



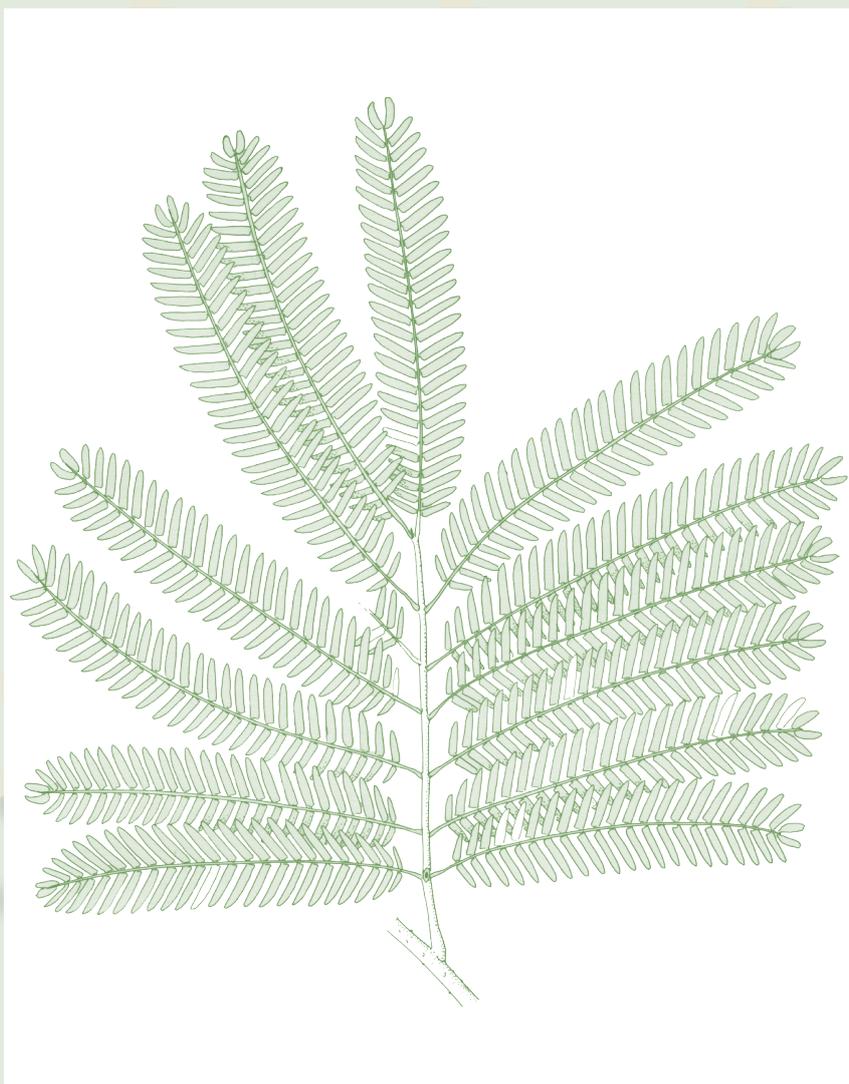
# MODULE 1

## Species conservation strategies

### Teacher's notes 1.1

# ***Leucaena salvadorensis*: genetic variation and conservation**

David Boshier



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## Module 1

### Species conservation strategies

#### Teacher's notes 1.1

### ***Leucaena salvadorensis*: genetic variation and conservation**

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#### Introduction

These Teacher's notes aim to assist teachers in using the **Case Study 1.1 *Leucaena salvadorensis*: genetic variation and conservation** in the classes. The notes:

- describe the key concepts covered in the case study, with references to forest genetic resources textbooks or papers where explanations 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](http://www.bioversityinternational.org)

- Teacher's PowerPoint presentation
- Video with general background to the genus *Leucaena* and the importance of species diversity
- The Case Study.

