CIPM Research Grant Final Report

Title - The Role of Hybridization in Biological Invasions: A Study with *Centaurea maculosa* and *C. diffusa* (awarded 2005)

Investigators: Dr. Ruth A. Hufbauer
Colorado State University
Fort Collins, CO

Amy C. Blair
Colorado State University
Fort Collins, CO

Proposal

Hybridization between distinct species or genotypes can lead to evolutionary innovation (Arnold, 1997; Rieseberg and Carney, 1998). When the hybridizing entities are noxious invasive weeds, a vital concern is whether individuals of hybrid origin will exhibit characteristics that stimulate invasion (Abbott, 1992; Ellstrand and Schierenbeck, 2000). There is substantial evidence that hybrids between many species can be more fit than the parental species in certain environments, often environments that are novel to the parent species (Moore, 1977; Arnold, 1997).

Hybridization may be particularly frequent and ecologically relevant for invasive species for two reasons: (1) when species are introduced into a new range, distinct genotypes or species that are geographically disjunct in the native range can have more opportunities to mate, and (2) invasive species, by definition, are entering new environments, which are novel in myriad ways. Indeed, in a survey of the literature, Ellstrand and Schierenbeck (2000) found 28 examples of hybridization preceding invasiveness in many diverse plant families. Despite the potential importance of hybridization in invasion success, few studies have closely examined the underlying mechanisms and consequences of post-introduction hybridization.

The goal of this proposal was to initiate research to evaluate the ecological consequences of hybridization between the noxious weeds spotted and diffuse knapweed (*Centaurea maculosa* and *C. diffusa*) for their invasion and biological control. For the immediate research objectives, we propose to conduct a combination of phenotypic surveys of hybrid populations and biological control agents; long-term research objectives include molecular surveys, and field and greenhouse experiments.

Results

During summer 2005, Blair surveyed 47 populations chosen in a stratified random manner (19 spotted and 28 diffuse knapweed) in 6 western states (CO, ID, MT, OR, WY, WA). At each site, she visually inspected the population to look for individuals with hybrid morphology. Floral characteristics are diagnostic for the parental species and the described hybrid, *C. x psammogena* (Ochsmann 2000). Thus, seven floral traits provided a consistent way to divide the plants among the 3 classes (Fig. 1). Twenty-nine of the 47 populations contained plants with hybrid characteristics, which ranged from 4-78.7% of the population. Interestingly, populations with parental diffuse-like individuals had higher levels of hybridization – 25 out of 28 popualtions vs. 4 out of 15 spotted populations. At 30 randomly selected sites, Blair conducted a more intensive survey, measuring plant height and diameter, stem number, polycarpy or monocarpy (evidence of last year's stalk), and floral traits of 30 randomly selected individuals. Using a model with the main effects of geographic region and classification (hybrid, spotted, or diffuse), hybrids show intermediate morphology for traits related to plant size (i.e. height, number of stems, and plant diameter).

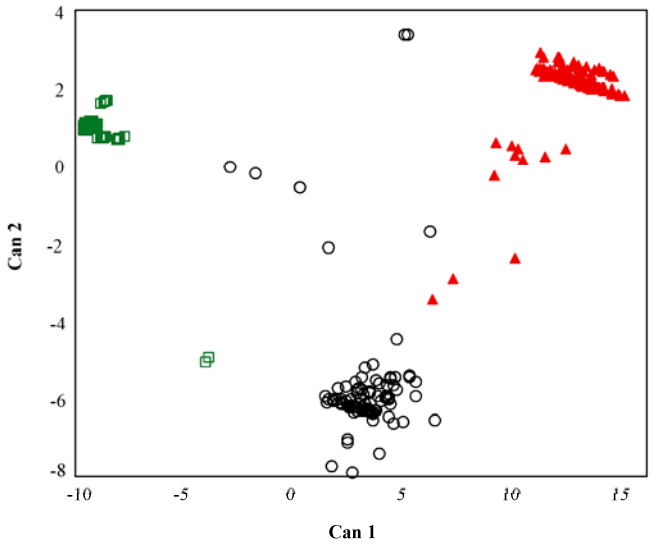

Fig. 1. A plot of the first two canonical variables. Floral characteristics used in this analysis include: flower color, capitula width, capitula length, spine length, presence or absence of vertical vein on bracts, darkness of spot on bract, and presence or absence of golden bracts. Red triangles=diffuse, open circles=putative hybrids, and green squares=spotted knapweed.

Additionally, Blair surveyed nine of the spotted-like and nine of the diffuse-like populations for the four common biological control seed feeders (5 seedheads per each of 30 plants). Interestingly, the proportion of putative hybrids with larvae in seedheads was less than spotted and diffuse plants, but only significantly less than spotted knapweed.

