interest switched to molecular approaches. Existing but dispersed data should be assembled, maintained and evaluated for potential to inform seed zone delineation, plans for assisting gene flow in response to climate change, identification of propagation material for restoration and conservation efforts for high-value populations.
8. Many of the species identified as priorities, especially for local use, have received little or no research attention, indicating a need for associating funding to priority setting exercises. This should be a starting point for expanding knowledge, particularly about genetic control of and variation in valuable traits.

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