

Seed Collecting for *Ex Situ* Conservation

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BOTANIC GARDENS

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RARE
PLANT
CARE
& CONSERVATION

Objectives of Seed Collecting for *Ex Situ* Conservation

- Capture the genetic diversity represented in wild populations
- Provide material for reintroductions
 - Genetically diverse
 - Ecologically appropriate
- Seeds to develop propagation protocols
- Provide material for conservation research
- Do no harm to the wild populations!

Each seed collection may be the founders of future populations. Because our collections are small, genetic considerations become paramount
- we follow special seed collection protocols

Species and Populations Most Likely to be Impacted by Seed Collections

- Small effective population size
- Short-lived individuals
- Sexual reproduction predominant
- Few individuals usually produce seed (are they the same every year?)
- Infrequent successful seed production (poor seed crop most years [quality & quantity])
- Short-lived seeds = little or no buffering via seed bank of gene pool contraction & drift

Plants of conservation concern often have one or more of these characteristics

Do the seeds tolerate desiccation?



Betty Swift

Follicles of *Coptis aspleniifolia*

- Orthodox seeds
 - seeds that tolerate desiccation
- Recalcitrant seeds
 - seeds that do not tolerate desiccation
- Intermediate
 - seeds that tolerate some desiccation (to ~20% water content)

Guide to Desiccation Tolerance

Trait	Likely to Be Orthodox (Desiccation and Freezing Tolerant)	Questionable Tolerance to Orthodox Storage
Habitat	Arid is especially likely; If it is not growing in a wetland, it is likely	Wetland, riparian
Conditions in nature	Seeds normally experience dry down and/or hard freezes	Seeds normally remain moist and do not experience hard freezes.
Season of seed production	Not spring	Spring
Life form	Not tree	Trees
Seed bank	Persistent	Not persistent
Dormancy	With dormancy	No dormancy
Seed moisture content at time of maturation	Dry when it is naturally shed from plant	High (30%–70%)
Seed size		Very large (avocado seeds aren't desiccation tolerant) or very small (orchid seeds and fern spores require storage in liquid nitrogen)

