A protocol for quantifying variability in plant–herbivore interactions

HerbVar: A collaborative network studying global patterns of variability in herbivory

1. Motivation: Published studies and personal observations suggest the distribution of herbivore feeding damage among individual plants within a population is often highly skewed such that most plants experience relatively low levels of damage, and a small fraction of plants experience disproportionately high levels of damage. Theory suggests that such variability can have dramatic ecological and evolutionary consequences. For example, variability among plants can lead overall herbivore population size to be greater or less than expected based on average plant quality and asymmetric fitness surfaces can lead to over-investment in defensive traits. Surprisingly, despite the theoretical importance and potential generality of variability in herbivory, it has received little empirical attention, limiting our fundamental understanding of how plants and herbivores interact.

We are forming a global collaboration to quantify the distribution of herbivory for diverse plant species in multiple ecosystems across the world. The goal of this work is (1) to assess if variability in herbivory is indeed a common feature of plant–herbivore interactions, and (2) to examine how the amount of variability and skew varies among different types of plant species, herbivore communities, and ecosystems. Quantifying general patterns in the distribution of herbivore damage within populations would be a major contribution to our fundamental understanding of herbivory. In addition, identifying the factors that correlate with variability in herbivory would provide the field with a new paradigm for describing plant–herbivore interactions and allow us to generate novel hypotheses about the ecology and evolution of plant–herbivore interactions.

2. Project goals:

1. Quantify the within-population distribution of plant damage and herbivore density across many systems
2. Quantify how within-population distributions of damage and herbivore density differs across
    a. Plant species
    b. Plant functional traits (from literature)
    c. Plant ecology (e.g., rarity)
    d. Herbivore species
    e. Herbivore functional groups
    f. Ecosystem type
    g. Latitude
    h. And many other potential factors (e.g., seasonality, precipitation...)

3. Overview:
Below, we provide a straight-forward and broadly applicable protocol to achieve these goals. This is the **Primary HerbVar Survey Protocol**. In brief, 30 randomly-selected plant individuals in a site (~population) are surveyed for herbivore damage and (possibly) herbivore abundance. Data are also collected on the nearest conspecific neighbor of each plant (for a total of N = 60 plants). These methods yield estimates of variability, skew, and spatial patterns (e.g., autocorrelation) in herbivore damage.

The HerbVar Primary Survey Protocol is designed to work for many common plant growth forms and contexts, so we expect most surveys to use this protocol. The primary protocol, however, will not work for every plant growth form or context, so HerbVar has multiple alternative survey protocols. Alternative protocols can be found in the shared Drive in the "Alternative protocols" folder. These include protocols for surveying **plants with low density or abundance**, **mature trees**, **cacti and other succulents**, **reproductive (flower/fruit/seed) damage**, and **vertebrate browsing damage**, as well as an **optional insect sampling protocol**. If the primary protocol is not feasible for a species or site, then we suggest one of these alternative protocols. If none of these alternative protocols fits the situation, then collaborators may deviate from the primary protocol. We trust collaborators to decide how to adapt the primary protocol in ways that works for their systems. We suggest, however, that collaborators strive to follow the spirit of the protocol below: randomly select at least 30 plants from a site and census them and their nearest neighbors for herbivory and herbivore data. For a dataset to be usable in the overall study, it will have to be comparable to data collected using this protocol. Collaborators who deviate from the HerbVar protocols should carefully record their methods.

The primary protocol works best for sites with at least ~90 plant individuals, such that it makes sense to sample individuals randomly. **If your site has fewer than ~90 individuals of your plant species, then please consider comprehensively censusing all individuals within the site** as suggested in our document on surveying low-density/low-abundance sites. A comprehensive census, when feasible, would be even better than the protocol below. If plants are far enough apart, please take GPS coordinates for each plant. If a comprehensive census is not feasible, then please modify the primary protocol or the low-density/low-abundance guidelines to work efficiently with your species and site. Please reach out to the HerbVar coordinators if you have questions or want to check that your modifications will lead to adequate data.

