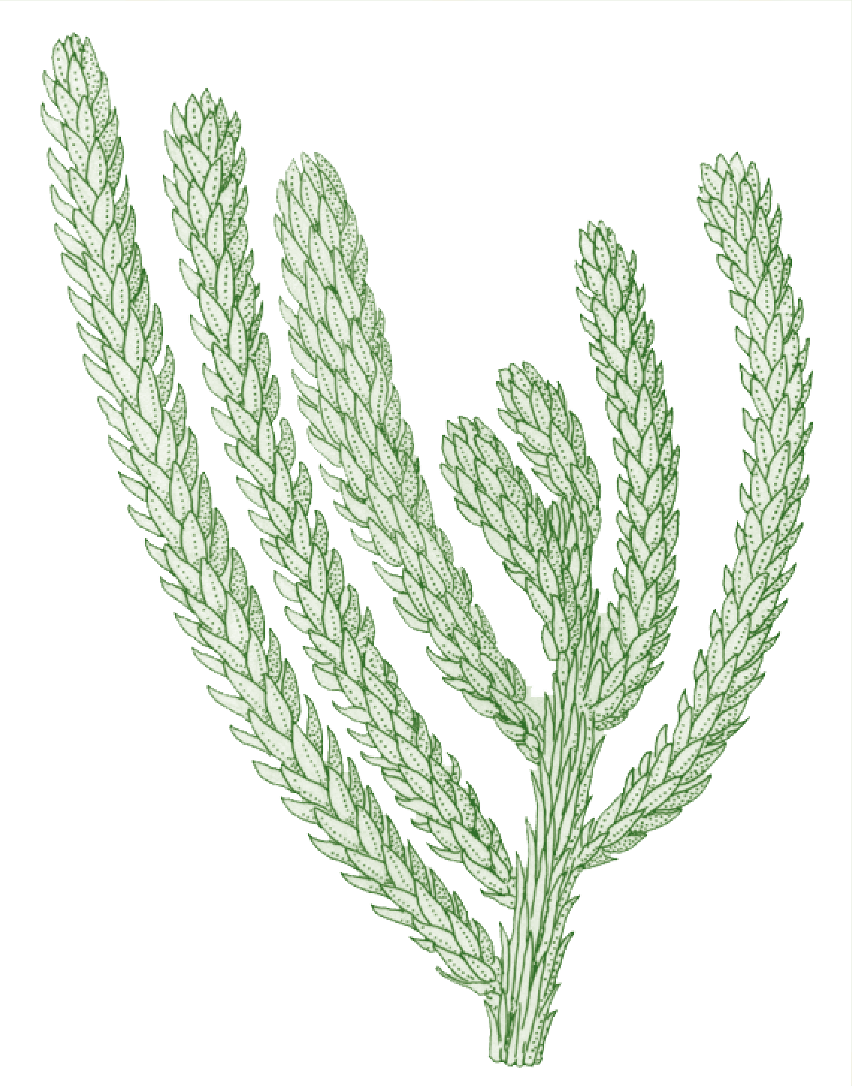
Bioversity International

MODULE 3
Tree seed supply chains

Teacher's notes 3.1

Genetic bottlenecks in the restoration of *Araucaria nemorosa*

David Boshier



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Genetic bottlenecks in the restoration of *Araucaria nemorosa*

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Introduction

These Teacher's notes aim to assist teachers in using the **Case Study 3.1 Genetic bottlenecks in the restoration of *Araucaria nemorosa*** in classes. The notes:

- describe the key concepts covered in the case study, with references to forest genetic resources textbooks or papers where explanations of these principles can be found (full references at the end of these notes).
- give tips on how to prepare and run the exercise and discuss the main learning points (genetic and other) that students should be able to derive from the case study.
- give an outline commentary to the PowerPoint presentation which is used to introduce the case study to the students. The presentation contains pictures of the species, sites where it occurs, relevant land-use issues in the area, and figures/tables from the exercise.

The following support materials can be found on the accompanying DVD or at the Forest Genetic Resources Training Guide webpage at www.bioversityinternational.org

- Teacher's PowerPoint presentation
- Short video
- The Case study.

Key concepts to cover/introduce in this Case Study

The exercise, as described in the case study, explores the following issues, with respect to how the seed/seedling supply chain can affect the genetic diversity in species.