From Center for Plant Conservation 2019

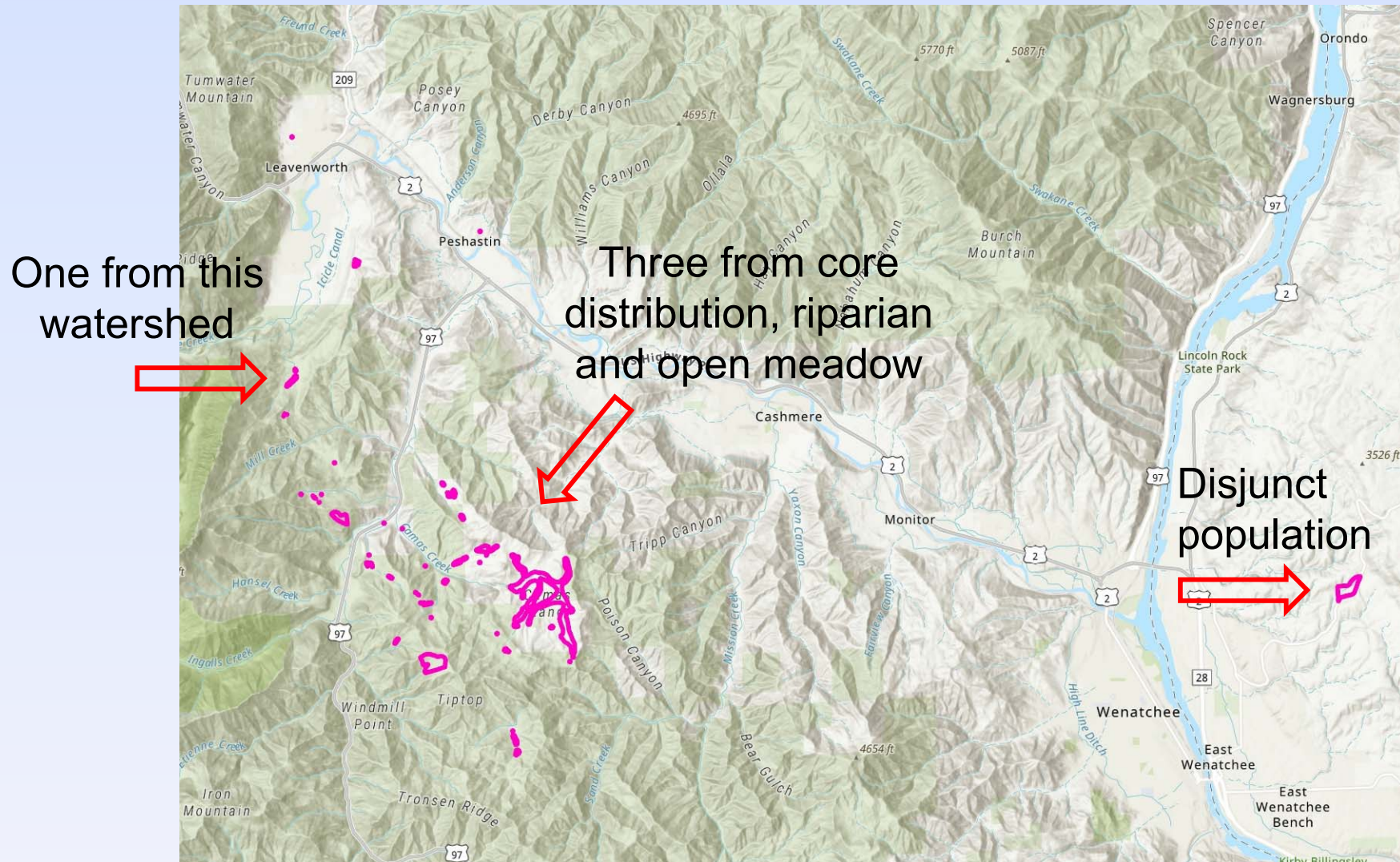
Population Selection Guidelines



Delphinium viridescens

- Collect seeds from at least five different populations that represent geographic range and ecological breadth of the species
- Collect from unique or disjunct populations
- Collect from populations at risk of extirpation
- Logistics and opportunity may be key determinant

Seed Collection Priorities Example



Collection Size

What is the purpose?

long-term *ex situ* conservation

future reintroductions

For long-term *ex situ* conservation:

- **Collect 3,000 seeds from at least 50 unrelated maternal plants**
- **Collect and store seeds along maternal lines**

Following the 10% rules

10% RULE

Only collect 10% of the viable seeds available from an individual or population in a year*

COLLECTION FREQUENCY

10% of seed should be collected no more than 10 out of 90 years

Considerations for Collection Size

What is the breeding system of the species?

- Does the species self fertilize?
 - Affects genetic diversity within and across populations
- Does the species reproduce asexually?
 - Affects genetic diversity, availability of fertile seed
- Is hybridization occurring or suspected?
 - Should seeds be collected?

Size of collection may be adjusted based on these considerations

Determine the number of seeds to collect based on seed availability

**Optimal collection size:
~ 3,000 seeds from
50+ maternal plants when possible
(up to 60 seeds collected from a single plant)
Following the 10% rule**

1. Check seed viability
 - Healthy, empty, malformed, predated, moldy?
 - Do a cut test on a few seeds, but use your judgment on how many to check
 - Sample from different locations in the inflorescence

Inspect seeds for damage, fill



Predation of *Sidalcea oregana* var. *calva* can reach 95% by a native weevil predator



Variability in seed fill of *Trifolium douglasii*

Determine number of seeds to collect

2. Determine number of viable seeds per fruit/head and number of fruit per plant
3. Calculate number of viable seeds per plant
4. Repeat for randomly selected 5 to 10 plants
5. Calculate number of viable seeds in population
6. Calculate 10% of the total seed output of the population



Ripening fruit of *Astragalus australis* var. *cottonii*

Example 1:

You estimate that there are about 300 plants that have ripe seeds available. You sample 5 plants and determine that on average plants holds 75 mature seeds on the day of the collection. How big will your seed collection be?

$$300 \text{ plants} \times 75 \text{ seeds/plant} = 22,500 \text{ seeds in the population}$$
$$10\% \text{ of } 22,500 = 2,250 \text{ seeds}$$

- ▶ Maximum no. of seeds collected is 2,250 (10% population rule)

$$10\% \text{ of seeds from an average plant} = 10\% \times 75 = 8 \text{ seeds}$$

- ▶ Seed collection based on 10% individual rule:
 $8 \text{ seeds} \times 50 \text{ plants} = 400 \text{ seeds}$

Therefore, collect on average 8 seeds from 50 (*or more*) plants

SEED COLLECTION LIMITED BY 10% INDIVIDUAL RULE

Example 2:

There are 40 plants with ripe seeds on the day of the collection, and approximately 20 fruit on each plant. Each fruit holds 40 seeds on average and approximately 20% of the seeds are empty.