Discussion

It is quite apparent that diffuse knapweed has experienced significant introgression from spotted knapweed. Recent work from collaborators in Europe suggests that the spotted knapweed in North America is completely tetraploid, while we are fairly certain that the diffuse is diploid. Thus, we believe that introgression is not ongoing, but instead diffuse knapweed was introduced as some type of a hybrid swarm. It is interesting that the hybrid-like phenotypes have been maintained in populations. It is quite plausible that the introgressed genes confer some advantage to diffuse knapweed, and that is currently where we are directing our efforts.

Surveys during summer 2006 in the Ukraine showed that spotted and diffuse knapweed distributions overlap frequently in that country, and when occurring in the same

population, hybrid swarms result. It seems plausible that diffuse knapweed was introduced from such a swarm. Collaborators in Turkey and Romania, where spotted and diffuse do not overlap extensively, have informed us that they do not see hybrid-like phenotypes in diffuse knapweed populations. Thus, it is interesting to ask the question if the hybridization of the species has resulted in these highly successful invasive individuals.

Publications

Currently N/A. At minimum, one or two papers will come out of this work. Potential titles and journals for submission are listed below.

- Morphological and Molecular Evidence that the Noxious Weed, Diffuse Knapweed, is of Hybrid Origin. Potential journals for submission – *Molecular Ecology, Biological Invasions, Oecologia*

- Is Hybridization a Stimulus for Invasion? An Experimental Study with Diffuse Knapweed. Potential journals for submission – *Evolution, Biological Invasions, New Phytologist.*

Literature Cited

Abbott RJ (1992) Plant invasions, interspecific hybridization and the evolution of new plant taxa. *Trends in Ecology & Evolution* **7**, 401-405.

Arnold ML (1997) *Natural hybridization and evolution* Oxford University Press, Oxford, UK.

Ellstrand NC, and Schierenbeck KA (2000) Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences of the United States of America* **97**, 7043-7050.

Moore WS (1977) Evaluation of narrow hybrid zones in vertebrates. *Quarterly Review of Biology* **52**, 263-277.

Ochsmann J. (2000) Morphologische und molekularsystematische Untersuchungen an der *Centaurea stoebe* L.-Gruppe (Asteraceae-Cardueae) in Europa. - Diss. Bot. 324 (Ph.D. Dissertation) 242 pp.

Rieseberg LH, and Carney SE (1998) Plant hybridization. *New Phytologist* **140**, 599-624.

Products

As Blair planned her trip around the west, she contacted over 50 local county weed agents in six different states. Additionally, she made contacts with local professors in several of the areas she visited. It is her current plan to send all published findings to

each of the contacts to help inform them on management decisions regarding spotted and diffuse knapweed. Both Hufbauer and Blair are committed to outreach activities; this past year, Blair gave a lecture to AP High School students about the importance of invasive species and also spent a morning teaching minority elementary students about basic molecular tools. The findings from this research will be incorporated in future outreach events.

Long-Term Goal/s and Continued Progress of Research

Blair collected leaf tissue from the same 30 individuals in each of the 30 populations mentioned above for DNA analyses aimed at exploring whether phenotypic evidence of hybridization is supported by the genotype. Taking a molecular approach also will allow me to identify introgression in the genome that may not be manifest in the phenotype. I will use both microsatellites and amplified fragment length polymorphisms (AFLPs). Blair has successfully made F1 hybrids between spotted and diffuse knapweed, and she is currently crossing these individuals in to diffuse knapweed to make Back Cross 1 (BC1) individuals. A greenhouse common garden with both BC1 and parental spotted and diffuse knapweed individuals will be conducted to elucidate if hybrids show traits that would confer an advantage to them in North America. For example, we will simulate competitive and ruderal environments and examine hybrid performance relative to parental phenotypes. Finally, we plan to mentor an honors student on project to ascertain if a common biological control agent favors hybrids or parents, or shows no preference.

Benefits of Seed Money

This seed money grant was critical to initiate work on the role of hybridization in the invasion of spotted and diffuse knapweed. This timely and important topic is the major component of Blair's PhD dissertation research, and without this money, she would have struggled to obtain as comprehensive data across the western United States. Without this field data as a foundation of knowledge about these species, proposed lab and greenhouse work would have little meaning. Additionally, the findings from this work have been incorporated in additional grants that are currently funding on-going projects. Hufbauer received an NSF Seed Grant and Blair received an EPA STAR Fellowship and an NSF DDIG. It was requisite in obtaining this funding to have solid, comprehensive data, which was only possible with the funding provided by the CIPM seed money grant.

Advancing This Research

This work is currently being advanced with the aforementioned greenhouse experiment and molecular work.

Website – N/A

Budget

Domestic Travel – $2883.52
A majority of the grant money went to pay for lodging, food, and transportation for Blair to visit populations of spotted and diffuse knapweed across the west. She traveled for a total of 6 weeks during summers 2005 and 2006.

Materials and Supplies - $198.60
Blair purchased flagging and other basic field materials (a cooler, ice packs, etc.).

Personnel - $1350
Because Blair did not use all of the funding for travel, an undergraduate student was hired to help process the samples that were collected. Opening seed heads of spotted and diffuse knapweed is very labor intensive.

Indirect Costs - $443.21