General conservation

- **Reproductive materials - source and collection:** see FAO et al. (2004a) pp. 47; Geburek & Turok (2005) pp. 569-570.
- **Conservation of species and genotypes:** see FAO et al. (2004a) pp. 11-14; FAO et al. (2001) pp. 7-10; FAO et al. (2004b) pp. 3-4; Finkeldey (2005) pp. 183-185; Geburek & Turok (2005) pp. 304-306, 391-399, 545-554.
- **Fragmentation, connectivity – gene flow and maintenance of viable populations:** see FAO et al. (2004a) pp. 43-44; FAO et al. (2001) pp. 45-47, 64; Boshier et al. (2004); Finkeldey (2005) pp. 93, 177-178; Geburek & Turok (2005) pp. 426-432, 440-443.
- ***In situ*, *ex situ* conservation and habitat restoration:** see FAO et al. (2004a) pp. 5-16, 33; FAO et al. (2001); FAO et al. (2004b); Finkeldey (2005) pp. 181-198; Geburek & Turok (2005) pp. 6-8, 535-562, 567-581.

Genetic concepts

- **Genetic processes associated with small populations - increased genetic drift, bottleneck, increased inbreeding:** see FAO et al. (2004a) pp. 43-44; Finkeldey (2005) pp. 75-76; Geburek & Turok (2005) pp. 426-432.
- **Effective population size compared to census size:** see FAO et al. (2004a) pp. 43-44; FAO et al. (2001) pp. 7, 10, 61; FAO et al. (2004b) pp. 10-12; Finkeldey (2005) pp. 177, 181-198; Geburek & Turok (2005) pp. 162-164, 420-431.
- **Population differentiation Gst/Fst:** see FAO et al. (2004a) pp. 41; Finkeldey (2005) pp. 111-115; Geburek & Turok (2005) pp. 264-266.

How to run the exercise

The exercise can be run in a number of ways depending on the time available and size of the class. The exercise works best if students work in groups of 4-5 (no more than six). It is best if the students have already read the case study before they start the exercise. *This way valuable class time is not lost with students reading the paper during the class.* So give the case study out in a prior class with instructions to read it before the next class. It perhaps goes without saying that it is vital that the teacher and any assistants are fully familiar with the whole text. NB: the exercise is set in the context of the mid 2000s, both in terms of the species status and the social context. Therefore, more recent information and changed status are not included as they are not relevant to the exercise.

Ideal number of students: 4-15

Ideal length of class: 3 hours, broken down as follows:

- ***Introduction***: use the video followed by the PowerPoint - *approx 30 minutes*.
- ***Group work***: suits 1-3 groups of 4-5. Each group devises an action plan. Each group tends to take a different approach and different issues are raised, such that overall, most points are covered. Students discuss the case study amongst themselves, responding to the specific points and developing their plan. The teacher should be around to answer any queries the groups have. However, it is not essential that all of the time is spent with the whole class together with the teacher. Once the teacher and groups are happy they understand the assignment and issues, each group could meet, discuss and prepare the map and strategy outside of class time – *1.5 hours*.
- ***Presentations***: each group presents its map and strategy verbally to the class (supported by the map written on large paper or in a PowerPoint presentation) – *10 minutes* per presentation, with *5 minutes* after each presentation for questions/comments by the rest of the class and teacher; *15-45 minutes,* depending on the number of groups.
- ***Final discussion***: led by the teacher allowing them to make general comments about what was good, what was missed, etc. – *10 minutes.*

Background information

Depending on the time and facilities available you can use any combination of the following resources: i) video on loss of forests in New Caledonia to present the general context of the island; ii) PowerPoint presentation.

***Video*:** this lasts seven minutes and is entitled: '*The world's most endangered forests*'. It is produced by Prof. Tom Gillespie (University of California) and National Geographic, and gives a brief view of New Caledonia, its culture, the importance of its forests in terms of world biodiversity and the threats.

PowerPoint**:** about 20 minutes to go through. It covers general concepts of Araucarias in New Caledonia, and in particular *A. nemorosa*. It also allows explanation of some specific issues raised in the case study, in particular understanding the data from the genetic studies.

Slide 1: title – shows the focus of the case study and the study species.

Slide 2: shows the location of New Caledonia in the world.

Slide 3: shows the image of New Caledonia as it portrays itself to the outside world. Emphasis is on beach tourism. But New Caledonia is a centre for biodiversity, in particular conifers. The images show stands of *Araucaria columnaris*, one of 13 Araucaria, all of which are endemic to the island.

Slide 4: contrasts the tourist image of the island – a tropical paradise with the reality of important forest ecosystems that have been and continue to be destroyed by mining and fires.