Calculate number of seeds per plant:

$$40 \text{ seeds/fruit} \times 20 \text{ fruit/plant} \times 80\% \text{ viable seeds} = \\ 640 \text{ viable seeds per plant}$$

Determine collection size based on 10% population rule:

$$50 \text{ plants} \times 640 \text{ seeds/plant} = 32,000 \text{ seeds in the population} \\ 10\% \text{ of } 32,000 = 3,200 \text{ seeds}$$

Determine collection size based on 10% individual rule:

$$10\% \text{ of seeds from one plant} = 10\% \times 640 = 64 \text{ seeds}$$

Seed collection based on 10% individual rule:

$$\sim 60 \text{ seeds} \times 50 \text{ plants} = 3,000 \text{ seeds}$$

► SEED COLLECTION MEETS 10% RULES

But.....

Example 2 continued

What if you encounter a plant with only two fruit?

What if you encounter a plant with 30 fruit?

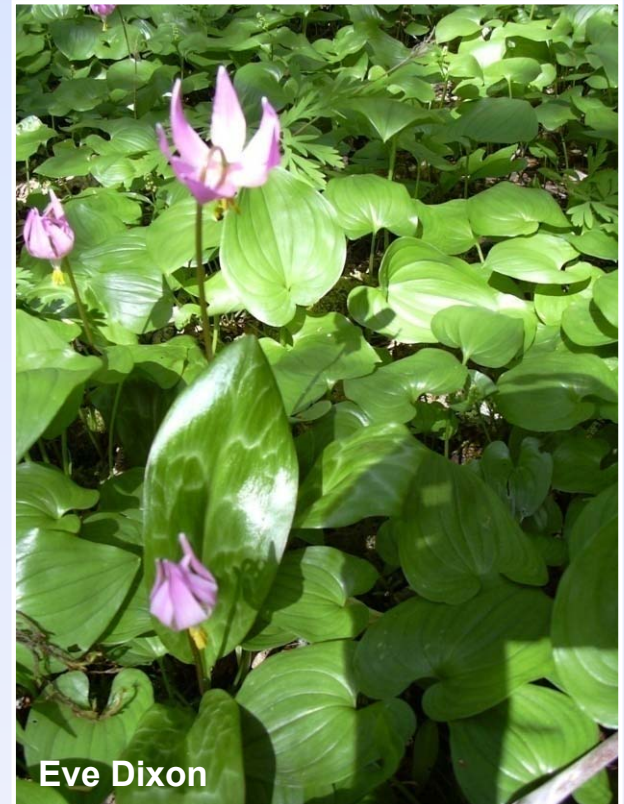
What if you can't tell if the seeds are viable without cutting them open (remember, 20% of the seeds are empty)?

Sampling Strategy

- Collect from multiple years (mark plants so they aren't sampled multiple times)
- Collect from multiple times over the season if possible
- Collect from plants that grow far apart
- Collect from large and small maternal plants
- Collect from plants in all microhabitats
- Collect from different parts of the inflorescence

Pre-Collection Activities

- Recommend you visit the population while it is in flower
 - Species may not look like themselves when they are fruiting
 - Determine what look alike species occur at the site
 - Use discrete flagging to help relocate fruiting plants
 - Gather data on population size and extent of occurrence



PINK FAWN-LILY
(Erythronium revolutum)

Timing Your Collection

- Collecting window will vary for different species, different locations, & different years
- Maturation time varies by species, weather, terrain, etc.
 - 6 weeks is good estimate for many species
 - Species formally known as lilies often take longer
 - Some species can be as short as 4 weeks (i.e. composites)
- How do you make sure you are there when the seeds are ripe?
 - Check herbaria records for phenology, adjust for climate change
 - Check phenology at other sites for same or other closely related species
 - correlate two sites using co-occurring species

Assessing Seed Ripeness

Definitely Ripe	Maybe Ripe	Probably Not Ripe (enough)
Fruit open & dry (judge by color, feel, firmness)	Fruit opening & drying (judge by color, feel, firmness)	Fruit closed & green (judge by color, feel, firmness)
Seeds easily dehisce from heads or are loose in fruit	Some seed dehiscing or loose	Seed firmly attached or not loose
Seed dark (not universal)	Seed darkening (not universal)	Seed light (not universal)
Seed well-formed	Seed well-formed	Seed poorly-formed
Seed hard, can't be dented (thumbnail test)	Seed firm, but can be dented with moderate effort (thumbnail test)	Seed mushy or easily dented (thumbnail test)

To Collect, or Not to Collect?

