A modified protocol for quantifying variability in herbivory for rhizomatous geophytes

By Yuval Sapir (YS), Tel Aviv University

When to use this protocol:

We suggest following this protocol when surveying herbivory on a rhizomatous plant species that meets two conditions: (1) it is feasible to determine what constitutes a genet by examining rhizomatous connections, and (2) genets are small enough at your study site that you could feasibly survey 30 genets and their nearest neighbors and estimate herbivory on each genet.

Background:

In semi-arid and arid climates, a considerably large number of plant species are rhizomatous geophytes. Their major characteristic is that they grow as patches of individuals, forming either dense (phalanx) or sparse (guerrilla) mats of individual ramets, each visible as a single leaf fan, and all connected through below-ground rhizomes and/or above-ground stolons into one plant (genet) (terms following Harper 1977 and Herben and Klimešová 2020). The extent of clonal growth defines the spread of the genet, and is on a continuous scale of density (Vallejo-Marin et al. 2010). See Figure 1 for examples of two density levels of genets in irises.

Figure 1 – Top left: Dense (“phalanx type”) genet of *Iris atrofusca*; Top right: Sparse (“guerrilla type”) genet of *Iris bismarckiana*; Bottom left: Compact rhizome of *Iris atrofusca* (this one has ~4 leaf-fan ramets); Bottom right: Stolons connecting ramets of *Iris bismarckiana*.

Note from MLR: Clonal plants present an interesting challenge and opportunity within the HerbVar Network. From a question-based perspective, we may be able to compare patterns of herbivory variability between clonal vs non-clonal plant species. These different modes of reproduction may confer different levels of genetic and phenotypic diversity within plant populations, which could affect patterns of herbivory. However, from a practical perspective, quantifying herbivory among plant 'individuals' is a challenge in these systems - what is an 'individual'?. In the interest of practicality, the primary protocol asks: "for clonal plants, we have been calling stems "plant individuals" if they are not connected aboveground. When looking for aboveground connections, we clear away detritus, but we do not dig or move soil." This means that, for some plant species, a plant individual will be equivalent to a ramet (e.g. if the connection is belowground; for example, Asclepias, Solidago). For other plant species, in which connections among ramets are visible aboveground, an individual will be equivalent to a genet. While we acknowledge that we would, in an ideal world, know the genetic independence of each plant "individual" to the same degree across systems, this can present a practical challenge, as digging up plants to establish connections would be infeasible and destructive. For clonal plants in our data, one way to address this will be to record whether plant individuals are more likely ramets or genets, as defined by the collaborator, and use this as a covariate in analyses. Finally, a key question we can answer using data produced by this protocol and the primary protocol is this: how do patterns of herbivory differ among non-clonal plants, clonal plants for which it was possible to determine rhizomatous connections (following this protocol), and clonal plants for which it was not possible to determine belowground rhizomatous connections.

In the case of geophytes such as irises, collaborators have developed the following approach, as this growth form challenges the estimation of herbivory and allows us to examine another level of variation in herbivory (variation among ramets within a genet). Practically, each ramet, observed as a leaf fan, may be considered a single individual. Genetically, as well as spatially, each such ramet is associated with its closest neighbor. In the smaller scale, each ramet consists of a few leaves of different developmental stage, and sometimes a reproductive unit (e.g. a flower). To account for herbivory in these different levels, I (YS) have developed a modified protocol for estimating variation in herbivory. This protocol was applied in five populations of irises of the section *Oncocyclus*. The species of this section are all rhizomatous clonal plants, growing in dry montane or semi-arid to arid habitats in the Middle East (Sapir and Shmida 2002, Wilson et al. 2016).

In this protocol, we consider a plant individual as a genet, consisting of one or usually multiple leaf fans (ramets) that are significantly distant from the next group of ramets. When first starting this on a new species or at a new site, we suggest spending time investigating what constitutes a genet. Follow rhizome connections from ramet to ramet to get a sense of what a single genet looks like.

Survey protocol for rhizomatous (clonal) geophytes

The first steps are identical to the regular protocol. I suggest a slight change in estimating radius of quadrat, but this depends on the average radius of genets. Modifications of the regular protocol are in red.