Slide 5: shows three of the Araucaria species, each of which has a different conservation status, in terms of IUCN categories.

Slides 6: shows the study species, its actual distribution (Figure 1 in the case study), its conservation status (IUCN category) and the main features of its reproductive biology.

Slide 7: shows an aerial view of the coastal part of the study species distribution. The remnant forests, which contain populations N1-3, N5, and N7, are visible. The inland population N6 (Forêt Nord) is not visible.

Slide 8: shows trees of the Port Boise populations and the main author of the study (Chris Kettle) with cutting pole.

Slide 9: shows the small Forêt Nord population before and after establishment of the Vale Inco nickel processing plant.

Slide 10: shows the Vale Inco mining company's nursery at Goro, which is the focus of their efforts to restore native vegetation after mining operations. Within this restoration, propagation of native species (including *A. nemorosa*) is emphasized.

Slide 11: summarizes what the objectives of the research were and shows plants of *A. nemorosa* in the nursery of IAC (Institut Agronomique Néo-Calédonien).

Slide 12: shows a mature cone of *A. nemorosa* and what fertile and infertile seeds look like.

Slide 13: Table 1 from the case study. The text related to Table 1 in the case study points out that cone production in all six populations was very low with a high proportion of adult trees bearing no mature female cones. Suggest that the groups should be thinking about what this variation among trees in female cone production means for genetic diversity. NB: how much you explain will depend on how much the groups are capable of understanding from their direct reading.

Slide 14: covers the issue of population size. Ask the groups to relate these to population sizes in Table 1 (case study) and to think about what this means for their viability. Also, how overlapping generations mean that effective population sizes of these remnants will be lower than the census number.

Slide 15: Table 2 from the case study – shows genetic diversity measures for three different cohorts (adults, wild and nursery seedlings) from the two populations at the centre of restoration efforts, as well as adult trees and wild seedlings for four other populations. NB: how much you explain will depend on how much the group is capable of understanding from their direct reading. Ask them to focus on where there are differences between the cohorts within populations for the five variables shown in the table. Also say that the text mentions that adult and wild seedling cohorts are genetically similar (i.e. low Fst values; see references on page 1 for detailed explanations of Fst), whereas the significantly high Fst values show the adult and nursery seedling cohorts, and wild and nursery seedling cohorts to be genetically dissimilar.

Slide 16: theoretical genetic impacts through human disturbance and fragmentation of forest. On the left of the slide, the direct impacts that are observable; on the right the expected genetic effects that follow different human impacts on tree populations. The study provides information on the first and third of these – ask them to think in their groups about what the study shows about the impacts on these.

Slide 17: allows explanation of the basic idea of a genetic bottleneck, where alleles are lost through sampling, but also through the increased impact of genetic drift.

Slides 18-22: allow the teacher to go over what the students should be doing in the exercise.

Slide 18: emphasizes the two parts of the exercise. The objective is given in the first paragraph of the case study and you can point out that it is relatively narrow in scope. Thus, proposed actions need to be within the context of on-going efforts, rather than entirely new initiatives.

Slide 19: gives the specifics of what is expected in step 1 - specifically what the map should consist of. Typically, students are good at identifying the various stages and actors in the current chain, but are often poor at identifying the genetic implications for each step, e.g. where bottlenecks are occurring. It is therefore worth emphasizing that they remember to identify these in their maps.

Slide 20: gives an example of a seed supply chain map from a similar exercise - you should stress that their map does not have to look like this. You can, however, go through it and point out the main issues, including the fact that in this case the group failed to identify the genetic issues associated with each step. This re-emphasizes the central part of the exercise.

Slide 21: gives the specifics of what is expected in step 2. This allows the teacher to emphasize the need to identify genetic risks (e.g. bottlenecks, inbreeding, rather than just loss of genetic diversity), and for recommendations to be specific within the current situation.

Slide 22: example of the second chart summarizing genetic impacts and recommendations for actions. This can be used to illustrate that the recommendations in this example are very general (e.g. technical support) and that the groups need to be more specific and practical, so that someone could see what they would actually do to improve the situation.

Important points to draw out in discussion and to cover in students' presentations

Comments about the questions

- *How is human disturbance influencing genetic diversity in A. nemorosa?*

Human disturbance has undoubtedly reduced population sizes, which leads to losses of genetic diversity. On-going restoration is also having an influence, with both nursery populations show evidence of being genetically compromised, but in different ways. The nursery stock derived from Kaanua is genetically impoverished (lower A_e, H_e, % rare alleles). The nursery stock derived from Forêt Nord does not suffer from low genetic diversity (A, W, N sources show similar values, Table 2), but is inbred (higher inbreeding coefficient F_{IS}).