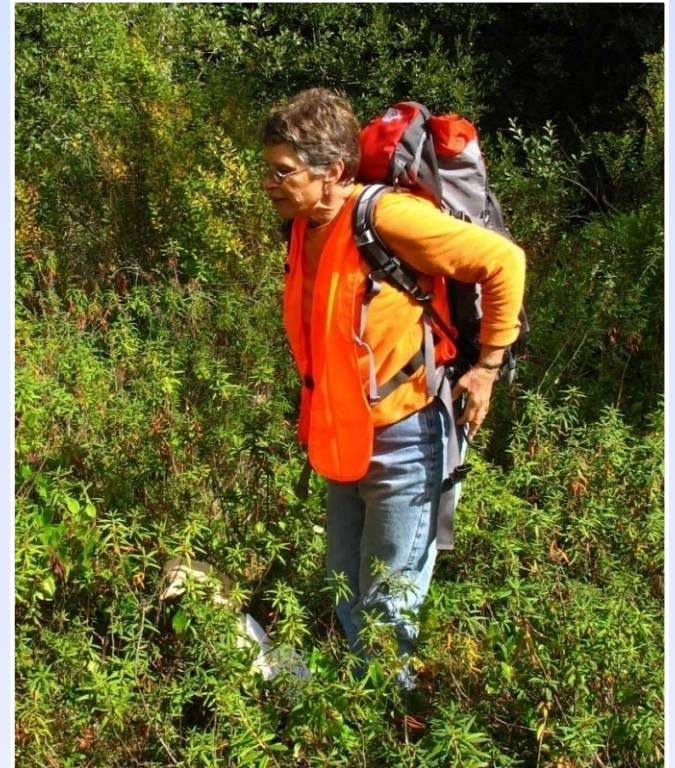
- How wet is too wet?
 - Rule of thumb: Not dripping
- Are the seeds ripe?
 - Most species ripen seed sequentially
 - Spot check several plants to evaluate ripeness
 - Determining ripeness: Table 5-1
 - Color, size, shape, firmness and ease of removing fruit/seed from plant
 - After-ripening: don't plan on it unless told otherwise
- Other factors – overall seed production, predation, etc.



TOO WET!

Field Gear

- Safety gear
- Botanical survey gear
 - GPS to document area of collection
 - Plant press for voucher
- Newspaper for drying out seeds
- Seed collecting tools
 - Fingers, clippers, pruners, scissors
 - Razor blade, hand lens to inspect seeds
 - Calculator
- Seed containers
 - Coin envelopes
 - Tupperware
 - Container to hold coin envelopes



Know when hunting season is, and dress appropriately!

Site Visit

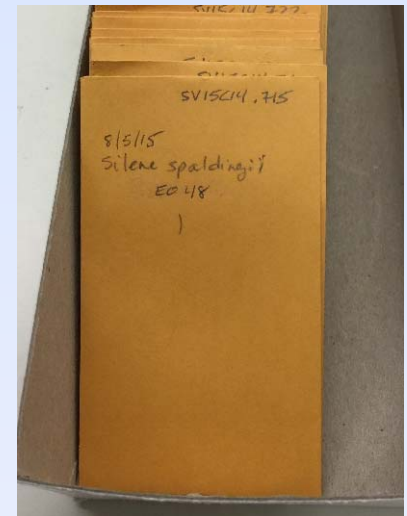
- Do not be a vector for invasive plants
 - Clean off all clothes and equipment that might be carrying seeds
 - Don't wear seed catching materials
- Map population to determine extent
- Evaluate ecological heterogeneity
- Determine effective population size
- Assess proportion of population with ripe seed
- Be ready to handle questions

**Don't let spotted knapweed
hitch a ride on you!**



Collecting the Seed

- Collect from ripe portions of the plant
- Keep the seeds from each maternal plant in separate containers/envelopes
- Label your envelopes first!
- Harvest the smallest possible amount of the plant
- If you need a fraction of the seeds in a fruit, disperse the rest on the ground
- Note other useful information for individual plants, such as:
 - GPS coordinates on plants (if large area)
 - Sub-site information
 - Unique plant attributes