- Pick a plant species (see "6. Guidelines for selecting plant species" below)
- Pick a site (see "7. Delineating a site" below for advice)
- Pick a time to sample (see "8. When to Sample" below for advice)
- Calculate a 'custom' radius for circular quadrats. We developed the following method to create quadrat sizes specific to each plant species and site, given that plant size and density vary immensely. This approach seeks an optimal, intermediate quadrat size that balances the costs associated with a small quadrat size (many empty quadrats) and a large quadrat size (quadrats that require counting many plant individuals).
    - Estimate mean density of genets per square meter by counting the number of plants in 1 m^2 at 10 random locations within the site; calculate mean density ( )
    - If genet area (clone/genet diameter) is >1 m and/or distances between genets are apparently irregular (that is, secondary dispersion of plants within population is patchy), count the number of genets in 1 m^2 every 5 meters along a 50 m transect.
    - Use to calculate a circular quadrat radius ( ) that would on average contain 4 genets:
        -
- Lay a transect through the middle of the site
    - Record GPS coordinates of origin, length (m), and compass direction (degrees) of transect (need to pick a coordinate system and precision)
- Select center points of circular quadrats. Randomly select 40+ points in the site by selecting pairs of random numbers. One random number represents distance along the transect (0–length of transect); the other represents distance left or right of the transect (left=negative, 0=center, right=positive). These are the center points of quadrats.

For each quadrat:

- Locate a quadrat center point using transect and measuring tape or stick
- Count and record the number of focal plants within meters of the center point (a circular quadrat)
- Record other quadrat level data:
    - Percent cover of focal plant (ignore non-focal species)
    - Percent cover of all non-focal plant species (ignore focal species)
        - These 2 percent covers could total more than 100% if they overlap
        - If surveying understory plants, ignore forest canopy when estimating percent cover

- If the circular quadrat has 0 plants, record a zero and continue to the next quadrat

If the circular quadrat has > 0 plants:

- Randomly choose 1 of the genets within the quadrat to survey
  - A quicker alternative would be to choose the genet closest to the quadrat center. But this is recommended only if you think it will produce an unbiased sample of genets from your site. Be careful about over-representing large and/or isolated genets (which will be closer to more points relative to small genets in crowded patches).
- Data to record for each selected genet (1 per quadrat):
  - Genet life stage: seedling, vegetative, reproductive
  - Genet size, measured as the height of the tallest leaf for plants in vegetative stage, or height of the taller flower for plants in reproductive stage.
  - Herbivore damage (see Damage estimation training document) in 3 ways:
    1) Total number of leaf fans (ramets); for genets with >100 ramets, write "100".
    2) Estimated percent damage across the whole genet. Visually scan all the green area of all ramets and all leaves, and estimate the percentage of damage.
    3) For each genet, choose 10 leaves randomly/ haphazardly. This could be stratified by ramet (e.g. one leaf per ramet, if the genet has 10 ramets). Whatever your method is, try to pick the 10 leaves to be a representative subsample of all leaves of the genet (e.g. include all leaf positions/ages as potential leaves to select).
    4) Record how many leaves (out of the 10 sampled) are damaged.
    5) In each damaged leaf, estimate percentage of damage. Note that tip of the leaf may be dry due to climate fluctuations in the arid regions. This area of dry leaf accounts for leaf area, but not account as herbivory damage.
    6) Record the agent of herbivory, if identifiable.
- Flower damage (florivory) – in plants at the reproductive stage.
  - Count number of open flowers (flowers that already underwent anthesis).
  - Record number of flowers damaged.
  - For each damaged flower, estimate the area damaged and the putative florivore.

- Data to record for the first nearest conspecific neighbor (genet) of selected genet:
  - All the same data as focal genet except nothing for neighbor's neighbor
- Continue visiting the randomly selected points until ≥ 30 focal genets and 30 nearest neighbor genets have been surveyed

References

Harper, J. L. 1977. Population Biology of Plants. Academic Press, London.

Herben, T., and J. Klimešová. 2020. Evolution of clonal growth forms in angiosperms. New Phytologist **225**:999-1010.

Sapir, Y., and A. Shmida. 2002. Species concepts and ecogeographical divergence of *Oncocyclus* irises. Israel Journal of Plant Sciences **50**:S119-S127.

Vallejo-Marin, M., M. E. Dorken, and S. C. H. Barrett. 2010. The Ecological and Evolutionary Consequences of Clonality for Plant Mating. Annual Review of Ecology, Evolution and Systematics **41**:193-213.

Wilson, C. A., J. Padiernos, and Y. Sapir. 2016. The royal irises (Iris subg. Iris sect. Oncocyclus): Plastid and low-copy nuclear data contribute to an understanding of their phylogenetic relationships. Taxon **65**:35-46.