- *What are the mating system, and seed and pollen dispersal mechanisms? What do these mean for conservation?*

Seed is gravity and wind dispersed. Pollen is wind dispersed. Trees are hermaphrodite (monoecious – male/female cones) so selfing can occur, and there is no self-incompatibility mechanism to restrict its occurrence. Inbreeding does occur naturally as is evident from the inbreeding coefficients of wild seedlings (Table 2). The lower inbreeding coefficient for wild seedlings compared to adult trees suggests that inbred material is normally selected against during regeneration. The implications for conservation are that increases in inbreeding should be avoided as they will reduce the quantity and viability of regeneration.

What are the threats to *A. nemorosa* (short-term/long-term)? List problems by type

Genetic

- *Population size*

Three of the populations are below 500 (Forêt Nord - N6, Mini Nuri - N5, Natasha's - N7, Table 1) and clearly too small to be viable in the long term. Effective population sizes (Ne) will be much lower, given the proportion of trees at reproductive size, the overlapping generations and unequal representation of seed trees in regeneration. The latter is also shown by low Ne values in Table 2.

- *Fertility/seed set*

The reproductive ecology survey suggests that only a small proportion of potentially sexually mature trees contribute to seed production in any one year and that this affects the diversity captured during collecting for nursery stock. Female cone production and seed set are very low and also show high variability between trees (Table 1). Although the data are based on one season, even if masting (mass coning) years occur, for restoration programmes there is rarely the luxury of waiting for such an exceptional cone production year to collect seed. Collections are undertaken when budgets are available for field work and growing space.

The low level of seed set may be due to pollen limitation. A more likely explanation is embryo abortion due to inbreeding depression. Severe inbreeding depression at the embryo stage is thought to be common in conifers, where controlled pollinations suggest that expression of deleterious recessives due to selfing is responsible for 80–96% of empty seeds. Availability of viable outcrossed pollen may be reduced where there is increased spatial isolation between fertile trees. Degradation and exploitation of the populations can thus lead to increased levels of selfing and reduced seed set.

An additional source of low seed set seen in other conifers is pollination by related species swamping reproductive structures with hetero-specific pollen and resulting in empty seeds. This is of potential relevance for *A. nemorosa* whose remnant populations are sympatric with very dense populations of the more widespread *A. columnaris*, which are likely to contribute significantly to the pollen cloud.

■ *Bottleneck*

The study showed that nursery seedlings from Kaanua (N1) are more genetically impoverished than their wild relatives despite being established from seed collected from the largest and one of the most genetically variable remnant populations. Allelic richness was dramatically lower in this nursery lot with loss of many rare alleles and an associated very low estimate of effective population size (Ne). These nursery seedlings captured <30% of the allelic diversity present in the source population, with one locus being monomorphic. By contrast, the nursery lot from Forêt Nord more effectively captured the available variation from its source. Although the Forêt Nord population is the most genetically impoverished, the nursery stock has more than twice the allelic richness of the seed lot from Kaanua (N1). One tree contributed over 50% of the total seed collection. This demonstrates dramatically how a cryptic genetic bottleneck can occur due to high variation in fertility.

■ *Inbreeding*

Wild adult and seedling populations of *A. nemorosa* exhibit some level of inbreeding, as indicated by significant positive inbreeding coefficients (F_{IS}), with the seedlings significantly more inbred than the adults. Nursery seedlings from Kaanua however, did not show significant inbreeding coefficients, but a slight heterozygous excess. An explanation for this result is that the seedlings are the offspring of very few outcrossed parents (e.g. the result is representative of a small number of events). This is consistent with these nursery seedlings being derived from a very small number of trees. In contrast, the nursery lot from Forêt Nord has a significant positive inbreeding coefficient, which is >50% higher than the wild seedlings and the adults in the same population. An explanation is that inbred progeny are under greater selection in natural forest conditions than in the comparatively benign nursery conditions (some inbred individuals survive in the nursery that would die in the wild).

Other types of problems

■ *Social, communication, resources*

Populations of *A. nemorosa* are subject to considerable threats due to habitat degradation related to increased fire frequency, introduction of mammals, logging and lack of controls on mining activities. Seventy per cent of the remaining stands of *A. nemorosa* (N 1-6) are on the land of one family. Although they are reported to be interested in the welfare of the populations and have a good working relationship with the Service de l'Environment, the future of a large proportion of the species is at the whim of one family.

