

Society for Range Management and Weed Science Society of America, Denver, CO. February 7-11 2010.

Keith B, Brummer T, Dyer W, Maxwell B, and Rew LJ

Deciphering dispersal patterns of Dalmatian toadflax.

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Deciphering dispersal patterns of Dalmatian toadflax

Barbara Keith, Tyler Brummer, William Dyer, Bruce Maxwell and Lisa Rew

Montana State University, Bozeman, MT



Introduction

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This study is creating, through herb-chronology (see poster PB-31) and genetic relatedness between individuals, a time-line for the invasion and expansion of a metapopulation of Dalmatian toadflax (*Linaria dalmatica*) located in the greater Mammoth region of Yellowstone National Park, WY. A metapopulation is defined as spatially separated patches of *L. dalmatica* whose proximity to one another allows interactions through cross-breeding. The degree of genetic variation within and among patches can indicate the directionality of the gene flow over the landscape¹. The work outlined here allows us to determine the contribution of vegetative growth and seed dispersal in population expansion for one site in the study area.

Methods- Sampling

Seventeen discrete patches of *L. dalmatica* were delineated and GPS mapped (~50 cm accuracy) within the study area (Fig. 3). Patches were defined by not having another individual within 2 m of any member of the patch².

Patch sampling:

- Perimeter of larger patches were GPS delineated.
- Individuals were mapped with GPS and GIS (~5 cm accuracy).
- Leaf tissue was collected from individual plants.
- For the largest patch "main", a 0.5 m wide area along the longest axis and two axes perpendicular to and bisecting the long axis were sampled.
- All other patches are considered "satellites".
- In the larger of the satellite patches, a single 0.5 m wide transect was sampled.
- All *L. dalmatica* plants were sampled in the smallest satellite patches.



Figure 1. Sample transect.

Methods-Molecular Markers

Inter-simple sequence repeat (ISSR) analysis was employed to develop phylogenetic maps of populations.

- Twenty ISSR primers were analyzed for the number of polymorphic markers generated.
- Genomic DNA from leaf tissue was subjected to duplicate polymerase chain reaction (PCR).
- Molecular markers were visualized through agarose gel electrophoresis (Fig. 2 and Fig. 4).
- Two primers³ were selected on the basis of the reproducibility and quantity of amplified bands (Table 1).

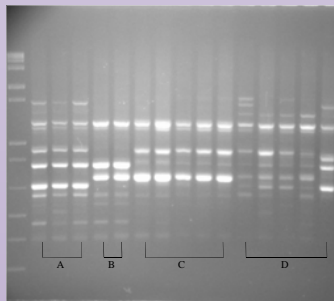


Figure 2. Inter-simple sequence repeat (ISSR) profile for 15 individual *L. dalmatica* stems PCR amplified with ISSR primer 5'-(AC)₆G-3' and visualized on a 1.5% agarose gel. For corresponding stem locations, see Figure 3 (A-C) and Figure 5 (D). The furthest left lane is the 1kb+ DNA marker (Invitrogen).

Table 1: Inter-simple sequence repeats (ISSR) primer sequences and amplification products

ISSR Primers	DNA fragment length (base pairs)	# bands	# polymorphic bands	% polymorphic bands
LR11; 5'-(AC) ₆ T-3'	325-1764	10	13	93
LR12; 5'-(AC) ₆ G-3'	400-1650	14	12	86

Results

Figure 4. Far right(A): Enlargement of a satellite patch. Sampled *L. dalmatica* stems were all part of the same genet (indicated by black circle). B) Right: Inter-simple sequence repeat (ISSR) profile for 8 individuals (positioned within the red circle (A)). Markers visualized as stated in Fig. 2.

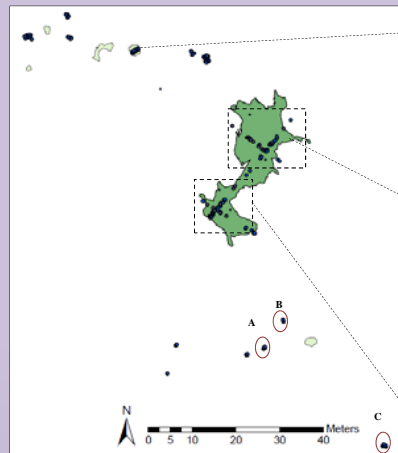


Figure 3. Distribution of *L. dalmatica* patches at study site. The main patch is shaded dark green. Larger satellite patches are shaded by light green. Blue dots represent individual stems sampled within the main patch and satellite patches. Red circles indicate location of individual stems profiled in Fig. 2.

Summary and Future Directions

- Both sexual and vegetative growth played a role in patch expansion of the main patch.
- Vegetative growth was predominant in the satellite patches (8/9 patches examined).
- The satellite patches represent separate genets.
- Due to the limited resolution of the agarose gel, we are currently size fractionating the PCR products on denaturing polyacrylamide gels. This will increase the number of molecular markers available to distinguish between true clones and very closely related individuals.
- Four sites within the metapopulation will ultimately be sampled to understand dispersal patterns across a larger landscape.

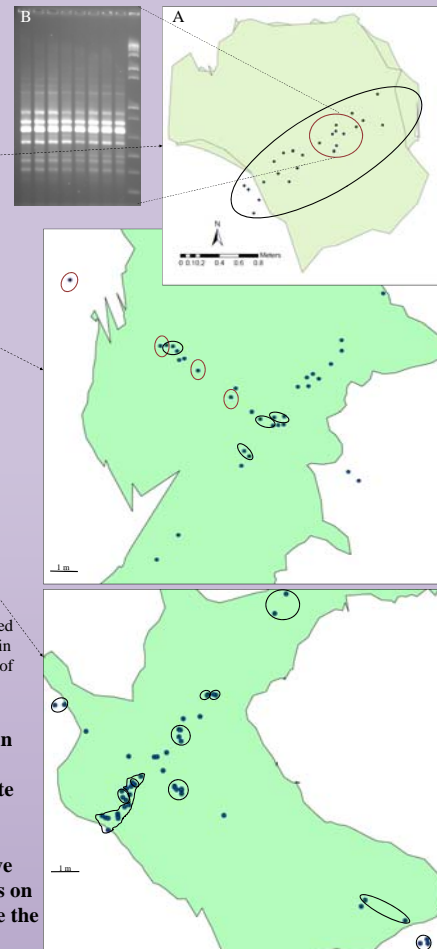


Figure 5. Enlargements of the main patch. Blue dots depict individual *L. dalmatica* stems. Genetically identical individuals are grouped into the same genet (black circles). Red circles indicate location of individual stems profiled in Fig. 2.