Complementary *Ex Situ* Conservation

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Reasons for Collecting Germplasm

- Necessary backup for *in situ* conservation
- Danger of genetic erosion/extinction
- Needed for immediate use
- Diversity is missing from *ex situ* collections "gap analysis"
- Research - we wish to know more about it
- Opportunism
- Emergency

Introduction

So what is actually collected on an expedition?

- **Living *ex situ***
  - Seed: sample of natural storage organ
  - Field gene banks: samples as cuttings or whole plants
- **Suspended *ex situ***
  - *In* vitro: samples of meristem or other tissue
  - Pollen: samples of a species pollen
  - DNA: samples of a species DNA
  - Passport data: site or taxon specific data

Exploration objective

- The object of plant exploration is to collect material with the maximum amount of useful genetic variability within a strictly limited number of samples

- The maximum variation in the minimum number of accessions

Expedition Timing

Hawkes (1980) points out that it is essential that the mission is undertaken at the most appropriate time, it will allow you to:

- Arrive in the various areas when the target taxon is ripe
- Collect from many distinct places within each region
- Cover local variation in soil, climate, altitude, varying agricultural practices, etc.,

Local Administrative Requirements

- Expeditions should always note and respect the legislation, regulations and customs of the countries in which they are undertaken (particularly post-CBD, Nagoya Protocol).

- When planning an expedition the appropriate authorities of the host country should be contacted and their assistance sort in the preparation of the mission.

Field sampling

- "Maximum diversity for minimum sampling“

- To achieve the conservationist needs to know:
  - the amount of genetic variation within and between populations
  - population structure
  - breeding system (inbreeder - outbreeder)
  - taxonomy
  - ecogeographic distribution of the target taxon in the target area

  - but this data may not be available.

  - Also the type of material being collected, seed or vegetative, will affect the sampling strategy, handling and storage techniques, quarantine and ultimately the method of distribution.

Wild Species Collecting

- Narrow collecting window - ripe seeds are quickly shed
- Fruiting times vary between and among populations - repeat visits may be necessary
- Difficult to find - populations often scattered and in remote places
- Commonly outbreeding - can sample fewer sites and fewer individuals

Field sampling

5 specific sampling questions

- Distribution of sites within the target area
- Number of sites to sample
- Delineation of a site
- Distribution of plants sampled at a sites
- Number of plants to sample per site

Distribution Of Sites Within The Target Area

- Governed by local ecogeographic conditions
  - If variable = more sites
  - If constant = less sites
- Always a conflict between collecting large samples at each site and fewer sites or smaller samples at more sites
- Covering larger area and collecting more samples will increase the likelihood of picking-up greater allelic variation
- Patterns of genetic differentiation within species are strongly correlated with environmental heterogeneity
- So where there is no information on the distribution of variation in nature, the collector should aim to sample as many habitat type as possible

Distribution Of Sites Within The Target Area

- Transect method
  - Capture maximum amount of variation associated with broad edaphic and climatic differences;
  - Samples every 50km or 200m change in elevation;
  - Favoured for annual crops, because annual mixing of the crop following harvest will not permit micro-geographic selection

Distribution Of Sites Within The Target Area

- Cluster method
  - Captures significant amount of variation associated with micro-ecogeographic factors
  - Favoured for wild or weedy species, they grow on a broader range of habitats than crop plants, gene flow is less and they show greater adaptation to local habitats
- Advantage of Cluster method
  - Saves overall travelling time between sites
  - Forces the explorer to sample diverse habitats
  - Increase the value of the collection for population studies

Number of Sites To Sample

- With no information distribution of genetic diversity in nature
- Assume each site gives the opportunity to sample a different set of alleles
- Thus optimum number of sites to sample is the maximum possible!
- Though in practice the number of sites will be restricted by the length of the collecting season, relative abundance of the target species, etc.

Delineation of the Site

- Crop relatively easy = farmers field
- Weedy species = in and around fields
- Wild species = more problematic
  - Area covered by interbreeding population
    - Breeding, pollen and seed dispersal system
    - Habitat barriers

Distribution of Plants Sampled Within Sites

- Random or non-selective sampling
- Systematic sampling
- Selective or biased (Marshall and Brown 1975)
- Enriched sample
- Bulked population or single plant
- Practical, keep off types separate (Frankel, Harlan, etc.)

