Environment and Natural Resources Trust Fund

Research Addendum for Peer Review

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Project Title: **Conserving prairie plant diversity and evaluating local adaptation:**

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1. Abstract

Tallgrass prairie, which extended across a major portion of Minnesota prior to European settlement, has been reduced to small remnants by agricultural and other uses. Recent and growing recognition of the prairie biome's importance of this biome for the ecological services it provides, for its evolutionary significance, and for its beauty, motivates large-scale restoration of prairie vegetation. Two serious problems hinder this effort: a) the seed available for prairie species does not meet the demand of restoration projects and b) there is limited understanding of the fitness consequences of transferring plants and micro-organisms among regions of the state. With this project, we will take major steps toward addressing both problems. For each of 16 species, we will conduct a large-scale program of gathering seeds from a dozen populations throughout the state and samples of these will be *archived for long-term conservation*. We will also conduct a three-pronged research program to inform practice in restorations. With six of these species, we will establish long-term studies of *the geographic scale of local adaptation.* Focusing on one of these species, we will conduct an intensive quantitative genetic study to assess the potential within multiple populations for *adaptation to ongoing environmental change.* We will determine the potential for symbiotic microorganisms on prairie plants to help or hinder successful prairie restoration. Results from these three studies will bear directly on choices of genetic sources for restorations and on decisions about managing micro-organisms in conjunction with restorations. Genetic material obtained for this project will be made available to seed producers for restorations.

2. Background

Since European settlement, the once vast expanses of MN tallgrass prairie totaling 18 million acres, have been diminished to small remnants totaling less than 1% of their former extent (see Fig. 1). Concomitantly, the tremendous genetic diversity within hundreds of prairie species has been drastically reduced. Plant populations in the scattered, prairie remnants are subject to severe inbreeding depression (e.g., Wagenius et al. 2010a), which can lead to further population decline and slow adaptation (Falconer and Mackay 1996, Shaw et al. 1998). This is of particular concern given ongoing climate change.

At the same time that prairies are declining, it is increasingly recognized that they play important roles for:

- support of diverse wildlife
- roadside stabilization and beautification
- sustainable harvest for biomass fuels
- improvement of water quality

In addition to valuing these essential ecological services, enthusiasm for preserving the natural beauty of prairies for future generations is spurring efforts to restore diverse prairie communities on extensive scales. However, such large-scale prairie restorations face daunting challenges.

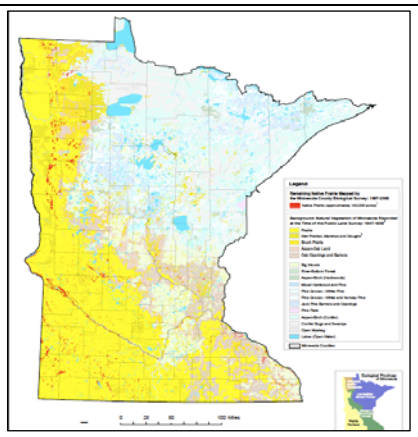

Figure 1. Vegetation types of Minnesota at the time of the Public Land Survey (1847). Original prairie is indicated by yellow and tan. Remaining prairie (Minnesota County Biological Survey; 2000-2010) is indicated in red.

To thrive, large-scale restorations require large quantities of seeds adapted to the environment in which they will grow (Hufford and Mazer 2003). Minnesota's prairie plants have been adapting to their local climates and soils since the glaciers receded 14,000 years ago. Thus even small remnants of prairie, scattered over the 4 subsections of MN's Prairie Parkland Province, contain valuable genetic resources. Associated pathogens and beneficial microbes such as nitrogen-fixing bacteria adapted along with the plants. However, climate change is now occurring at a rate too fast for plants to adapt (Etterson and Shaw 2001). Consequently, it is likely that climate change will be accompanied by losses of native plants, possibly in part due to disease, with the invasion of noxious weeds filling the space left behind. Taking steps now to protect and preserve the remaining genetic resources will help to insure the availability of genetically variable seed as germplasm for massive restorations, for adaptation to climate change, and for new uses yet to be discovered.

With the goal of preserving prairie plant diversity in Minnesota, we propose to accomplish the following outcomes: 1) conserve germplasm of prairie species from locations throughout the prairie region of Minnesota, 2) elucidate the scale of local adaptation in these plants, 3) assess prairie populations' potential for adaptive evolutionary response to current and novel selection, and 4) determine effects of microorganisms on prairie plants transferred between sites. Together, these results will provide information necessary to the state's effort to establish scientifically sound and economically feasible criteria for use of these prairie resources, while addressing questions of fundamental interest. Because the generation time of prairie plant species is typically long (e.g. greater than 20 yr estimated for *Echinacea angustifolia*, Hurlburt 1999), we envision continuation of this program well beyond the three-year period of funding from LCCMR to begin in 2011. We here provide detailed plans for conducting this project in its first three years.

3. Premises and Hypotheses:

I. Efforts to collect and preserve genetic diversity of Minnesota's prairie plants are essential both to avert their loss altogether and to support prairie restoration throughout the prairie region of the state.

II. Populations of prairie species are adapted to local abiotic conditions, as well as to the other organisms in their local communities. However, the geographic scale of local adaptation is not known and must be experimentally determined to inform the choice of germplasm for prairie restorations.

III. The genetic variation residing in populations of prairie species is the basis for ongoing adaptation. The amount of genetic variation, which determines rates of adaptation under natural selection, must be determined experimentally.

IV. Symbiotic microorganisms of plants are most beneficial when orginating from the same locality as the plant population. Microbes are more often pathogenic when orginating from a different location than the plant population.

4. Methodology

<u>Activity I. Seed collection and conservation</u>

Overview: We will make extensive collections of seeds of at least 16 species characteristic of Minnesota prairies for conservation and research. We will sample moist and dry habitats in at least 3 populations in each of 4 ecologically defined subsections of the state (Fig. 2), taking care both to ensure that genetically representative samples are obtained for each population and to avoid severely reducing natural seed input to the site. This component of the project parallels the current Seeds of Success (SOS) program of the Bureau of Land Management, involving many organizations and individuals within the US (www.nps.gov/plants/sos). The goal of the SOS program is to gather seeds for many plant species throughout the US and archive them for conservation. Our effort is distinct from SOS, because we will sample the genetic diversity of Minnesota intensively and systematically, as required to comprehensively evaluate the geographic scale of local adaptation (detailed in 2 below) and to establish a germplasm repository that will support prairie restoration specifically within and throughout the state of Minnesota. Samples will be stored in the state-of-the-art facility at the USDA National Center for Genetic Resources Preservation in Fort Collins, Colorado, at no cost to the state of MN. This federal facility has developed best practices to maximize seed viability over long-term storage, and facilities and staff there cannot feasibly be duplicated in MN. Seed resources will not be deployed to Colorado or any other state or private agency, and the state of MN and this project will retain ownership of all seeds. Our colleagues at the seed facility will assess viability and study the longevity of seeds.