4. The Primary HerbVar Survey Protocol

There is a template data sheet for this protocol, and example of a completed datasheet in the HerbVar shared Google Drive

- 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 plants per square meter by counting the number of plants in 1 m^2 at 10 random locations within the site; calculate mean density (D)
    - Use D to calculate a circular quadrat radius (r) that would on average contain 4 plants:
        - $r = \sqrt{4/(\pi D)}$
- 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 r 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 plants within the quadrat to survey
    - A quicker alternative would be to choose the plant closest to the quadrat center. But this is recommended only if you think it will produce an unbiased sample of plants from your site. Be careful about over-representing large and/or isolated plants (which will be closer to more points relative to small plants in crowded patches).
- Data to record for each selected plant (1 per quadrat):
    - Plant life stage: seedling, vegetative, reproductive
    - Plant size, use judgement to pick best measure for your species
        - E.g., standing plant height (ground to tallest living part), stem length, foliage diameter, stem diameter
    - Herbivore damage (see Damage estimation training document) in 3 ways:
        - (1) Presence/absence of leaf damage: *If a plant has ~60 leaves or less in total*, please record the total number of leaves on the plant, and the number of those leaves that have damage (count leaf as damaged if it has > 0.5% herbivory). *If a plant has more than ~60 leaves*, record presence/absence of herbivory on 60 randomly (arbitrarily) chosen leaves and please note you stopped at 60.
            - If plants have reproductive parts (flowers/fruits/seeds) that could have been damaged by herbivores, please see the HerbVar Flower/Fruit/Seed Damage Protocol. This is optional, but encouraged.
        - (2) Estimated percent damage on 10 randomly (arbitrarily) chosen leaves
            - One estimate per leaf (for a total of 10 estimates)
            - Ideally chosen leaves will be representative of all leaves (e.g., sample young and old leaves in proportion to frequency on plant)
        - (3) Estimated percent damage across the whole plant, optionally also breaking apart damage by type or even species of herbivore if possible (e.g., sucking damage versus chewing damage, add columns as needed)
            - E.g., 4 leaves of equal sizes with 2 leaves 50% eaten = 25% total
            - But take leaf size into account when leaves vary in size
    - Presence of plant diseases
    - Number of leaf mines and galls per plant (= herbivory + herbivores).

        - If there is reason to believe that galls or mines have accumulated through multiple years (e.g. stem galls on woody perennials), please note this
        - If there are too many mines or galls to count individually, estimate the number per plant by tallying the number per module (e.g. stem, branch) and multiplying by number of modules
    - Optional: abundance of other externally-feeding herbivores (standardized approach; see Herbivore sampling protocol to decide if/how to collect these data)
    - Distance to nearest conspecific neighbor (where the nearest neighbor is the plant with the closest aboveground tissue to any aboveground tissue on the focal plant)