■ *Policy, law and awareness*

The absence of a clearly articulated environmental policy, lack of effective management for protected areas and poor enforcement of legislation are major obstacles to effective long-term conservation. There is a lack of recognition of conservation issues/actions amongst the population, particularly with respect to terrestrial habitats. There is a need to control and inform landowners. The political status of New Caledonia means that international agencies will generally not fund conservation work there and only recently did France make the conservation needs of the island a priority.

Students' map and analysis should indicate:

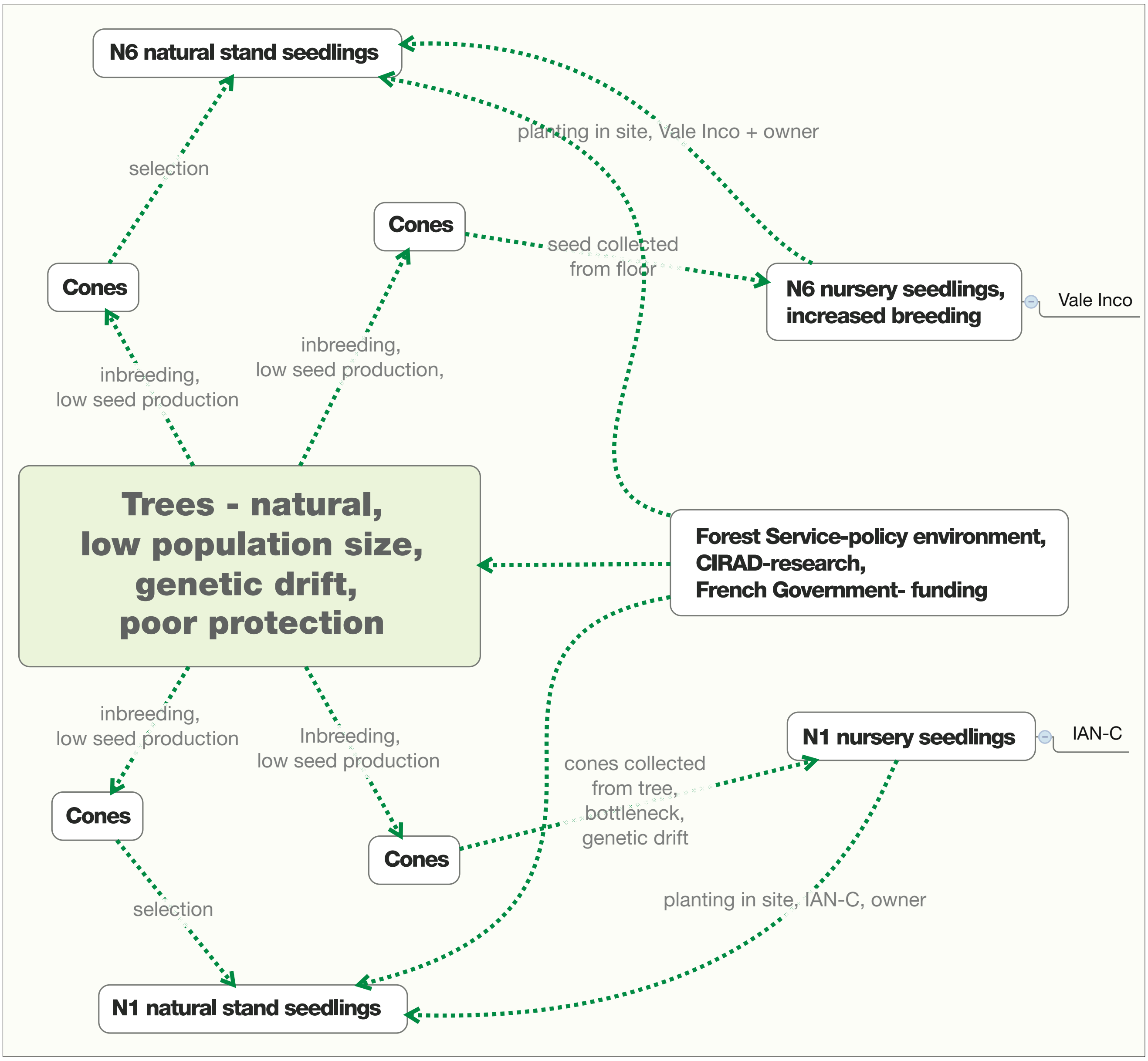
Step One - *A. nemorosa* seed/seedling supply chain mapping

1. Draw a map (flow-chart) of the seed/seedling supply chain, from the source to end-use in restoration (from mother tree to a new seedling at the end-use).