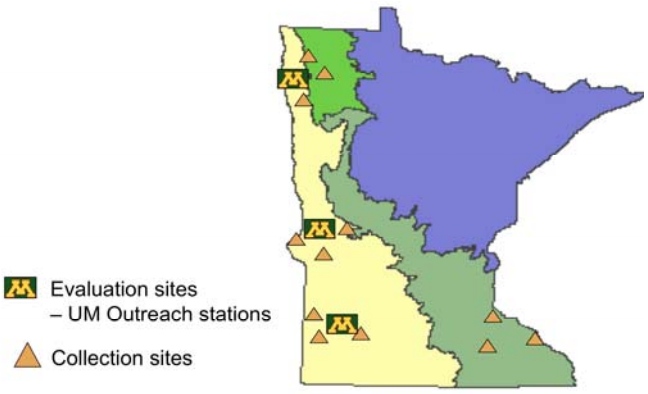

Figure 2. Minnesota's 4 ecoregions with collection sites (orange triangles) and experimental evaluation sites (green rectangle) shown.

Plans in Detail: During the initial period of funding, we plan to obtain genetically representative samples of seeds of at least 16 species, chosen from those documented to occur throughout the prairie region of the state (MacDonagh and Hallyn 2010, pp. B-7 - B-14). Among the species chosen, we will include early, mid- and late-flowering species. The set of species we collect will be chosen from those listed in Table 1; the final list will necessarily be determined by availability of populations that meet the criteria specified below.

Table 1. Potential species for seed collection

Grasses:	Forbs:
Bromus kalmii-Kalm's brome *Carex brevior*-Short sedge *Elymus canadensis*-Canada Wild Rye *Elymus trachycaulus*-Slender wheatgrass *Koeleria macrantha*- Prairie Junegrass *Panicum virgatum*-Switchgrass *Schizachyrium scoparium*-Little bluestem *Sorghastrum nutans*-Indian grass *Spartina pectinata*-Prairie cordgrass *Sporobolus heterolepsis*-Prairie Dropseed <u>Legumes:</u> *Amorpha canescens*-Lead plant *Astragalus crassicarpus*-Groundplum milkvetch *Astragalus canadensis*-Canada milk vetch *Dalea purpurea* – Purple prairie clover *Glycyrrhiza lepidota*-Wild licorice *Psoralea esculenta*-Prairie turnip *Vicia americana*-American vetch	<u>Composites:</u> *Aster sericeus* *Coreopsis palmata*-bird's foot coreopsis *Echinacea angustifolia*-narrowleaved purple coneflower *Helianthus pauciflorus*-stiff sunflower *Heliopsis helianthoides*-smooth oxeye *Liatris ligulistylis*-northern plains blazing star *Solidago rigida*-stiff goldenrod <u>Others:</u> *Allium stellatum*-prairie wild onion *Anemone cylindrica*-long-headed thimbleweed *Anemone patens*-pasque flower *Asclepias syriaca*-common milkweed *Galium boreale*-Northern Bedstraw *Gentiana andrewsii*-bottle gentian *Geum triflorum*-prairie smoke *Heuchera richardsonii*-alumroot *Lobelia spicata*-rough-spiked lobelia *Monarda fistulosa*-wild bergamot *Oenothera biennis*-common evening primrose *Phlox pilosa*-prairie phlox *Thalictrum dasycarpum*-Purple meadow rue *Verbena hastata*-Blue Vervain *Viola pedatifida*-bearded birdfoot violet *Zizia aurea*-Golden Alexander

We plan a stratified sampling scheme: three populations within each of four major regions of the original extent of tallgrass prairie in MN (Fig. 2) following a protocol based on that

of the SOS program (http://www.nps.gov/plants/sos/protocol). The sampling plan is motivated by two goals: 1) to capture a large proportion of the genetic diversity of each species and 2) to make our experimental evaluation of local adaptation more rigorous. We plan to sample across the greatest distances (up to 800 km) and across the extremes of MN's climates (for example, drought stress varies threefold across the prairie region, Thompson et al. 2000). Accordingly, we will gather seeds in four regions of the state (northwest, west-central, southwest and southeast), from three mesic sites in each region. As sampling sites, we will exclusively use locations harboring original, unploughed prairie. Some of the species listed (Table 1) have been widely planted in Minnesota from non-native sources. We will take particular care to avoid sampling populations likely to be genetically mixed. The Scientific and Natural Areas administered by the MN Department of Natural Resources are important reservoirs of native prairie, as are additional land holdings of the MN DNR; we have obtained provisional agreement from the DNR to collect seeds. We also have agreement, in principle, that The Nature Conservancy will allow us to collect. We will formalize these agreements and seek additional population sources.

To the extent possible, we will make seed gathering efficient by focusing our efforts in sites that harbor populations >200 flowering individuals for each of several of our target species, as established in preliminary visits to sites while plants are flowering. If populations this large are not available, we will gather seeds from populations no smaller than 60 individuals, both to avoid depleting populations and to ensure that samples are representative. In order to secure genetic diversity within populations, we will collect seeds from plants that are minimally 3 m apart. As possible within each mesic site, we will sample seeds from plants occupying more moist low-lying areas and also those occupying drier hilltops within each site, keeping these collections distinct. We will also gather seeds on multiple dates throughout the period of seed maturation to ensure that plants spanning a wide range of phenologies are represented. In preliminary visits to the sites, we will mark plants that satisfy these criteria.

Seeds will be collected as they mature. Those from a single species, site, habitat (moist vs. dry), and gathered on a given date will be bulked together and mixed thoroughly. Each sample will then be divided, and half shipped as soon as possible to the USDA National Center for Genetic Resources Preservation in Fort Collins, Colorado. There, they will be archived in conditions chosen to maximize the longevity of seeds. A portion of the samples will be available to researchers at NCGRP for investigations of seed viability and longevity. Half will be retained as the basis for the experiment described below (2). Altogether (i.e., over multiple collecting dates), we will plan to gather at least 6,000 seeds from 60 individuals (but minimally, 30 individuals) from each population in order to include 95% of alleles having frequencies at least 0.05 (Brown and Marshall 1995). For each population, a voucher sample of leaves and flowers will be collected, pressed and archived in the University of Minnesota Herbarium.

Both the collections of seeds archived for the long-term at NCGRP and the plants growing in the large-scale, long-term experiment proposed here will constitute a lasting repository of genetic variability for a substantial number of MN prairie species. Samples of this genetic material, primarily as seeds produced by plants in the experiment and secondarily from NCGRP, will be made available to MN seed producers at nominal cost; however, due to policy established for SNAs, germplasm drawn from SNA sites must be excepted.

Activity II. Establishment of long-term studies of local adaptation.