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

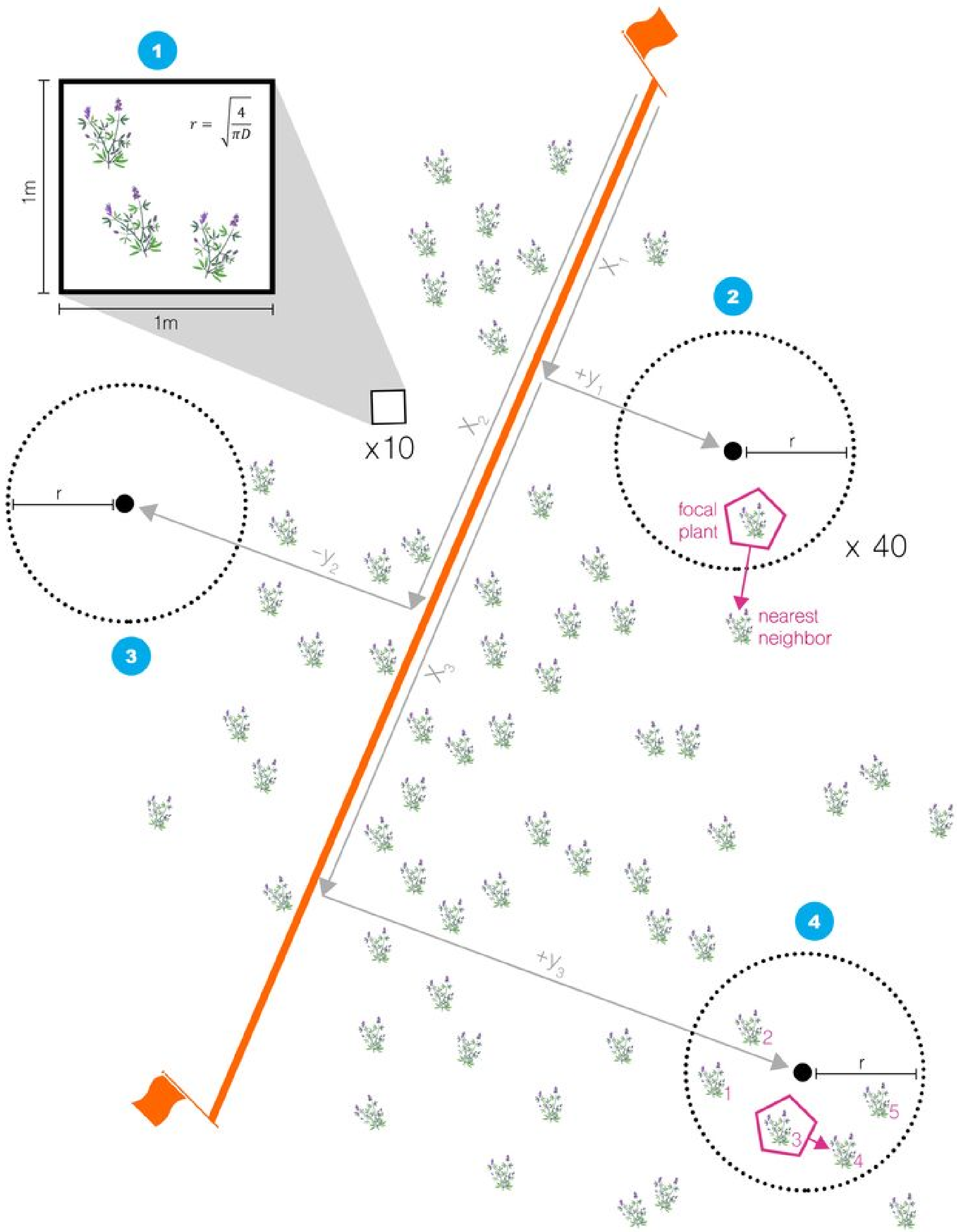

Fig. 1. A diagram of the sampling scheme described in the text. (1) Record plant density in 10 randomly located 1-m^2 areas to estimate plant density D, which is used to calculate quadrat radius r. (2) A quadrat with one focal plant and its nearest neighbor (outside quadrat). (3) A quadrat with no focal plants. (4) A quadrat with 5 focal plants; plant 3 is randomly selected for data collection, and its nearest neighbor is plant 4. Diagram by Moria Robinson.