#### Key concepts to cover/introduce in this Case Study

##### General conservation

- ***In situ, ex situ* conservation:** 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, **and conservation through use on farms - *circa situm*:** Boshier et al. (2004).

##### Genetic concepts

- **50/500 rule and 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) 10-12; Finkeldey (2005) pp. 177, 181-198; Geburek & Turok (2005) pp.162-164, 420-431.
- **Genetic processes associated with small populations – increased genetic drift, bottlenecks, increased inbreeding and consequently homozygosity:** see FAO et al. (2004a) pp. 43-44; Finkeldey (2005) pp. 75-76.
- **Self-incompatibility mechanisms:** see Finkeldey (2005) pp. 91-93; Geburek & Turok (2005) pp. 177-180, 428.

- **One migrant per generation rule -  $Nm > 1$**  (see Geburek & Turok 2005 pp 203, 442).

## 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 previous 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 1990s, both in terms of the species status and the country profiles; recent information and changed contexts are **not** included as they are not relevant to the exercise.

Ideal number of students: 12-20.

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

- **Introduction:** use the video followed by the PowerPoint – *approx 30 minutes.*
- **Group work:** suits 2-4 groups of 4-5. Each group devises a strategy for a different country and one works as an international conservation organization. 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 strategy. 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 strategy outside of class time – *1.5 hours.*
- **Presentations:** each group presents its strategy verbally to the class (supported by main points written on large paper or on a **PowerPoint** presentation) – *10 minutes per presentation*, with 5 minutes after each presentation for questions/comments by the rest of the class and teacher.
- **Final discussion:** led by the teacher allowing them to make general comments about what was good, what was missed, etc. – *10 minutes.*

## Background information

**Video:** this lasts 18.5 minutes and gives a general background to the genus *Leucaena* and the importance of using a diversity of species. *L. salvadorensis* is specifically mentioned and it shows the conditions under which the species occurs.

**PowerPoint:** about 20 minutes to go through. This re-emphasizes some of the points from the video, but also allows explanation of some specific issues raised in the case study.

*Slide 2* – map and table from exercise showing species distribution and population sizes (also *slides 9-10*).

*Slides 3 to 5* – photos of specific *L. salvadorensis* populations – they illustrate the lack of forest in the landscape and the maintenance of trees on farms by farmers. The Nueva Esparta population consists of only 16 trees found on one farmer's land. Of these, three are old trees, the rest much younger suggesting much lower effective population size.

Slides 6 to 7 – show how self-incompatibility leads to low pod production in *L. salvadorensis*, compared to the self-compatible *L. leucocephala* and consequent weediness issues for the latter.

Slide 8 – shows the genetic basis for the self-incompatibility (SI) mechanism in *L. salvadorensis*. The teacher can explain that the system (gametophytic SI) depends on SI alleles. Large numbers of SI alleles are present in most populations, so most crosses are compatible and produce seed (NB: *this is optional depending on whether the class is capable of dealing with this level of complexity*).

Slide 9 – covers alternative objectives for conservation – the teacher must stress the need for students to first define an objective for their strategy. It is very common for students to not define a strategy or to forget to explain what it is. Without this, it is impossible to judge the efficacy of a conservation strategy. Objectives should be pertinent and realistic and avoid the “operation was a success, but the patient died” syndrome i.e. we carried out all activities successfully, but it did nothing to help.

Slides 10 to 11 – cover the issue of population size. Relate these to the population sizes in Table 1 (Case Study) and how overlapping generations mean that the effective population sizes of these remnants will be lower than the census number.

Slides 12 to 15 – summarise different approaches to conservation and associated problems. The emphasis should be on their complementary nature, rather than *either/or*. The emphasis will, however, shift depending on the characteristics of the species and the population of concern.

Slides 16 to 18 – introduce the idea and debate around whether trees found in agricultural landscapes may be important for conservation of some species (sometimes known as *circa situm* conservation) and the negative view that they are not (for more detail see introduction to Unit 2: Trees Outside of Forests).