Overview: Experimental studies spanning over a century have established that it is common for local populations to exhibit greater fitness, contributing more offspring to the next generation, in the environment in which they originated, compared to populations introduced from elsewhere (reviewed most recently by Hereford 2009), yet the scale of local adaptation is unclear (Mackay et al. 2005). Experimental designs used to assess such local adaptation typically do not reveal its geographic scale, nor do they indicate the roles of particular habitat

characteristics, both abiotic and biotic, in performance of organisms in their home and distant sites. No systematic, experimental comparison of the degree of local adaptation of species differing in key respects has been conducted, although Leimu and Fischer (2008) found that small populations (< 1000 flowering individuals) are less likely to be locally adapted than larger ones. Consequently, it is not clear whether species having outcrossing breeding systems are likely to be locally adapted on a finer or coarser scale, and to what degree. Moreover, theory shows that prediction of this relationship is not straightforward (Lopez et al. 2009). To the extent that prairie populations are highly locally adapted, the success of prairie restorations will depend critically on the genetic sources chosen. It is, thus, urgent to increase understanding of the degree and geographic scale of local adaptation of prairie plants.

Plans in Detail: To rigorously evaluate the degree of local adaptation, we will initially focus our efforts on 6 species that typify MN prairie making use of the seeds collected described above. In future years, we plan to expand this experiment to include a considerably larger array of species, but, given the resources we anticipate will be available during the initial period of funding, it is feasible to carry out an informative study on a maximum of six species. These will be chosen to include at least one representative of each of the groups: grasses, forb, legume. Species flowering early and late in the season will also be represented. Few species characteristic of tallgrass prairie are strongly inbreeding, however, we will make an effort to include a species representing this breeding system to contrast with a high degree of outcrossing, which is more typical of prairie species.

We will plant seeds from all 12 populations sampled for each species at all three experimental evaluation sites (Fig. 2) corresponding to three of the four sampling regions represented in the seed collections. Because this approach imposes common environmental conditions on samples of distinct populations, it provides a rigorous framework for inference of the extent to which differences among populations in growth and other traits are attributable to genetic differences. Moreover, because replicate populations from each region will be sown into each of the sites, such that each will be grown in its own 'home' region, as well as in the two alternate ones, see Fig. 2), the study will be exceptionally informative with respect to local adaptation. We will significantly reduce costs by utilizing existing University of Minnesota Research and Outreach Centers (ROCs). We will monitor survival and growth of plants from each sampled population at each site in order to determine the relationship between plant survival and growth, on the one hand, and, on the other, the experimental factors: region of garden, region of origin, and soil moisture in location of origin.

The locations for the experiment are Crookston MN, representing the northwest, Morris MN, in the central west, and Lamberton, in the southwest. Within each ROC, chosen to avoid areas that have recently received fertilizer applications. Sites will be prepared in advance, as follows. To reduce the abundance of non-native vegetation, the plots will be tilled in the spring. At that time, seeds of the prairie grass, *Bouteloua curtipendula*, obtained from a local source, will be sown into each site as a natural matrix within which seeds of the focal species will be sown.

At each location, we will establish arrays of plots in a randomized complete block design. Each of the 12 populations of each of the six species will be represented once in each block. The 72 species-populations will be assigned to random positions within each block and, at each position, a random sample of 100 seeds will be sown in rows into plots, 20 cm x 20 cm. Ten replicate blocks will be established according to this design. Altogether 2160 plots will be sown (3 planting sites x 10 blocks/site x 6 species x 12 populations/species). To allow comparison of accessions from moist vs. dry sites, this basic design will be expanded to accommodate these samples according to their availability. For example, if there are samples from both dry and moist habitats for 10 source locations for 3 of the species, 30 plots will be added to each block, resulting in altogether 2460 plots.

We plan to establish the experiment from seed for two reasons. First, restorations are most often carried out with seed; to the extent that traits of seeds and juvenile plants contribute to local adaptation, comparison of populations that take into account differences in performance at the seed and seedling stage will bear most informatively on the importance of local adaptation for restorations. Second, a new approach to analyzing data on life history, aster, recently developed at the U of MN (Shaw et al. 2008), makes joint analysis of binary outcomes (like survival) and measures (like size) straightforward.

Our recent experience indicates that the size of plots and groups of 100 seeds are appropriate. In field experiments to evaluate recruitment of *Echinacea angustifolia* from seeds, emergence of seeds gathered from the wild in four different years ranged from 1% - 20% (Wagenius et al. 2010b). At this range of germination and somewhat greater, the plots of area 400 cm^2, a size that is relatively easy to search for tiny, inconspicuous seedlings, can be expected easily to accommodate plants that emerge and through 10 years of establishment and early growth.

Seeds will be sown into the experiment in the fall so that they will undergo natural field conditions through the winter. It is common that seeds of prairie species are dormant at the time they are released from the plant and break dormancy only after freezing or nicking of the seed coat, which in nature occurs over the winter.

We will monitor seedling emergence in the plots early in the following year, late May/early June. Seedlings will be individually ringed with birdbands, and their locations will be precisely mapped. In the first summer after emergence and each subsequent summer, the survival and size (leaf number and length of longest leaf) of each seedling will be recorded. In the event of essentially complete failure of germination or establishment of any of the species, we will sow in seeds of another species in the following fall. Occurrence and abundance of introduced species in plots will be recorded so that we can account for variation in competitive conditions in our analyses. Additional seedlings that emerge in subsequent years will also be recorded and measured.

For each species, the fitness of the populations in the three locations will be evaluated and compared using aster analysis. The basic analysis will take into account the numbers of seedlings that emerge in each plot, their survival at each observation time, and the size of each. Fitness differences among populations growing in common conditions are likely due to genetic divergence among the populations. However, it is possible that differences in the conditions at the collecting sites where the seeds developed contribute to phenotypic differences among the populations. Such environmentally induced maternal effects are generally found to be greatest during the earliest life stages, dissipating with age (Roach and Wulff 1987). We will evaluate the role of maternal effects and distinguish genetically based population differences by testing for differences in fitness among populations once the earliest stages are taken into account using nested models (see first example in Shaw et al. 2008). We will evaluate the degree of local adaptation by comparing the overall fitness of the populations sampled nearest to each experimental site with those from more remote locations. We will also test for the role of source habitat (moist vs. dry) in the expression of fitness in each location.

Because many prairie species are long-lived, this experiment will become more informative the longer it runs. We plan to monitor it for at least a decade in order to make fitness comparisons that take into account fecundity variation in reproductive output, in addition to survival and size. If necessary, we will thin plots at random to maintain manageable, biologically realistic densities. Beyond this, we will allow access to the experiment to colleagues who can use it to address further scientific questions for which it is well suited. For example, to what extent do populations differ within each site with respect to herbivory? In this way, the experiment can be viewed as a scientific observatory that can offer insights into questions well beyond the particular ones that are our foci.

