

Genetic analysis of common reed (*Phragmites australis*)

Tippery Lab, University of Wisconsin - Whitewater

262-472-1061

tipperyn@uww.edu

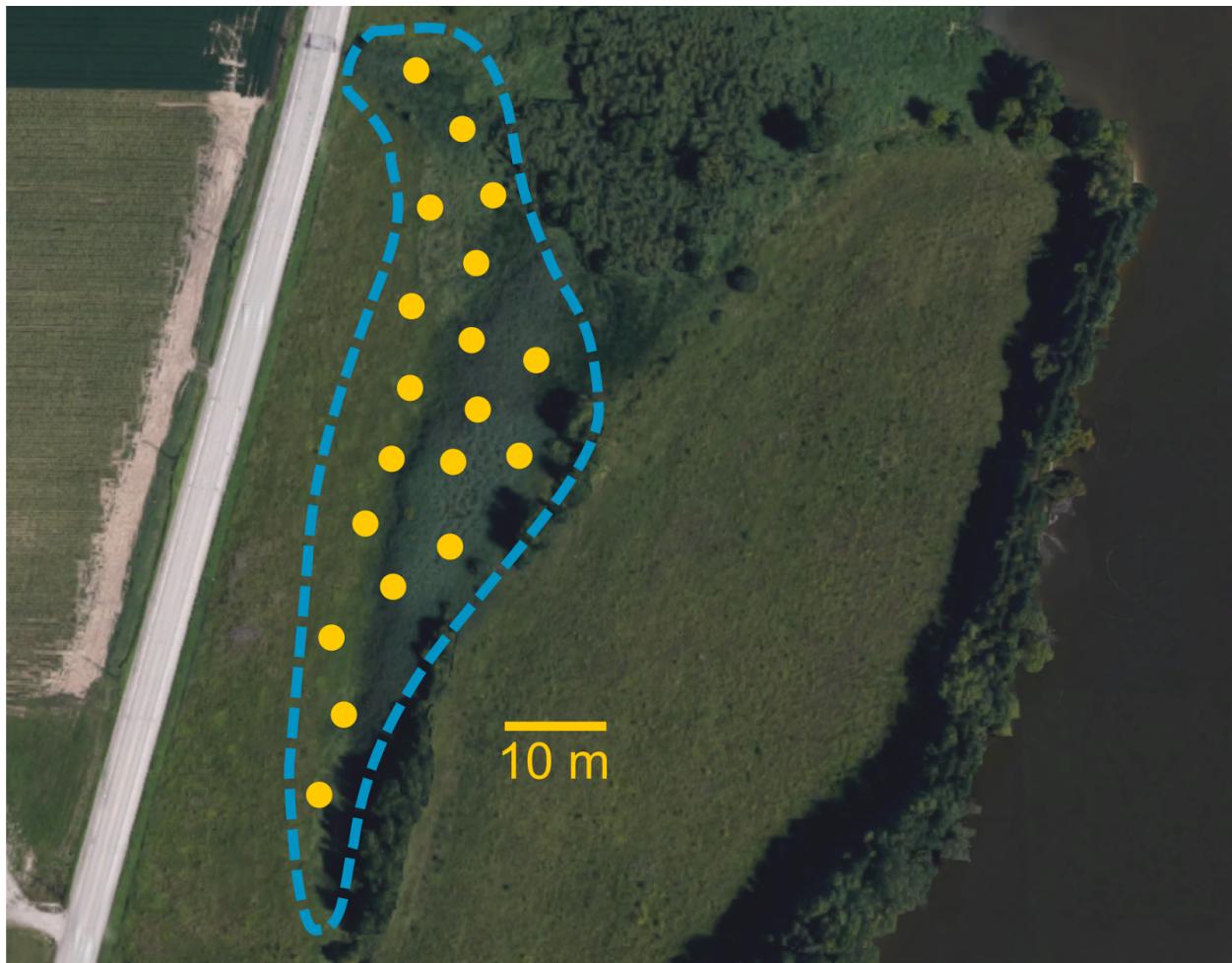
Overview—*Phragmites australis* (common reed) exists in northern North America as two subspecies: *P. australis* subsp. *americanus* is native and relatively rare, whereas *P. australis* subsp. *australis* is non-native, arriving originally from Eurasia and continuing to increase its range here. Our lab is able to assess the genetic diversity of common reed plants belonging to the native and non-native subspecies. We use the microsatellite technique, which analyzes specific pieces of DNA that vary in length among individuals. Microsatellite markers potentially can identify the source of introduction for plants of the non-native subspecies. In addition, microsatellite markers can assess genetic variation within a population. For example, populations that are spreading vegetatively (i.e., clonally) by rhizomes would have a uniform genetic signature, whereas populations that arose from seed would be expected to have more genetic variation. Microsatellite markers also offer the potential to detect hybrids between two *Phragmites* subspecies. For samples that we receive, we will analyze seven genetic markers to determine the similarity of plants to one another and to other local populations. Because plants can be genetically variable within a population, we recommend that you send material from 5–10 plants in the same population, making sure the individuals are separated from each other by at least 3 m (10 ft), to avoid the possibility of sampling genetically identical plants that are connected underground.