Slides 19 to 20 – allow the teacher to present the concept of common/rare and widespread/localized alleles (see Box 1 below).

Slide 21 – allows the teacher to explain what the dendrogram in the exercise shows, i.e. which populations are more closely related genetically to each other. You can point to the idea that this can help in prioritising which populations to conserve.

Slide 22 – allows the teacher to explain the significance of Table 4 and Figure 3 for the exercise, i.e. the geographic distances between each of the populations, how small they are in most cases and that the overall distribution only covers 160 km. Figure 4 uses the data from Table 3 and shows that gene flow decreases with distance between the populations. The concept of one migrant per generation ( $Nm > 1$ ) being sufficient to prevent population differentiation can be introduced and used to help decide conservation priorities.

Slides 23 to 24 – allow the teacher to go over what the students should do in the exercise. The teacher should stress: a) the need to be specific in what the strategy includes – students tend to be too general in their recommendations; b) the need to prioritise – students tend to recommend doing everything, failing to recognise that resources for actions are extremely limited; c) they should indicate what information/evidence they have used to justify each recommended action; d) they need to present a convincing case that would sway a donor/government to give them funds and/or enact policy/legislation to conserve the species.

## Important points to draw out in discussion and to cover in students' strategies

### Comments about the questions

Think about:

- *How has human disturbance shaped the genetics of *L. salvadorensis*?*

From the text, students should be able to identify that there is no evidence that the overall distribution of the species was much larger than is currently shown in Figure 1. However it is now fragmented due to human intervention and population sizes are greatly reduced.

- *What is the mating system, seed and pollen dispersal mechanism?*

See Phenology section, slides 6-7.

- *What are the levels of genetic variation and how are the alleles distributed across populations? List the localized but common alleles?*

See Box 1.

- *Are the provenance regions suggested in the map valid - which populations are different?*

Clearly the provenance regions in the map are not valid. The *Distribution* section indicates that the original provenance regions shown in the map were defined on the basis of principal watersheds, with the exception of the western and eastern extremes, where the Honduras/El Salvador and Honduras/Nicaragua boundaries were used respectively. The dendrogram (Fig. 2) is the easiest way to see which populations are genetically similar or different. The dendrogram shows that the populations can be grouped into 3-5 provenance regions. This allows the students to think about which populations should be conserved i.e. it is not worth putting scarce resources into conserving two populations that are essentially genetically the same and contain the same alleles (e.g. La Garita and Rio Nacaome). Nueva Esparta (El Salvador) groups genetically with San Antonio (Honduras) i.e. pollen flow does not respect political boundaries (see also FAO et al. (2004a) p. 31).

- *Original collectors grouped the Calaire and Charco Verde sites together as one provenance - is this valid?*

Not from the genetic data – Charco Verde groups genetically with La Garita and Rio Nacaome. However, the small size of Charco Verde (79 trees) and in particular, the small number of trees (six; Table 1) from which the seeds were sampled mean the relationship will be highly influenced by sampling/drift effects. It would therefore be unwise to regard them as genetically distinct and group them together as they are only 11 km apart and from climatically highly similar areas.

- *The ability to draw definitive conclusions from the genetic data is limited by small sample sizes from some populations, although the use of seed means that more than just the seed trees within each population were sampled.*

Ecological information, such as the rainfall and altitude in Table 1, can also aid genetic conservation by indicating populations likely to experience similar/different environmental selection pressures and therefore be genetically distinct. In this case the information in Table 1 suggests similar relationships to those shown in Figure 2, with only differentiation between La Galera, Calaire and Charco Verde not apparent.