Activity III. <u>Evaluation of the potential of prairie populations to adapt.</u>

Overview: The rate at which populations adapt to change in their environment depends on the amount of genetic variation for fitness in novel conditions. Quantitative genetic approaches are designed to rigorously evaluate precisely such properties of populations; molecular genetic variation poorly represents genetic variation for quantitative traits, including fitness (Reed and Frankham 2001). Etterson (2004 a, b, Etterson and Shaw 2001) employed a suite of quantitative genetic methods to assess the adaptive potential of an annual species of the prairie, *Chamaecrista fasciculata*. She detected considerable genetic variation for fitness in each of three populations drawn from widely distant locations. Nevertheless, she inferred that adaptation is likely to lag considerably behind the predicted changes in climate because of adverse correlations between traits under selection.

To obtain the pedigreed population required for the quantitative genetic study, we plan to carry out crosses on plants growing within natural populations, as we have previously done successfully (Montalvo and Shaw 1996, Heiser and Shaw 2006). This plan will achieve the greatest realism, while also producing the pedigreed population as quickly as possible. We will also grow samples of our focal species from seed in a greenhouse. If these reach reproductive maturity within a year, they can more conveniently be used as the parents of our crosses instead. Below, we focus on the more challenging plan of field crosses.

In our studies of adaptive potential, we will focus initially on geographic location and key aspects of changing climate, including soil moisture. However, we will also be able to extend these studies to evaluate genetic variation in response to particular micro-organisms (4. below). We will assess genetic variation by conducting quantitative genetic studies of plant traits likely to be under selection; for example, leaf thickness is often important for adaptation to drought. Given the labor-intensive nature of this kind of study, we will focus it on one species that is also represented in the local adaptation experiment (2. above). As candidates for this component of the project, we are considering *Aster sericeus, Coreopsis palmata, Lobelia spicata, Thalictrum dasycarpum.* The final choice of focal species will be based on availability and size of populations.

Plans in Detail: For the focal species, we will obtain a pedigreed sample of seeds from each of three populations by carrying out formal genetic crosses on plants *in situ*, as follows. Within each population, at least 60 individual plants will be chosen at random to serve as parents from among those that are initiating flowering. Each will be marked and its location recorded using GPS. To make the crosses maximally informative, we plan to conduct them as 10 independent reciprocal factorials each involving 6 parental plants (each of 3 plants pollinated by each of 3 other plants, and vice versa). Our goal will be to obtain 150 seeds per mating. To prevent access of insect pollinators and herbivores to the flowers, we will cover them with netting while the flowers are receptive. Thereafter, the netting will be removed during the period of seed maturation. Flowers may be covered again toward the end of this period so that seeds are not lost to natural dispersal.

The resulting progeny will be grown at three sites, one near each source population. Within each site, the family groups of all three populations will be assigned to random locations within each of five blocks. Seeds will be sown in groups of 30 during the fall. The following spring, we will monitor seedling emergence, including timing. During the summer, survival and size of individuals will be recorded, as above, as will measures of plant size.

Analysis of the data will employ aster modeling to evaluate the genetic variation in fitness for each population in each location. It will be of particular interest to compare the genetic variation in fitness of populations growing in warmer, drier sites than their source locations, as a basis for inferring the potential of those populations to adapt to warming climate. Supplementary studies of a subset of individuals will be undertaken to evaluate selection on traits that likely bear on drought tolerance, including leaf thickness and density of stomata.

Activity IV. <u>Determination of effects of microorganisms on prairie plants transferred between sites.</u>

Overview: Pathogens, or the lack of suitable mutualistic symbionts can be a major constraint on the establishment of new natural or agricultural plant populations (Burdon 1987). Moreover, seed cleaning typical of restoration efforts removes neither pathogenic nor beneficial fungi within seeds. In this research, we will determine the positive and negative impacts of microbes on the process of prairie restoration and prairie plant adaptation. Information on local adaptation in plant-associated microbes will help to manage these resources to enhance restoration efforts.

Plans in detail: We hypothesize that symbiotic microorganisms of plants are most beneficial when originating from the same locality as the plant population. Microbes are more often pathogenic when originating from a different location than the plant population. To test this, we will examine the effects of microbes on plant survival, in local and more distant populations.

In the above local adaptation experiments, 6 focal plant species from each of 12 populations will be planted into 10 replicate blocks at each of the three experimental evaluation sites giving a total of 2160 experimental plots.

a) As these are being monitored for seedling emergence and plant survival, data will be collected on pathogens occurring on aerial leaves and stems. Pathogens will be identified visually and by the molecular sequence methods described in b).

b) Using results from a), we will choose 3 plant species for more intensive study - likely a legume or forb demonstrating variable levels of disease across plots, and two grass species. Small portions of 3 plants per population, for 3 replicate blocks (324 plants total per species) will be removed to the lab to determine plant-associated bacteria and fungi. These microbes will be isolated from the plant and identified using molecular sequencing methods as previously (Pan, Baumgarten et al. 2008; Pan and May 2009).

c) In these experiments, we will test the central hypothesis that microbes from "home" sites are more often beneficial to plant growth and reproduction than are microbes from "away" sites. In addition, we want to explore whether some microbes might provide "protective" mutualisms - microbes protect against disease or herbivory by insects and mammals. We will germinate seeds for the 3 focal species in porous fabric "pots" in the greenhouse to obtain 60 plants per species per evaluation site, or 180 plants per species in total. For each of the three experimental "home" sites, 10 plants grown from seed of each experimental site (30 total) will be retained in the greenhouse as controls, and 10 plants will be planted out (outplants) into that "home" site experimental garden, plus 10 outplants to each of the other two "away" site experimental garden. These outplants will grow at each site for 6 weeks to "collect" symbiotic microbes. We then plan to return all experimental plants to a UM St. Paul greenhouse where we will subject both controls and microbe-associated plants will be subjected to the most common pathogens from each "home" site described in a) and b). For example, the plants grown at the Lamberton site will be subjected to Lamberton-collected pathogens, and both the controls and the outplants will include plants from the "home" site and plants from the "away" sites. We are bringing all plants back to the St. Paul campus to maintain quality control of pathogen applications, to monitor results on a daily basis, and to minimize travel time to complete the experiments.

Analysis: **a)** A list of the most common pathogens per plant species and per site will be recorded. For many prairie plants, pathogen species are unknown. We will also evaluate the level of disease incidence in each plot, thus gaining valuable information regarding differences in the distribution of pathogens across sites. **b)** The combination of culturing and molecular sequence information will yield a broader assay of the microbes in plants, regardless of whether we observe visible disease symptoms in a). We will then use analysis of variance and other approaches such

as principal components to test whether their distribution differs significantly across sites and plant species. **c)** In the above two evaluations, we will gain insight into the microbial community associated with each of the focal species, and will identify pathogens that apparently limit seedling establishment. In c) we will test whether some of the most important pathogens may cause plant death, and whether their impact on the plant depends on the microbes associated with the individual plant. Using analyses of variance, if we find that microbial symbionts significantly reduce disease severity (Lee, Pan et al. 2009), we will gain insight into the potential use of these microbes as natural protective mutualisms to prevent plant disease and enhance seedling establishment in prairie restorations.

