

Final Report: The genetic structure of populations of a threatened leafhopper,
Aflexia rubranura

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Aflexia rubranura is a threatened, prairie-restricted leafhopper that feeds on grasses in the genus *Sporobolus*. This insect was thought to be extinct, but a few years ago, Ron panzer found several populations in northern Illinois prairies. The purpose of this study was to determine whether the genetic composition of this species was similar in the few remaining populations. Knowledge of the genetic structure can be used to inform management for this species with respect to genetic diversity of the species, and may also allow us to choose the best source of insects for use in re-introduction efforts. Knowing the degree to which populations have diverged genetically may also give us a better idea of the likelihood of exchange of individuals among populations. This, in turn, may allow us to evaluate the possibility that populations may 'rescue' one another through immigration.

If populations are genetically distinct, then this finding would suggest that one of two scenarios is likely to be occurring. First, it might indicate that gene flow (i.e., the exchange of genes between populations as a result of immigration, mating and survival) is sufficiently limited, relative to the strength of selection, that populations are diverging as a result of adaptation to different environments. A second possibility is that differentiation among populations is not adaptive and reflects the combination of low gene flow between populations coupled with founder effects (the founding of a population by a small number of individuals) and/or genetic drift (the loss of genes from populations as a result of random processes such as independent assortment, demographic stochasticity and other random events). Finally, populations could be similar in their genetic composition, a fact that would suggest that gene exchange between populations is common, and/or that differences among habitats are not sufficiently strong to cause genetic divergence among populations in the presence of gene flow.

We had reason to believe that populations could be differentiated in this species. First, gene flow might be limited because *A. rubranura* populations are comprised of approximately 90% brachypterous (short-winged, flightless) individuals, and 10% macropterous (long-winged dispersive) ones. Given the fragmented nature of prairie habitats in this region, we felt that the success of migrants might be greatly decreased. In addition, we also know from surveys that populations are severely depressed by fire, at least in the first few generations following a burn. Reducing populations to very small sizes (putting them through 'bottlenecks') increases the likelihood that random effects such as founder effects or drift play a role in determining genetic composition of populations.

We collected individuals of *A. rubranura* from five locations in Illinois: Goose Lake Prairie (50 individuals), Lake of the Hills Fen (3 locations with 31, 20 and 4 individuals, respectively), and Illinois Beach State Park (33 individuals) using permits issued to Ron Panzer. In addition, 108 individuals from Goose Lake Prairie were collected to use for exploratory runs of gels. Three populations were collected at Lake of the Hills Fen because a fire in one area had depleted the population and a road may have served as a barrier to recolonization from other areas within the park. Thus it was possible that populations might have diverged genetically even at this very fine geographic scale.

Genetic analysis was done using starch gel electrophoresis. This procedure uses variation in respiratory enzymes to assay differences among populations. Variation at these loci is thought to be selectively neutral. Individual leafhoppers were collected live and frozen in a -70 degree C freezer until used. Dr. Ed Lisowski, then of the Illinois Natural History Survey (Biodiversity) used the 108 individuals from Goose Lake Prairie to survey the population for enzymes that would show variability and that could be reliably scored. We screened 35 enzymes and found 23 that were usable, but only four that were variable among these populations (Table 1).

All individuals from the three sites were then run on gels and scored for alleles, which are expressed as having differential mobility on the gels. Allele frequency data were analyzed using BIOSYS, as well as with a modification of this program that uses a bootstrapping technique to estimate the 95% confidence levels around Wright's F_{ST} statistic, which indicates the degree of differentiation among populations.

The results showed that frequency of homozygote and heterozygote individuals was in accordance with Hardy-Weinberg equilibrium; thus, not much inbreeding was occurring within these populations. Populations in the larger collected from Lake of the Hills Fen were not significantly different in allele frequencies (based on Wright's F_{ST}), so they were all lumped into a single population for the larger analysis. When all three locations were considered, we found significant population differentiation (Table 2.). We examined exactly where those differences were occurring using a Monte Carlo simulation devised by Dr. Stewart Berlocher to compare expected values of the Fisher's Exact test to observed values. These differences were due primarily to differences in frequency between alleles in the Lake of the Hills Fen population versus the Goose Lake Prairie and Illinois Beach populations at the HAD locus. In addition, the Goose Lake Prairie population differed from the other two at the IDH locus.

Our results show that these leafhopper populations are differentiated genetically. There are several implications of these results. First, gene flow between populations is not sufficient to override effects of either selection and/or genetic drift at these sites. Second, a follow study using reciprocal transplant experiments within and among sites could be conducted to determine whether differences among populations have adaptive value. If they are adaptive, then caution must be taken if individuals from one population are wanted to bolster populations at another location. Outbreeding depression may occur in the target

population, if genes that are not locally adaptive are introduced into an adapted population. Finally, each of these populations has some unique alleles and serves as a reservoir of genetic diversity in this species. It is possible that a combination of individuals from all these populations might serve as the best inoculum for founding new populations by maximizing the amount of genetic diversity upon which selection could act. This suggestion should be taken with caution, however, as theory with respect to how genetically divergent populations should be used as inocula has yet to be developed.

The data from these analyses will be combined with natural history data gathered by Ron panzer on this species. We anticipate publishing these results in a conservation-oriented journal in the next year.

Table 2. Analysis of population differentiation using BIOSYS

Locus	F(IS)	F(ST)	F(IT)
Acon-2	.0000	.0000	.0000
AOX	.0000	.0000	.0000
Ald	.0000	.0000	.0000
Dia-1	.0000	.0000	.0000
Dia-2	.0000	.0000	.0000
Fum	-.0094	.0087	-.0007
GOT-1	.0000	.0000	.0000
GOT-2	.0000	.0000	.0000
G3PDH	.0000	.0000	.0000
a-GPDH	.0000	.0000	.0000
Hex-1	.0000	.0000	.0000
Hex-2	.0000	.0000	.0000
HAD	.1174	.1398	.2408
IDH	-.0380	.0353	-.0013
LAP	.0021	-.0033	-.0011
MDH	.0000	.0000	.0000
MPI	.0000	.0000	.0000
6PGDH	.0000	.0000	.0000
PGI	.0000	.0000	.0000
PGM	-.0156	.0039	-.0116
SOD	.0000	.0000	.0000
TPI	.0000	.0000	.0000
XDH	.0000	.0000	.0000
Mean	.0697	.1013	.1640

Jackknife
Estimates over loci

Mean	.1236	.1542	.2693
S.D.	.0918	.0838	.1676

95% Conf. limit by bootstrapping over loci: (.0034, .1332) (for F_{ST}).