### List problems by type

#### Genetic

- *Which populations are too small?*

**Box 1** Common/rare and widespread/localized alleles

In an attempt to define genetic sampling/conservation priorities and 'useful variation', Marshall and Brown (1975) defined four conceptual classes of alleles based on allele frequency and distribution within and between populations. Each allele is classified into whether it is rare (frequency of <0.05 or <0.10), in contrast to when it is common and exceeds that frequency at least once. These two classes are then divided into two subclasses based on their geographic distribution, recognising whether an allele occurs in many populations (widespread) or in only one or a few (localized). This gives the following matrix:

	Widespread	Localized
Common	easy to collect	priority
Rare (<0.05)	sample size dependent	chance

Collecting/conserving the 'common-widespread' class, which presumably includes broadly adapted alleles, presents no problem as they are inevitably collected regardless of the strategy. Conservation of the 'rare-widespread' alleles will depend on the total collecting effort (i.e. sample size) and not how that sample is deployed across populations. Marshall and Brown (1975) argued that 'common-local' alleles merit priority in conservation strategies as they presumably include alleles that confer specific adaptation to local conditions. Specific alleles in the 'rare-localized' group are extremely difficult to collect (i.e. chance), and include variants that are very rare in the whole species (e.g. recent or deleterious mutants). A fraction of this class will be included, but conservation of every existing specific 'rare-localized' allele will always be beyond the available resources.

Using the concepts of allelic richness (frequency is common >0.05, or rare <0.05) and allelic evenness (occurrence is widespread >0.25 populations or local <0.25 populations), students should be able to identify the following alleles (see Table 2) as being:

- Localized but common e.g. *Pgm-2b* Calaire, *Pgi-3b* La Galera, *Pgd-1b* San Juan Limay, La Garita.
- Localized and rare e.g. *Pgm-1a*, *Pgi-3e*, *Idh-2d*.
- Widespread and common e.g. *Pgm-1b&c*, *Pgm-2a&c*, *Pgi-2a,b,c*, *Pgi-3d*, *Pgd-1a&c*, *Idh-1a,b,c*, *Idh 2a,b,c*.
- Widespread but rare e.g. *Idh-1d*.
- *Pgi-3a* and *Pgi-3c* may be thought of as localized but common, but their occurrence is roughly in half the populations and hard to categorise.

There may be recognition that the limit of what is rare and common is arbitrary (generally set at 0.05 or 0.1). So *Pgm-1a* with 0.085 frequency at Rio Nacaome and absent from all other populations would probably be best thought of as localized and rare.

Most populations are too small – only three (Calaire, La Garita, San Juan de Limay) are at or above 500. Effective population sizes are likely to be much lower due to overlapping generations (e.g. Nueva Esparta is mentioned as having only 3 large trees in the remaining 16 trees). The self-incompatibility (SI) mechanism in *L. salvadorensis* is also likely to reduce effective population sizes; in small populations, SI alleles will be lost through drift, as with other alleles. With fewer SI alleles in a population, more crosses will be incompatible and fail to produce seed, while any tree with a unique SI allele will have a reproductive advantage (being compatible with most other trees) and therefore dominate reproduction, reducing the effective population size and genetic diversity of any regeneration.

### Others

■ *What are the threats to L. salvadorensis (short-term and long-term)?*

Short-term threats are mainly deforestation, a lack of knowledge about *L. salvadorensis* and preferential planting of *L. leucocephala* in reforestation programmes within the natural distribution of *L. salvadorensis*. Long-term, the small population size of almost all populations threatens the species in terms of both stochastic threats and evolutionary adaptability.

■ *For which populations is action a priority and of what type should this be?*

Priority should be to conserve those populations that have the greatest chances of viability (genetically and in the social context), that together cover the range of genetic diversity within the species.

■ *Which conservation methods - in situ, ex situ, on-farm (circa situm)?*

See *General points* below.

■ *What are the limiting social factors to conservation, and planting?*

Human pressures on remaining forests and trees. The lack of knowledge about *L. salvadorensis* and the availability of seed/plants of the species for those working in tree planting.