5. Results and Deliverables

This project will establish a lasting repository of genetic variability for a substantial number of MN prairie species, including seeds archived to preserve their viability and plantings growing at three locations in Minnesota. This germplasm will be made available to other researchers and to producers of seed for prairie restorations within the state. Microbes isolated in these experiments will be maintained as living cultures in the May Lab. Pending approval by appropriate regulatory procedures, those microbes proving to be natural protective mutualists will be available to the public. We will seek additional funding to provide training to the public on the use and maintenance of these microbes.

The research based on the seed collections will yield recommendations about appropriate choice of germplasm for prairie restorations. These recommendations may differ for different species or groups of species. Our work will also inform prairie restoration workers about the potential of native plant populations to adapt to changing environment and the rate of adaptation that can be expected in response to prevailing and novel natural selection. Our studies of micro-organisms associated with prairie plants will lead to recommendations regarding the need to prevent transfer of microbes with seeds or, conversely, the importance of ensuring transfer of beneficial microbes to ensure that populations will thrive in their target locations.

This project will also provide training that will help undergraduates, graduate students, postdocs and, eventually, members of government agencies and the public develop expertise in evolutionary genetics and restoration biology and capabilities to advance restoration efforts in the future.

6. Timetable

2011 July – October
Activity I: Collect seeds. Archive samples at NCGRP.
Activity II, IV: Prepare field sites and samples for sowing into them. Sow seeds.

2012 May – June
Activity II: Monitor emergence of seedlings and map them.
Activity IV: Monitor disease symptoms on seedlings (a).

July – August
Activity III: Conduct crosses.
Activity II: Record survival and size of recruited seedlings.
Activity I: Supplementary gathering of seeds.

August – October
Activity III: Harvest seeds from crosses. Sow them into three sites.
Activity I: Supplementary sowing of seeds.

October – 2013 May
Activity IV: Identify micro-organisms. Choose focal species of plants and microbes (a).

2013 May – June
Activity II: Assess overwinter survival and additional emergence.
Activity III: Monitor emergence of seedlings.
Activity IV: Obtain tissue samples from plants in the field (b).

2013 July – September
Activity II: Record survival and size of established individuals.
Activity III: Record survival and size of recruited seedlings.
Activity IV: Plant experimental outplants into the field (c). Monitor micro-organisms.

2013 October – 2014 April
Activity IV: Return outplants to the greenhouse. Pathogen inoculation and monitoring.
Activity II, III, IV: Draft manuscripts reporting results.

2014 May – June
Activity II: Record survival and size of plants.
Activity III: Record survival and size of plants.
Activity II, III, IV: Finalize manuscripts reporting results.

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Hurlburt, D. P. 1999. Population ecology and economic botany of *Echinacea angustifolia*, a native prairie medicinal plant. PhD thesis. University of Kansas, Lawrence, KS.

Lee, K., J. J. Pan, et al. (2009). "Endophytic *Fusarium verticillioides* reduces disease severity caused by *Ustilago maydis* on maize." FEMS Microbiology Letters **299**(1): 31-37.

Leimu, R., and M. Fischer. 2008. A meta-analysis of local adaptation in plants. PLoS ONE 3: e4010.

Lopez, S., F. Rousset, F. H. Shaw, R. G. Shaw, O. Ronce. 2009 Joint effects of inbreeding and local adaptation on the evolution of genetic load after fragmentation. Conservation Biology 23: 1618–1627.

McDonagh, P. and N. Hallyn. 2010. Site Specific Native Grassland Seed Mix Design Methodology for Minnesota. MN Department of Transportation.

McKay, J.K., C. Christian, S. Harrison, and K.J. Rice. 2005. How local is local? – Practical and conceptual issues in the genetics of restoration. Restoration Ecology 13: 432-440.

Montalvo,A.M. and R.G. Shaw. 1994. Quantitative genetics of sequential life-history and juvenile traits in the partially selfing perennial, *Aquilegia caerulea*. Evolution 48: 828-841.

Pan, J. and G. May (2009). "Fungal-fungal associations affect the assembly of endophyte communities in maize (*Zea mays*)." Microbial Ecology **58**(3): 668-678.

Pan, J. J., A. M. Baumgarten, et al. (2008). "Effects of host plant environment and *Ustilago maydis* infection on the fungal endophyte community of maize (*Zea mays*)." New Phytologist **178**(1): 147-156.

Reed, D. H. and R. Frankham. 2001. How closely correlated are molecular and quantitative measures of genetic variation?: a meta-analysis. Evolution 55: 1095-1103.

Roach, D. A. and R. Wulff. 1987. Maternal effects in Plants. Ann. Rev. Ecol. Syst. 18: 209-235.

Shaw, R. G., D. L. Byers, and F. H. Shaw. 1998. Genetic components of variation in *Nemophila menziesii* undergoing inbreeding: morphology and flowering time. Genetics 150: 1649-1661.

Shaw, R.G., C.J. Geyer, S. Wagenius, H.H. Hangelbroek, J.R. Etterson. 2008. Unifying life history analyses for inference of fitness and population growth. American Naturalist 172: E35-E47.

Thompson, R. S. *et al.*, U.S. Geological Survey Professional Paper, 1650 A-B (2000).

Wagenius, S., A. Dykstra, C. E. Ridley, and R. G. Shaw. 2010b. Seedling recruitment in the long-lived perennial, *Echinacea angustifolia*: a ten year experiment. Restoration Ecology, accepted.

Wagenius, S., H. H. Hangelbroek, C. E. Ridley, and R. G. Shaw. 2010a. Biparental inbreeding and interremnant mating in a perennial prairie plant: fitness consequences for progeny in their first eight years. Evolution 64: 761-771.

8. Credentials

RUTH GEYER SHAW
Department of Ecology, Evolution and Behavior
University of Minnesota

Education

Oberlin College	Biology	B.A. 1976
Duke University	Botany, Genetics	Ph.D. 1983
University of Washington	Genetics	1984-1986

Appointments

2000-present	Professor, University of Minnesota
2002-2003	Visiting Professor, Universite de Montpellier II
1995-1996	Visiting Professor, University of Edinburgh
1993-2000	Assistant, Associate Professor, University of Minnesota
1987-1992	Assistant Professor, University of California, Riverside.
1978-1979	Teaching Assistant, Duke University.

Academic Honors and Fellowships:

1975	Phi Beta Kappa
1979-1982	National Science Foundation Graduate Fellowship
1984-1986	National Institutes of Health Individual Post-doctoral Fellowship
1995-1996	Bush Sabbatical Fellowship, University of Minnesota
2002-2003	Fellowship, John Simon Guggenheim Memorial Foundation
2002-2003	College of Biological Sciences (UM) Sabbatical Supplement
2009	President's Award, American Society of Naturalists

Selected recent publications:

Davis, M.B. and R. G. Shaw. 2001. Range shifts and adaptive responses to quaternary climate change. Science 292: 673-679.

Etterson, J. R. and R. G. Shaw. 2001. Constraints to adaptive evolution in response to global warming. Science 294: 151-154.