Label Each Envelope

Your name

Date

Species Latin name

plants sampled

Obtaining a Genetically Diverse Sample

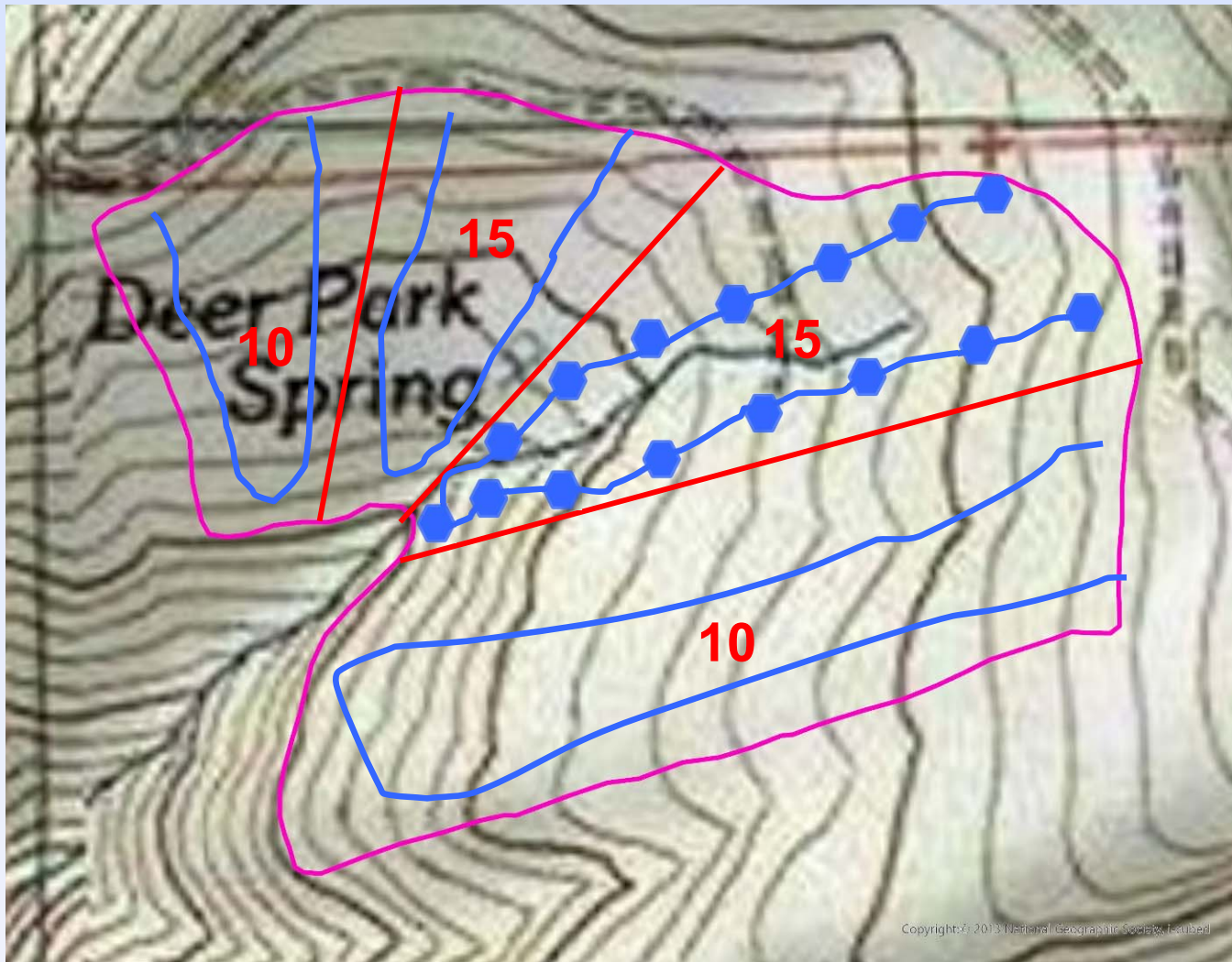
- Sample variety of plants based on vigor, size, location, seed productivity, disease, etc.
- Resist temptation to collect from plants with the most seed
- Sample from plants throughout entire area of population
 - Subdivide area of population
 - Allocate seed packets by subarea
 - Strategy for dispersing your collection plants, i.e. points on transect



Earl Doan

SEVERAL FLOWERED SEDGE
(*Carex pluriflora*)

Collection Strategy for *Delphinium viridescens*



Taking Care of Your Collection

- Do not cook the seeds on hot sunny days
 - Do not leave seeds in a hot, unventilated car
 - Do not place in direct sunlight, i.e. dashboard
- Many critters hang out in fruit, but they will crawl out as the fruit dries
- Allow seeds to dry
 - If damp or wet, lay out on newspapers
 - Do not put in front of heater or vent or in oven/stove
 - Keep packets in cool, dry area, out of direct sunlight, with good air ventilation



Rod Gilbert

FRINGED KITTENTAILS
(*Veronica schizantha*)

Documenting Your Collection

- Date of collection, name and contact info of collections
- Detailed directions and precise mapping of population and collection area
- Population size estimate
- Habitat description and associated species
- Estimates of seed production
- Observations on seed quality and concerns
- Threats to populations, management concerns
- Photos

**Provide seeds to seed bank
AS SOON AS POSSIBLE**

Questions?

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