2. The map should identify:
   - Key actors (individuals, institutions) and processes in the *A. nemorosa*

chain (what actors do).
- Influences of the seed/seedling supply chain on its genetic variation (bottlenecks, selection, genetic drift).
- Social limits in the seed/seedling supply chain (e.g. policy/law, trade, institutional or capacity issues).

Clearly, there are many ways in which a map can be drawn up. The example below shows the main features that should be included in their maps, with the main actors, genetic influences and social limits identified.



Step Two - analysis

3. What are the genetic risks associated with the current seed system?

   Bottlenecks, inbreeding, increased genetic drift – all leading to reduced genetic diversity and increased inbreeding depression (reduced fertility and reduced vigour of trees).

4. Make specific recommendations to improve the situation, addressing diversity issues in the seed/seedling system (e.g. practical ways to collect seed that ensure genetic diversity in restoration).

There is a need to develop restoration strategies that will augment current population numbers, re-establish populations on former sites and prevent escalation of associated genetic problems in wild seedling cohorts. The conservation of *A. nemorosa* is to some extent dependent on establishment of nursery-reared seedlings. It is therefore vital that this stock is genetically robust. It is possible to make some common sense observations from the results. The most striking genetic problem was in nursery stock derived from cones collected from trees in Kaanua. Climbing trees is costly, time consuming and requires specialist equipment, limiting the potential to sample many trees. Furthermore, the high variability in cone production is likely to lead to sampling only trees with large numbers of cones, which coupled with high variability in seed viability, results in a severe cryptic bottleneck. Where direct cone collection is used for establishing nursery stock, tracking the progeny sampled from different trees would be a practical method of identifying cases where stock becomes dominated by a small number of families. Sampling over multiple years would also help to maximize the number of trees contributing to nursery stock

In contrast to the direct collection of cones, the nursery stock from Forêt Nord was collected directly from the forest floor. There was no evidence of a genetic bottleneck in this sample. Adopting this sampling approach is likely to result in a more comprehensive sample of seed from the entire population as it is easy to cover a wide area that contains seed dispersed from many trees. However, as this seed showed a higher level of inbreeding than in the wild seedling population, inferior stock in the nursery should be culled, or there will be high mortality in restoration plantings.

An alternative strategy for sourcing nursery stock is sampling established wild seedlings directly from the forest floor and rearing them on in nurseries. Wild seedlings in all six populations studied contained levels of variation comparable to the adult populations and are likely to be the product of a much larger effective population of adults than in a single season's seed. Although the wild seedling populations are generally more inbred than the wild adults (higher F_{IS}), these seedlings will already have been subject to some selection (Table 2), with some highly inbred seeds selected out. The fitness of established seedling populations is therefore expected to be higher than that of the seed population. As mortality levels in *A. nemorosa* seedlings are high, especially in old growth forests because of competition, collection of some of these seedlings may represent an efficient use of this material. This needs balancing against the risk of negatively affecting any natural regeneration that may occur, as well as the cost and practical challenge of translocating seedlings. Such considerations will place a limit on the sample sizes that can be collected in this way.

Further information

FAO, DFSC, IPGRI. 2001. Forest genetic resources conservation and management. Vol. 2: In managed natural forests and protected areas (*in situ*). International Plant Genetic Resources Institute, Rome, Italy.

FAO, FLD, IPGRI. 2004a. Forest genetic resources conservation and management. Vol. 1: Overview, concepts and some systematic approaches. International Plant Genetic Resources Institute, Rome, Italy.

FAO, FLD, IPGRI. 2004b. Forest genetic resources conservation and management. Vol. 3: In plantations and genebanks (*ex situ*). International Plant Genetic Resources Institute, Rome, Italy.

Finkeldey R. 2005. An Introduction to Tropical Forest Genetics. Institute of Forest Genetics and Forest Tree Breeding. Georg-August-University, Göttingen, Germany.

Geburek T, Turok J, editors. 2005. Conservation and Management of Forest Genetic Resources in Europe. Arbora Publishers, Zvolen, Slovakia.

Forest Genetic Resources Training Guide

Other modules to be published among the following:

Plantation forestry, Tree domestication, Forest restoration, Genetic modification