Heiser, D.A. and R.G. Shaw. 2006. The fitness effects of outcrossing in *Calylophus serrulatus*, a permanent translocation heterozygote. Evolution 60:64-76.

Lau J.A., Shaw R.G., Reich P.B., P. Tiffin. 2007. Strong ecological but weak evolutionary effects of elevated CO2 on a recombinant inbred population of *Arabidopsis thaliana*. New Phytologist 175: 351-362.

Shaw, R.G., C.J. Geyer, S. Wagenius, H.H. Hangelbroek, J.R. Etterson. 2008. Unifying life history analyses for inference of fitness and population growth. American Naturalist 172: E35-E47.

Ronce, O., F. H. Shaw, F. Rousset, R. G. Shaw. 2009. Is inbreeding depression lower in maladapted populations? A quantitative genetic model. Evolution 63: 1807-1819.

Lopez, S., F. Rousset, F. H. Shaw, R. G. Shaw, O. Ronce. 2009. Joint effects of inbreeding and local adaptation on the evolution of genetic load after fragmentation. Conservation Biology 23: 1618–1627.

Wagenius, S., H. H. Hangelbroek, C. E. Ridley, R. G. Shaw. 2010. Biparental inbreeding and inter-remnant mating in a perennial prairie plant: fitness consequences for progeny in their first eight years. Evolution 64: 761-771.

Ossowski, S., K. Schneeberger, J. I. Lucas-Lledo, N. Warthmann, R. M. Clark, R. G. Shaw, D. Weigel, M. Lynch. 2010. The rate and molecular spectrum of spontaneous mutations in *Arabidopsis thaliana*. Science 327: 92-94.

Wagenius, S., A. Dykstra, C. E. Ridley. 2010, and R. G. Shaw. Seedling recruitment in the long-lived perennial, *Echinacea angustifolia*: a ten year experiment. Restoration Ecology, accepted.

Related activities

- Computer programs for maximum-likelihood analysis of quantitative genetic data. (Shaw, R.G. and F.H. Shaw.1994, freely available: http://biosci.cbs.umn.edu/eeb/quercus.html). In addition to making these programs available, both R.G. Shaw and F.H. Shaw assist interested colleagues by providing advice on the use of the programs and by tailoring the programs for particular designs and statistical models.
- Maintenance of mutation accumulation (MA) lines of *A. thaliana* for the past ten years. This includes advancement of one set of MA lines by single-seed descent from generation 15 to generation 30, as well as delivery of samples of the MA lines to colleagues interested in working with them (J. Conner, C. Fenster, J. Kelly, T. Mitchell-Olds, S. Henikoff, E. Richards).
- Service on grant panels: most recently, NSF: Population and Evolutionary Processes, Spring 2004, 2006. NIH: Genetic variation and evolution, Jan 2006. Member of Editorial Boards: Journal of Heredity, 1992-1995; The American Naturalist, 1993-1997; Evolution, 1995-1997; Genetics, 1994-2002; New Phytologist, 2004-2009; The American Naturalist, Editor, 2008- .
- Participation in governance of Society for the Study of Evolution (Council, 1997-1999, Vice President, 2005) and American Genetic Association (Council, 1999-2001).
- Consulting with Minnesota state agencies about genetic sources for restorations of prairie plant populations on state lands.

Throughout my career, my research has addressed fundamental questions regarding adaptation in native plant populations and has also yielded guidance for managing impacts of human disturbance, including climate change, introduction of invasive plants, and fragmentation of populations into small remnants. In over 17 yr at U of Minnesota, I have mentored graduate students' experimental studies of adaptation in prairie plant populations, and for 10 yr I have led UM's participation in an NSF-funded long-term experimental study investigating the evolutionary consequences of severe fragmentation of prairie populations of purple coneflower, *Echinacea angustifolia* (collaboration with Dr. S. Wagenius of the Chicago Botanic Garden, see http://echinacea.umn.edu). Among the key results of these studies are demonstration of: degree of local adaptation to present-day habitats and limits to rates of adaptation to climate change in partridge pea, *Chamaecrista fasciculata*, dramatic reduction in seed production of progeny from crosses between prairie plant populations, large differences in survival and fecundity among remnant populations, and exceptionally severe inbreeding depression affecting growth and survival in purple coneflower. Moreover, my colleagues and I have recently developed an approach for analyzing data on individual survival and fecundity, the fundamental measures of adaptation (references in leading scientific journals below). This new approach provides far more precise inferences about adaptation than previously possible, and it will be crucially important to the success of the proposed research. I have been honored with positions of leadership in major scientific organizations, including the Society for the Study of Evolution, the American Genetic Association, and the American Society of Naturalists.

DONALD L. WYSE
Department of Agronomy and Plant Genetics
University of Minnesota, St. Paul, MN 55108
Phone: 612-625-7064, E-mail: wysex001@umn.edu

EDUCATIONAL HISTORY

The Ohio State University, 1970, B.S., Agronomy
Michigan State University, 1972, M.S., Crop Science (Weed Science)
Michigan State University, 1974, Ph.D., Crop Science (Weed Science)

PROFESSIONAL POSITIONS

Founding Director, Minnesota Institute for Sustainable Agriculture, U. of Minnesota, 1992-2000
Co-director, Center for Integrated Natural Resources and Agricultural Management, 1995-present
Professor, Dept. of Agronomy and Plant Genetics, University of Minnesota, 1986-present
Associate Professor, Dept. of Agronomy/Plant Genetics, University of Minnesota, 1980-1986
Assistant Professor, Dept. of Agronomy and Plant Genetics, University of Minnesota, 1974-1980

PROFESSIONAL ORGANIZATIONS AND HONOR SOCIETIES

North Central Weed Science Society, Weed Science Society of America, Sigma XI, Plant Physiology

HONORS AND AWARDS

Co-author of the Outstanding Paper published in Weed Science, 1987
Weed Science Society of America Outstanding Young Weed Scientist, 1987
Outstanding Teacher Award in the College of Agriculture, 1988
Weed Science Society of America Outstanding Teacher Award, 1991
Outstanding Faculty Performance Northrup King Award, 1991
CIBA-GEIGY Award for Outstanding Achievement in Agriculture, 1991

PROFESSIONAL ACTIVITIES

Univ. of Minnesota, Member, State Pesticide Impact Assessment Team, 1976-2000.
Univ. of Minnesota, Member, Water Quality Advisory Board, Lead Scientist on Pesticides, 1986-2000.
Univ. of Minnesota, Organizing Member of the Biological Pest Control Center, 1988-present.
Univ. of Minnesota, Director, Minnesota Institute for Sustainable Agriculture, 1992-2000.
State of Minnesota, Member of the Sustainable Development Initiative—Agriculture Team, Appointed by the Governor of Minnesota, 1993-95.
Univ. of Minnesota, Founding Member, Steering Committee, Kellogg Food Systems Initiative, 1994-
Univ. of Minnesota, Member of College of Agriculture Legislative Relations Working Group, 1994-2000.
Univ. of Minnesota, Co-director, Center for Integrated Natural Resources and Agricultural Management, 1995-
Univ. of Minnesota, Founding Member, State-wide Coordinating Committee, Regional Agricultural and Natural Resources Sustainable Development Partnership, 1998-2001.
North Central Weed Science Society, Board of Directors, 1984-87.
CSRS, Chairperson, NCT-160, Weed Management Model, 1988-90.
Weed Science Society of America, Member of CSRS/WSSA Committee on the Future of Weed Science, Washington, D.C. – 1992.
Weed Science Society of America, Chair, WSSA Sustainable Agriculture Committee, 1995-2008.