Number of Plants Per Site

- Require the number of plants that will contain 95% of all the alleles at a random locus occurring in the target population with a frequency greater than 0.05 Marshall and Brown (1975)

- Changed to allele frequency > 0.10 (Brown, 1978)

- Collect from 50-100 individuals per site and collect 50 seeds from each plant (50 x 50 = 2500 seeds per sample)

Methods For Seed Collections of Wild Species

1. Collect from (30 -) 50 (-100) individuals per site (50 seeds of each) as one sample, or less if necessary, At Random. One inflorescence per plant is generally suitable.

2. Sample as many sites as possible in time available.

3. Choose sampling sites over as broad an environmental range as possible.

This should capture all alleles with frequency of 5% or more in the population.

Wild Species Collections

Wild species differ from cultivated species in that:

1. Wild species generally have a broader genetic base than the allied crops, due to the founder effect of domestication and disruptive selection.

2. Wild plants show more genetic variation, heading, flowering, seed set.

3. Natural populations show greater tolerance of geographic and ecological conditions, because they are not bred for uniformity in annual sowing or bulk harvesting.

4. Population density is less than cultivated species, they are found as part of a mixed communities.

5. Wild plants display a range of breeding systems, there is a higher proportion of outbreeding species.

Wild Species Collection (Cont.)

6. Most crop plants are annual, but most wild plants are perennial and their population possess a complex age structure.

7. Any crop may be represented by one or two species, the wild relatives are more numerous and priorities need to be established for collection.

8. Cultivated material is highly mobile and can be spread rapidly by man, whereas wild material is unlikely to be spread by man and so can develop highly localised patterns of gene distribution.

Material collection

- Material gathered during germplasm collecting:
  - passport data
  - voucher specimens
  - vegetative plants
  - seed

Collection forms

- E.G. Maxted (1989)

Site Information:

Country ..... Province ..... Date.../.../92

Site Number ..... Nearest Village.....

Location .....

Altitude(m).....Latitude.....N Longitude.....E Rainfall.....cm

Site Physical .....

Site Vegetative .....

Coded Environmental Information:

PR	TS	pH	ST	AS	SL	%C	DS	WR	AP	GP	%R	RT	%T	TT	PN

Collection forms

- E.G. Maxted (1989)

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Taxon Accession Information:

Coll. Nos .........  Name ..............................

Petal Colour Standard ........ Wing .........  Keel ..........

Habitat ..................... Pop. Character ...................

Herb. Spec. Y/N Nos. Duplic ..... Date .../.../92 Rhizobia Y/N

Seed Coll. Y/N Coll. Size ....... Nos. Plants Sampled ........

Date Of Seed Coll.  1  .../.../92  2 .../.../92   3 .../.../92
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Voucher specimens

- Good quality voucher specimens should be collected, especially when collecting wild species, because it facilitates identification and records features of the particular accession.
- Representative flowering and, if possible, fruiting specimens should be pressed.
- Good specimens should be representative of population, contain all parts of the plant and be accompanied by detail passport data.
- Always collect more than one set of material, extra sets can be sent to specialists or sent for exchange

Germplasm

- Collect fruits, seed heads or seed from populations that have been identified
- Seed collected of doubtfully identified or intra-specific variants plants should be collected under a different accession number
- Each bulked seed accession or individual plant's seed place in a separate bag (paper or cotton, depending on the size of the collection) with site number, field identification and accession number on outside (written in pencil)
- Normally seed is collected as bulk accessions, but if genetic studies are to be undertaken then seed from each plant should be collected separately

Germplasm

- Bulked accession needs to be thoroughly mixed before any division to ensure each sample has the same genetic profile
- Alpha tag with the site number, field identification and accession number should be placed inside bag and on outside of the bag
- All accessions collected at a particular site inside one larger bag with the site number marked on the outside of the bag
- Whenever possible samples should be collected from disease free plants.
- Collect sufficient samples to allow splitting of collection

Germplasm

- Fleshy fruited plants seeds require extraction from the fruit collected, ripe fruits should be harvested and the seeds should be squeezed out and then left to dry on blotting paper.

- Seed collected in the rain may require threshing and drying to avoid the growth of moulds be using artificial heat, but the temperature should not exceed 40˚C.

Seed collections should be:

- fumigated (if required)
- threshed
- cleaned
- divided
- dried

Ingredients for a Successful Expedition

- Planning
- Flexibility

- Involve local people
- Develop a search image
- Choose appropriate techniques
- Document the collecting
- Safety first
- Follow up