■ *What do end-users need to know and how will you communicate that?*

Those involved in tree planting and land management within the species' natural distribution (e.g. NGOs) need to know about the differences between *L. salvadorensis* and *L. leucocephala*. The communication strategy requires production of targeted information (e.g. posters/leaflets).

■ *Who will do, what and where?*

(See *General and Specific points* below).

■ *How will you pay for it?*

It is vital students understand that resources for conservation are limited and therefore require a prioritization of actions depending on what is available. The main actions outlined below are limited, but feasible. Seed collection would require additional funds, while communication of the importance and advantages of *L. salvadorensis* requires a modest budget that would also benefit from redirection of existing resources.

### Students' strategies should indicate:

#### General points

Most populations are too small. Only three (Calaire, La Garita, San Juan de Limay) are at or above 500. Effective population sizes are likely to be much lower due to overlapping generations (and loss of SI alleles).

Conservation efforts need to be targeted at where scarce resources can be most effective. It is not practical, nor cost-effective to recommend activities for all populations.

Strict *in situ* conservation options are very limited. Only in Nicaragua is the remaining population associated with remnant forest. All the other populations occur as trees within human modified agro-ecosystems (i.e. farmers' fields), such that a combination of on-farm conservation (*circa situm*) and *ex situ* actions will be the most effective.

#### El Salvador

There is only one population. This is at Nueva Esparta and the population is far too small. Table 1 shows 16 trees and effective population size is likely to be much smaller (nearer to three - the number of large trees, see *Seed collection* section).

There is evidence of a genetic bottleneck effect (from small population size) with fixation of some alleles (e.g. *Pgm-1b*, *Pgd-1c*, Table 2).

Seed collection from the remaining Nueva Esparta trees may also lead to the use of inbred material.

The San Antonio population in Honduras is the closest in distance (18 km, Table 4) and genetically (Fig. 2). The value of  $N_m$  (3.6, Table 4) indicates historically extensive gene flow between the two populations.

Conservation action must increase both the size of the population, and also genetic diversity. San Antonio is the most closely related (see above) and therefore the best place from which to bring seed to restore the genetic diversity of the Salvador population. Typically in the class exercise, the Salvador group recommends this action but fails to discuss the possibility of seed exchange with the Honduran group during the exercise!

The list of endangered species in the El Salvador country profile does not include *L. salvadorensis*, indicating an information gap that the conservation strategy needs to address.

The students often show great concern/enthusiasm for conserving the remaining trees at Nueva Esparta. However, although these are the only trees left in El Salvador there is no evidence they are genetically distinct/unique i.e. losing them will not lose anything that cannot be replaced. Resources would be better spent in bringing in closely related material with a broader genetic base that gives greater prospects for long-term population viability. There is also a high priority for addressing the lack of knowledge about the species within the country (both policy level and local level). A conservation strategy should link to local community reforestation efforts to ensure *L. salvadorensis* is planted in preference to *L. leucocephala*. This should involve actions to make *L. salvadorensis* seed/plants easily available (e.g. use of some of the imported seed to plant an *ex situ* stand that can be used as a seed stand within 2-3 years).

### **Honduras**

Honduras contains the most populations (six) of the species and the widest range of diversity.

Only Calaire and La Garita populations have more than 500 trees (Table 1), the others being currently too small for long-term viability.

There is evidence of genetic bottlenecks (from small population sizes) with fixation of a range of alleles (e.g. La Galera *Pgi-2c*, *Idh-1b*, San Antonio *Pgi-3c*, Rio Nacaome *Pgm-2c*, Charco Verde *Pgi-3d*; Table 2).

Concentrating conservation efforts on La Galera, La Garita and Calaire will ensure conservation of genetically distinct populations which are the most viable in terms of population size and contain between them all the common but localized alleles.