TEACHING EXPERIENCE

My responsibilities include teaching and supervising graduate student research in weed science and cropping systems.
AGRO 4503 (3 credits), Biology, Ecology and Management of Invasive Plants

RESEARCH AND MANAGEMENT EXPERIENCE

Donald Wyse is a Professor in the Department of Agronomy and Plant Genetics at the University of Minnesota, St. Paul, where he teaches and conducts research in weed management, cropping system development, and plant breeding and selection. His research concentrates on biological weed management, development of multifunctional agricultural systems, perennial crop breeding, and legume and grass seed production systems. He has focused his research efforts on the development of perennial cropping systems, cover crop systems, biomass prairie polycultures, and has studied their impact on soil and water quality. He has lead several multi-disciplinary research teams composed of university faculty and scientists from both state and federal agencies. He has experience in managing large multi year grants. Dr. Wyse was the founding Director of the Minnesota Institute for Sustainable Agriculture and currently serves as Co-director of the Center for Integrated Natural Resources and Agricultural Management at the University of Minnesota. Recent activities of the Center have led to the development of the Mississippi River—Green Land, Blue Water Initiative that includes universities, state and federal agencies, and NGO's that have organized to deal with the landscape issues that impact water quality in the Mississippi River and Great Lakes Basin. He was one of the founding organizers of the Midwest Cover Crops Council and is an active member of the Executive Committee.

SELECTED PUBLICATIONS (past 3 years)

Jordan, N., G. Boody, W. Broussard, J.D. Glover, D. Keeney, B.H. McCown, G. Mclasaac. M. Muller, H. Murray, J. Neal, C. Pansing, R.E. Turner, K. Warner, and D. Wyse. 2007. Sustainable development of the agricultural bio-economy. Science 316: 1570-1571

Moncada, K.M., N.J. Ehlke, G.J. Muehlbauer, C.C. Sheaffer, D.L. Wyse, and L.R. DeHaan. 2007. Genetic variation in three native plant species across the state of Minnesota. Crop Sci. 47:2379-2389

Hulke, B.S., E. Watkins, D. Wyse, and N. Ehlke. 2007. Winterhardiness and turf quality of accessions of perennial ryegrass (*Lolium perenne L.)* from public collections. Crop Sci. 47:1596-1602

Borchardt, J.R., D.L. Wyse, C.C. Sheaffer, K.L. Kauppi, R.G. Fulcher, N.J. Ehlke, D.D. Biesboer and R.E. Bey. 2008. Antioxidant and antimicrobial activity of seed from plants of the Mississippi River Basin. Journal of Medicinal Plants Research 2:81-93

Harbur, M.M., C.C. Sheaffer, K.M. Moncada, and D.L. Wyse. 2009. Selecting hairy vetch ecotypes for winter hardiness in Minnesota. Online. Crop management doe:10.1094/CM-2009-08XX-01-RS.

Sheaffer, C.C., D.L. Wyse, and N.J. Ehlke. 2009. Palatability and nutrient value of native legumes. Native Plants J. 10 (3):225-232

Valentas, K.J., G. Garto, P. Gillitzer, M. von Keitz, C. Lehman, s. Taff, and D.L. Wyse. 2009. Chisago/Isanti/Pine Biofuels Feasibility Study. http://www.bti.umn.edu/WE_CIP/images/CIPreprot200.pdf

Valentas, K.J., G. Garto, P. Gillitzer, M. von Keitz, C. Lehman, s. Taff, and D.L. Wyse. 2009. White Earth Biofuels Feasibility Study. http://www.bti.umn.edu/WE_CIP/images/WEreprot200.pdf

Thelemann,R.,G. Johnson, C. Sheaffer, S. Banerjee, H. Cal, and D.L. Wyse. 2010. The effect of landscape position on biomass crop yield. Agron. J. 102(2):513-522

Glover, J.D., J.P. Reganold, L.W. Bell, J. Borevitz, E.C. Brummer, E.S. Buckler, C.M. Cox, T.S. Cox, T.E. Crews, S.W.Culman, L.R. DeHaan, D. Eriksson, B.S. Gill, J. Holland, F. Hu, B.S. Hulke, A.M.H. Ibrahim, W. Jackson, S.S. Jones, S.C. Murray, A.H. Paterson, E. Ploschuk, E.J. Scaks, S. Snapp, D. Tao, D.L. Van Tassel, L.J. Wade, D.L. Wyse, Y. Xu. 2010. Increasing food and ecosystem security via perennial grains. Science 328:1638-1639

GEORGIANA MAY

Current appointment: Department of Ecology, Evolution and Behavior
Department of Plant Biology
University of Minnesota
Current position: Professor

Education

Bowling Green State University	B.S. Biology	1971- 1975
University of Georgia, Athens	M.S. Botany	1977 - 1980
University of California, Berkeley	Ph.D. Botany	1981 - 1987

Advisor: Dr. John W. Taylor

Appointments, fellowships, awards

Bowling Green State University
Tinappel Award (Botany honors) 1974 - 1975
University of Georgia, Athens
Teaching assistantship 1977 - 1980
University of California, Berkeley
Teaching assistantship 1981 - 1984
Research assistantship with Dr. John Taylor 1983 - 1984
Graduate Fellow, University of California 1984 - 1986
Mycological Society of America Fellowship 1985
NSF Dissertation Improvement Grant (BSR 8514309) 1985
San Francisco Mycological Society Fellowship 1986
University of North Carolina, Chapel Hill 1987 - 1990
N.I.H.-N.I.G.M.S. Postdoctoral fellowship (P.J. Pukkila)
University of Minnesota
Assistant Professor, Plant Biology 1991 - 1997
Associate Professor, Plant Biology 1998 - 2000
Bush Sabbatical Leave 1998 - 1999
Associate Professor, EEB/Plant Biology 2000 - 2009
Professor
Mycological Society of America
Alexopolous Prize (outstanding young mycologist) 1997

Selected Recent Publications

Baumgarten, A., Cannon, S., Spangler, R., May, G. 2003.Genome-level evolution of NBS-LRR resistance genes in the *Arabidopsis thaliana*. *Genetics* 165:309-319.

Forche, A., Beckerman, J, Kauffman, S., Becker, J., May, G., Magee P. 2003 A system for studying genetic changes in *Candida albicans* during infection. *Fungal Genetics and Biology* 39: 38-50.