Deliverables—We will conduct microsatellite analysis on the samples you send and make the results available to you. *Unless otherwise specified, you acknowledge that samples and data belong to Dr. Tippery, who reserves the right to publish and otherwise disseminate results.*

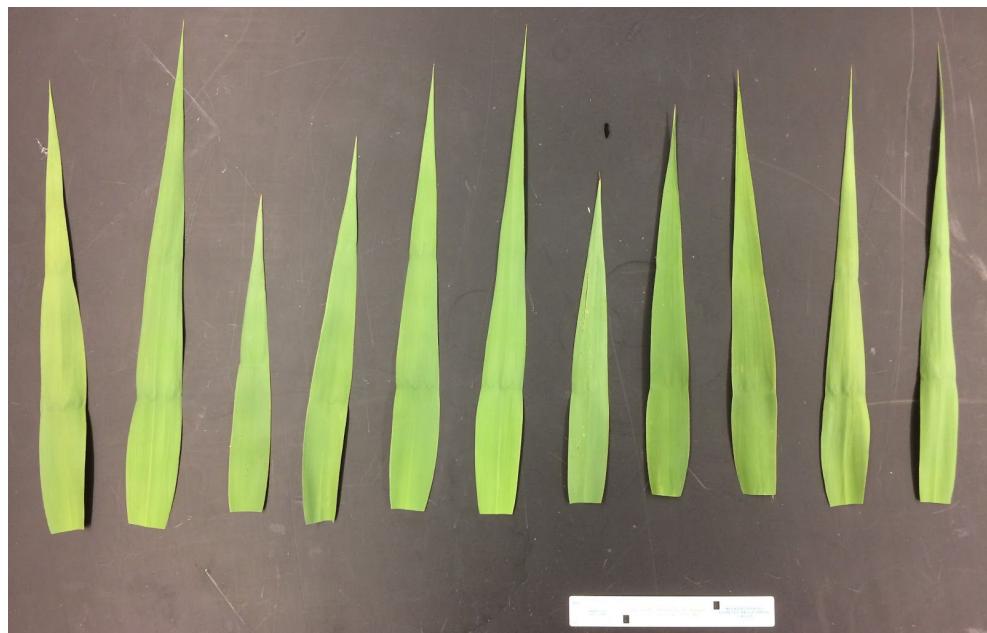
Service Agreement—You must complete the Service Agreement document before any analysis can be done. In the document, be sure to specify the number of samples to be analyzed and calculate the cost (see Pricing Schedule at the end of this document). Initial payment (50% of analysis cost) must be received before samples will be processed.

Collection protocol—

1. At each sample location ('population'), get a rough idea of how much area the population occupies. Ideally you would collect plants evenly throughout the population, but you may be limited by the degree to which plants can be accessed (totally understandable!). In the example population below, bordered by a blue dotted line, 20 yellow collection points are evenly spaced throughout. It is not necessary to keep track of the exact location where each plant was collected. Collect a maximum of 20 plants per population. Plants should be separated from one another by 3–10 m. If the population is so small that 20 plants cannot be collected at 3 m intervals, then please collect fewer plants.



2. For genetic analysis, we will need at least one leaf per plant. Leaves should be in good condition, mostly free of herbivore and fungal damage, tears, wilting, browning, etc. Younger leaves are best. The image below shows several leaves in good condition. Larger portions of plants are preferred, as these resist deterioration better and allow us to choose the best tissue for DNA extraction. **Note: We will assume that separate leaves came from separate plants, so please do not send multiple leaves from the same plant unless you clearly group them together (e.g., by putting them into the same bag).*



3. In addition, we will need a larger portion of one plant per population, to serve as a voucher specimen. This portion should constitute the top 50 cm or so of the plant. Plants in reproductive condition (flowering and/or fruiting) are optimal, but reproductive organs are not required. In the image below, the ruler is 15 cm (6 in) long.



4. For each plant to be sampled, remove either one high-quality leaf or the top portion of the plant. Younger plant tissues are located closer to the top, and these are preferred for DNA extraction.
5. Enclose leaves and upper stems in an airtight ziploc bag. Take care to avoid conditions that will hasten decay:
 - a. Keep the bag interior dry. Standing water will facilitate rapid decay
 - b. Keep the bag in a refrigerator until you are ready to mail the plants
 - c. Mail the plants as soon as you can after collecting them (1–2 days in the refrigerator should be fine)
 - d. Choose a shipping option such as USPS Priority Mail that will send the package to us in 1–2 days
 - e. Mail the plants earlier in the week so that they do not sit in a postal facility over the weekend
6. Complete the Chain of Custody Record on the following page (one per population), and enclose this with your plant samples
7. Mail the samples and email Dr. Tippery so that he knows to expect the samples. You can send a tracking number if you like, but this is not necessary
8. Data and analysis should be available within one month from the time samples are sent.
Summary files will be sent by email

Chain of Custody

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Please complete one form for each population.

Mail samples to:

Contact person: _____

Tippery Lab
Department of Biological Sciences
University of Wisconsin - Whitewater
800 W Main St
Whitewater, WI 53190

Organization name: _____

Email address: _____

Telephone: _____

Collector name: _____

Locality description:

Collection date: _____

County: _____

City or township: _____

Water body: _____

Latitude: _____

Longitude: _____

Site identifier (if used): _____

Number of plants sampled: _____

Notes:

Pricing Schedule

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Analysis	Price per unit	Quantity	Line total
Population genetic analysis – setup charge	\$95	n/a	\$95
Population genetic analysis – per sample charge	\$65		
		TOTAL:	