surveyID	date	site	transect_dist	subtransect_dist	plantID	focalPlantCover	otherPlantCover	numPlantsinQuad	plantStage	ht_cm	numLeaves	numLeavesHerb	percHerbPlant	percLf1	percLf2	percLf3	percLf4	percLf5	percLf6	percLf7	percLf8	percLf9	percLf10	NNdist	NNangle	pathogen	mines	galls
hv2019_5	2019.06.24	Kellogg	0.4	3	1	10	15	7	v	9.5	14	3	2.5	1	1	0	0	0	0	0	0	0	0	19	255	0	0	15
hv2019_5	2019.06.24	Kellogg			1.1				v	5	7	0	0	0	0	0	0	0	0	0	0	0	0			0	0	0
hv2019_5	2019.06.24	Kellogg	0.9	-7.5	2	25	30	17	v	6	8	3	25	5	80	30	0	0	0	0	0	0	0	0	331	0	0	0
hv2019_5	2019.06.24	Kellogg			2.1				s	2.5	7	1	15	55	0	0	0	0	0	0	0	0	0			0	0	5
hv2019_5	2019.06.24	Kellogg	1.7	-5.4	3	10	15	12	v	5.5	19	1	1	10	0	0	0	0	0	0	0	0	0	8	34	1	0	0
hv2019_5	2019.06.24	Kellogg			3.1				v	7.5	13	3	10	70	10	1	0	0	0	0	0	0	0			1	0	0
hv2019_5	2019.06.24	Kellogg	1.9	1.8	4	20	22.5	23	v	10	20	6	25	25	20	15	0	0	0	0	0	0	0	5	193	0	3	0
hv2019_5	2019.06.24	Kellogg			4.1				v	10	25	8	15	35	5	7.5	0	0	0	0	0	0	0			0	0	0
hv2019_5	2019.06.24	Kellogg	2.4	-2.1	5	20	30	8	v	10	14	7	15	2.5	2.5	5	2.5	1	0	0	0	0	0	8	252	1	0	0
hv2019_5	2019.06.24	Kellogg			5.1				v	5	10	4	7.5	15	2.5	1	0.5	0	0	0	0	0	0			0	0	0
hv2019_5	2019.06.24	Kellogg	3.1	-6.4	6	10	15	7	v	6	13	3	5	85	2.5	10	0	0	0	0	0	0	0	11	18	0	0	0
hv2019_5	2019.06.24	Kellogg			6.1				v	10.5	21	8	2.5	10	2.5	0.5	0.5	0	0	0	0	0	0			1	0	0

Fig. 2. An example spreadsheet with data. Meta-data with coordinates, transect length, quadrat radius, etc. recorded in a separate tab. See Datasheet template for HerbVar in the HerbVar shared Google Drive.

5. Methods notes:

- Modifications of this protocol may be necessary to adapt it to different systems (see "3. Overview" above). If this protocol won't work for your system, please first consult our alternative protocols (see page 2 above and Alternative protocols folder). If our alternative protocols do not solve the issues, then you may adapt the primary protocol as needed. Whatever you do, please record methods carefully and strive to follow the spirit of the protocol and produce comparable data.
- In our experience, 1 survey (of 1 site of 1 plant species) takes 2 well-trained undergraduates 2-4 hours to complete using the methods above (after a species and site have already been selected). This is in old fields, prairies, and deciduous forests in Michigan. Could take longer in other systems.
- We select 40 quadrat center points (instead of 30) so that we have extra points ready in case some quadrats are empty. If you predict that many quadrats will be empty (e.g., in a very spatially clumped population of plants), then select more points (e.g., 60 points). (Remember the goal is to have 30 focal plants sampled).
- Sometimes, especially in small populations, a focal plant ends up being another focal plant's neighbor. This is fine. Just note and keep going. If you have time, you can add an extra focal plant at the end (but this isn't totally necessary).
- 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.
- Please see our Damage estimation training document for guidelines on how to estimate herbivore damage. Here are two tips:
    - Sometimes discerning herbivore damage from physical damage (e.g., wind, trampling) is tricky. We do the best we can. We look at things like how jagged the cut edges are and if they travel past the missing area into the remaining leaf tissue (which would suggest the damage may have been physical).