Neuhauser, C., Andow, D., Heimpel, G., May, G., Shaw, R., Wagenius, S. 2003 Community Genetics: Expanding the synthesis of ecology and genetics. *Ecology* 84: 545-558.

Cannon, S. B., Mitra A., Baumgarten, A.,Young N.D., May, G. 2004. The roles of segmental and tandem gene duplication in evolution of large gene families in *Arabidopsis thaliana. BMC Plant Biology* 4:10-16.

Barnes, C. W., Szabo, L. J., May, G., Groth, J. V. 2004. Inbreeding levels of two

Ustilago maydis populations. *Mycologia* 96 : 1236-1244.
Forche, A., Magee, B., Magee, P., May, G. 2004. Genome-wide single-nucleotide polymorphism map for Candida albicans. Euk Cell. 3:705-14.
Couch, B., Spangler, R., Ramos, C. and May, G. 2006. Pervasive Purifying Selection Characterizes the Evolution of *I2* Homologues. Mol. Plant Microb. Interact 19:288-303
Voth, P.D., L. Mairura, B. E. Lockhart and G. May 2006. Phylogeography of Ustilago maydis virus H1 in the USA and Mexico. J Gen Virol ; 87: 3433-3441.
Munkacsi, A.B., S. Stoxen, and G. May. 2007. Domestication and cultivation of crop plants did not drive diversification of their pathogens. Evolution 61:388–403.
Baumgarten, A., Suresh, J, May, G., Phillips, R. L. 2007. Mapping QTLs contributing to *Ustilago maydis* resistance in specific tissues of plants using two recombinant inbred populations of maize. Theor Appl Genet. 114:1229–1238 DOI 10.1007/s00122-007-0513-5
Pan, J., Baumgarten, A., and May, G. (2008) The effects of host plant environment and *Ustilago maydis* infection on the fungal endophyte community of maize (*Zea mays*) New Phytologist **doi**:10.1111/j.1469-8137.2007.02350.x
Munkacsi, A. B., S. Stoxen, and G. May (2008) The domestication of maize left behind little record of ancestral interactions with its obligate pathogen, Ustilago maydis. Proc. Royal Acad. Sci. B doi:10.1098/rspb.2007.1636
Bentley, S. …9 authors…May, G., …2 authors …Ishimaru, C. A. (2008) Genome of the actinomycete plant pathogen *Clavibacter michiganensis* subspecies *sepedonicus* suggests recent niche adaptation. J. Bacteriology 190: 2150–2160.
Forche, A., Magee, P. T, Selmecki, J. Berman, May, G. (2009) Evolution in *Candida albicans* populations during a single passage through a mouse host. Genetics 182: 799-811.
Pan, J. and G. May (2009) Fungal-fungal associations affect the assembly of endophyte communities in maize (*Zea mays*) Microbial Ecology 58:668-678.
Lee, K., J. J. Pan and G. May (2009) Endophytic *Fusarium verticillioides* reduces disease severity caused by *Ustilago maydis* on maize. FEMS Microbiology 299:31–37.

Current federal grants

NSF-Environmental Genomics $900,000 09/2007 - 08/2010 (0723451) Pathogen evolution in complex microbial communities May, G., PI; Kistler, C., coPI

Hatch Funds

Evolution of pathogen virulence $150,000 09/2009 - 08/2011

8. 2011-2012 Detailed Project Budget

IV. TOTAL TRUST FUND REQUEST BUDGET 3 years

BUDGET ITEM	AMOUNT

Personnel: - Prof. Ruth Shaw - One month summer salary per year is requested for supervision of the seed sampling and field experiments investigating local adaptation component of the project. 76% salary, 24% benefits	$44,000
Personnel: Prof. Georgiana May - One month of summer salary per year is requested for the microbial studies component of the project. 76% salary, 24% benefits.	$40,000
Personnel: Prof. Donald Wyse - No salary is requested. He will supervise the seed sampling and field experiments investigating local adaptation component of the project (along with Prof. Ruth Shaw).	$-
Personnel: PostDoc - One full-time position for two years. This position will be responsible for seed sampling and field experiments investigating local adaptation. 83% salary, 17% benefits	$98,000
Personnel: Graduate Student - Two half-time positions for two years. One position will be responsible for seed sampling and field experiments investigating local adaptation. The other position will work on the microbial studies. 62% salary, 38% benefits	$192,000
Personnel: Research Scientist - One half-time position for two years and one quarter-time position for three years. 73% salary, 27% benefits	$70,000
Personnel: Undergraduate Student - Four students for two months each during the summer. These positions will assist with fieldwork. 93% salary, 7% benefits.	$28,000
Contracts:	$-
Equipment/Tools/Supplies: Supplies - Field supplies include materials for gathering seeds; pots, soil, and tags for establishing plants in the greenhouse; and stakes for delineating plots in the field. Lab supplies include petri dishes, media and reagents.	$20,000
Acquisition (Fee Title or Permanent Easements):	$-
Travel: Travel within MN to sites for collecting seeds and to locations of experimental plots ($0.50 per mile).	$21,000
Additional Budget Items: DNA Sequencing - to identify microbes associated with plants; includes sample prep and analyses @ $10/sample x 1200 samples (300 per site).	$12,000
TOTAL ENVIRONMENT & NATURAL RESOURCES TRUST FUND $ REQUEST	$525,000

V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ Being Applied to Project During Project Period:	$-	
Other State $ Being Applied to Project During Project Period:	$-	
In-kind Services During Project Period:	$-	

Remaining $ from Current ENRTF Appropriation (if applicable):	$-	
Funding History:	$-	

Ruth Shaw will take primary responsibility for coordinating the project. She and Don Wyse will together lead the efforts to gather seed and to establish the experiment evaluating local adaptation (Activities I and II). Shaw will lead the study of adaptive potential (Activity III). Georgiana May will lead the microbial studies (Activity IV). Each component of the project is labor-intensive and will demand the efforts of a postdoc, graduate students, research scientists, and undergraduate students. Extensive travel to the collection sites and the research sites will be required. Funding for supplies for the field and for the lab, as well as for DNA sequencing, is also included.

9. Dissemination and Use:

The results of this research will be prepared for publication in the peer-reviewed scientific literature. In addition, we will continue to consult with state agencies, producers of seed for prairie restoration and others involved in restoration, basing our recommendations on the findings that arise from this project. Beyond the plans outlined here, we will invite colleagues to use our experiments to address further scientific questions for which they are well suited. In this way, the experiments can be viewed as a scientific observatory that can offer insights into questions well beyond the particular ones that are our foci.

Both the collections of seeds archived for the long-term at NCGRP and the plants growing in the large-scale, long-term experiment proposed here will constitute a lasting repository of genetic variability for a substantial number of MN prairie species. Samples of this genetic material, primarily as seeds produced by plants in the experiment and secondarily from NCGRP, will be made available to MN seed producers at nominal cost; however, due to policy established for SNAs, germplasm drawn from SNA sites must be excepted.