As the seed can remain viable for many years, seed collection from the La Galera, La Garita and Calaire populations and its storage in a seed bank would ensure *ex situ* conservation of the majority of the genetic diversity within Honduras in case the remaining populations are destroyed *in situ*. The strategy should specify details of the seed collection e.g. within each of the La Galera, La Garita and Calaire populations, collect equal quantities of seed from at least 20 trees separated by 100 m.

### **Nicaragua**

There is only one population, but it is the largest of the species (>1000, Table 1). There is therefore no evidence of a genetic bottleneck effect (from small population size) with no fixed alleles for the loci studied (SJL, Table 2). This is the most viable of the populations. Conservation actions could be relatively small, given that the population is large and there is no evidence of any immediate threat to it. Some form of monitoring is required to check that the population is being maintained and not decreasing in size. A seed collection to conserve the population *ex situ* (as for Honduras) would be worthwhile to avoid loss of the genetic resources in the face of any catastrophic destruction of the population *in situ*.

The low priority accorded the species (see Country notes) within Nicaragua suggests that resources for conservation will be limited and one should therefore make use of existing tree planting initiatives.

### **International**

International organisations aim to support activities that will lead to conservation of globally threatened species. From the information provided on FAO, it is unlikely that a large amount of money would be forthcoming through the Field Programme, given its lack of action in the dry regions of Latin America. It is more likely that a small grant (note the mean, maximum and minimum figures) would be assigned from the Regular Programme. Funds are typically limited and therefore they must be assigned where they have the greatest chance of maximum impact, rather than the politically easy option of being equally shared amongst countries. Therefore, there is no justification for allocating any funds to El Salvador, the population there being too small to be viable, nor does it contain any unique genetic diversity. The priority should be in aiding the conservation of the genetically distinct populations in Honduras and Nicaragua. The most cost-effective support is likely to be in the form of financial aid for *ex situ* seed collections and storage (see Honduras and Nicaragua country strategies). A higher proportion of funds should go to Honduras where there are more populations (three in Honduras, one in Nicaragua) to collect from. The group could also promote the exchange of seed between the countries so that the seed is held in more than one seed bank to help ensure conservation should one bank fail.

Typically, the international group recommends actions for each country but fails to discuss the proposals with the other country groups during the exercise. Something that is perhaps not atypical in the real world! If this group does indeed fail to discuss with the other groups, the teacher can use this in the final discussion as an example of the importance of involving all stakeholders (and effective communication between them) in drawing up and implementing realistic conservation efforts.

### **Follow-up**

If the teacher finds that one or more group has recommended every conceivable action as part of their strategy one can do a follow-up short exercise with the aim of getting them to reconsider and prioritize their proposed actions. Say to the groups that they will have only US\$10 000 to implement their strategy and ask them to indicate which of their proposed activities they would fund with those resources. Usually this quickly results in groups seeing the reality of what is normally available and many proposed actions are eliminated as superfluous. Examples of approximate costs of possible activities are given below. NB: these are not the only options and others could be added.

Activity	Cost US\$
Seed collection per population	1000
Establish <i>ex-situ</i> conservation stand, per population	500
Provenance trial, per site	2000
Maintenance of conservation stand or trial for first 3 years	500
Monitoring, per site per year	100
Dissemination material per publication (includes distribution)	1000
Inter-country workshop to promote <i>L. salvadorensis</i> conservation	3000
New molecular marker study (assumes material already collected)	5000

## Further information

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## Forest Genetic Resources Training Guide

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- 1.1 ***Leucaena salvadorensis*: genetic variation and conservation**
- 1.2 *Talbotiella gentii*: genetic variation and conservation
- 1.3 *Shorea lumutensis*: genetic variation and conservation

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### MODULE 3 Tree seed supply chains

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### MODULE 5 How local is local? – the scale of adaptation

- 5.1 Selecting planting material for forest restoration in the Pacific north-west of the USA
- 5.2 Local adaptation and forest restoration in Western Australia

*Other modules to be published among the following:  
Plantation forestry, Tree domestication, Forest restoration, Genetic modification*