    - Another challenge is old damage that occurred when leaves were still expanding. This could potentially make area removed seem larger than it was. If we suspect something like this happened, then we try to bend the leaf back into shape to see if it seems like the missing area expanded over time.
- We will accept surveys that only assess damage and do not identify herbivores. This will allow people without insect ID skills to participate in the study.

6. Guidelines for picking plant species:
We are hoping for a broad sampling of plant species, so data on any plant species will be valuable. However, we have developed a sampling plan structured around 1) gathering data for as many plant families as possible; 2) in-depth sampling of plant species within five focal families (Apocynaceae, Asteraceae, Fabaceae, Rubiaceae, and Solanaceae); and 3) sampling of three globally-distributed taxa: *Taraxacum officinale* (dandelion), *Plantago lanceolata* (narrowleaf plantain), *Plantago major* (broadleaf plantain). You can read more about our sampling plan on our website (https://herbvar.org/protocols.html; “”HerbVar species selection plan”).

Thus, contributed surveys would ideally include one one new family that is not currently in the database, one species from a focal family, and one survey of a focal species. Additional surveys would be the collaborator’s choice and could include re-sampling the same species through time or across a gradient. While this stratified sampling approach is preferred, all plant populations are of interest and collaborators are welcome to select plants based on criteria that make sense to them (familiarity with taxa, location & feasibility, etc). Also, feel free to re-sample species that have already been sampled. It will be interesting to have estimates of how consistent our data are within species. But once a species has been surveyed 2-3 times, it’s probably preferable to survey a new species.

We have charts in tabs in the Completed surveys document that are constantly updating to indicate gaps in sampling. In addition to the guidelines above, other features of a plant species that would make it a valuable addition to the dataset include:
- Occurs in a novel ecosystem
- Possesses a novel or underrepresented growth form, life history, or other set of traits

Other species selection notes:
- We have been surveying both native and non-native plant species.

- We are interested in agricultural and other cultivated plants and have already sampled a handful. When surveying cultivated plants, make sure the plants have been free of insecticides for an ecologically meaningful time before your survey.

7. Delineating a site:
We realize that defining the ‘edges’ of a site can be subjective and not easy. We search for an area where a given plant species occurs at a high enough density to easily select 30 focal plants and 30 unique neighbors with our method. This is usually a relatively dense patch. Walk around and see if you see the density drop off to well below the mean density that is used to calculate radius size. This is usually quite simple, e.g., when we walk out from the center of a “site” and don’t see any individuals of the focal species within 5 m, we decide we’re at the edge of a patch. In some systems, delineating a single, sampleable population simply might not be possible (e.g., where a species covers a vast area). In these cases, collaborators should simply do their best to select a reasonable, representative area to sample.

8. When to sample:
This will depend on the natural history of the system. We will accept data sampled at any time as long as there has been some herbivory. We will use sampling date to examine how herbivory changes seasonally (please note approximate dates for beginning and end of growing season for each survey, see siteData sheet in datasheet template). However, the most valuable surveys will be after enough time has passed for an ecologically meaningful amount of herbivory to accumulate. In strongly seasonal systems, this will be in the latter half of the growing season. But it could also be once leaves have reached maturity (e.g., for species in which most herbivory is on expanding leaves). In other systems, the best time to sample might be during or after a key life history stage (e.g., flowering). All that said, there is no perfect time to sample. Collaborators should use their knowledge to decide when to sample (and sample when is feasible… some data is better than no data!). And repeat sampling is acceptable.

9. Common garden data:
Common gardens are a powerful tool for studying plant–herbivore interactions. Several collaborators have proposed including them in HerbVar, and we would like to try if we can get enough data. To be applicable to this study a common garden’s design would have to be random with respect to genotype. If a garden was somehow stratified with blocks containing repeated instances of, e.g., different levels of leaf toughness, then damage distributions will not be comparable to damage from wild populations. We may still be able to use such datasets, but only if we have enough to use them in a separate analysis. Please get in touch if you would like to contribute common garden data.