

GRANT NARRATIVE

PROJECT TITLE: Implementation of Recovery Actions for the Hine's Emerald Dragonfly (*Somatochlora hineana*) in Illinois, and Wisconsin

Project period: October 1, 2007 to February 28, 2009*

PURPOSE/NEED:

The Hine's emerald dragonfly (HED) is one of North America's rarest dragonflies (Bick 1983, Cashatt 1991). This dragonfly is currently known from Illinois, Michigan, Missouri, and Wisconsin. Studies to date show that the Illinois population is the most genetically diverse and the Wisconsin population is the largest and presumably the most secure. Historically known from northern Ohio and Indiana, it is now thought to be extirpated from these states (USFWS 2001). Quantitative information on the population status across its range is very limited with only two sites (Lockport Prairie in Illinois and Mud Lake North in Wisconsin) being extensively studied Soluk et al. 1998, 2000. Most of the sites for this species are described from the presence of adults alone making the status of many sites as breeding habitat uncertain.

The HED larvae live in small temporary streams and flowage areas of fens and sedge meadows, fed by calcareous groundwater associated with dolomitic bedrock (Soluk et al. 1998; Cashatt and Vogt 2001). Known occupied habitats are currently restricted to public and privately owned land in Cook, DuPage and Will Counties, in Illinois; Door and Ozaukee Counties in Wisconsin; Alpena, Mackinac, and Presque Isle Counties in Michigan; and Dent, Iron, Morgan, Phelps, Reynolds, Ripley, Shannon, Washington, and Wayne Counties in Missouri. Habitat loss due to agriculture, development, limestone quarrying, and groundwater extraction are the probable cause of this species' decline. Loss of remaining habitat through disruption of ecological and hydrological processes is the primary threats to surviving populations.

To develop appropriate conservation and recovery efforts for the HED, information on its current distribution, population status and viability are crucial. Due to the fact that adults are highly mobile, and limited systematic survey work has been conducted. Identification of viable, reproducing populations is also particularly important for assessing the species' status and long-term viability in each state and prioritizing sites for conservation and recovery efforts. Larval studies can provide information useful in the quantification and identification of trends in HED populations. Adult dragonflies often are found some distance away from what is believed to be suitable larval habitat. More information on ecological requirements of the HED is needed, including determining larval habitat requirements. Thus, a search for HED larval habitat within existing sites was considered a high priority by the recovery team. For these reasons, we are proposing to identify new breeding locations and quantify known populations in Door and Ozaukee Counties in Wisconsin and Cook, DuPage and Will Counties in Illinois by surveying for HED larvae.

*WI grant E-1-41 end date is 12/31/2008, IL grant E-40-R-1 end date is 2/28/2008

Population genetic data are essential to the management and recovery of endangered species. It

has long been recognized that the presence of genetic diversity within species allows for future adaptation in a changing environment (e.g., Frankel and Soulè 1981, Avise and Hamrick 1996). Furthermore, habitat fragmentation can reduce gene flow between habitat patches leading to reductions in heterozygosity and allelic diversity via genetic drift (e.g., Frankham et al. 2001). Disruption of natural population structure and dynamics across the occupied landscape can accelerate declines in genetic diversity within already depauperate and declining populations. The major goals of the proposed study are to: 1) determine levels of genetic variability in HED sites in Cook, DuPage, and Will Counties in Illinois and Door and Ozaukee Counties in Wisconsin, 2) estimate the population genetic structure (level of genetic connectivity) among sampled locations within each state or geographical cluster of sites, 3) delineate populations, subpopulations, metapopulations, or other appropriate units within the sampled areas based on frequencies of genetic markers, 4) estimate genetically effective population sizes of these units, 5) estimate levels of genetic similarity across the sampled HED sites.

OBJECTIVES:

The primary objective of this study is to determine the current status of the HED at sites where it has been observed, and to gather information on new sites that will contribute to the long-term viability and recovery of the species. New information will contribute to the recovery of the species. Specific objectives of this project are listed below with the priorities addressed by each objective:

1. Conduct larval and exuvia surveys at known sites within the historical range of the species to obtain estimates of the current population status needed to determine recovery, protection, and restoration priorities. Develop and obtain an index of relative abundance at occupied sites to allow comparisons between sites and help prioritize future conservation activities. Surveys will be conducted from June to November 2008 on public property in Cook, DuPage and Will Counties in Illinois and Door and Ozaukee Counties in Wisconsin from June to November 2008. This proposed work will address priority 1 recovery tasks 3.4, 3.5, and 3.10 and priority 2 recovery task 1.2.1.2.
2. Identify reproducing populations by documenting presence of larvae or exuviae. Identify and characterize larval habitat used at occupied sites. Characterize habitat at landscape level to better understand this species' distribution within sites. Surveys will be conducted from June to November 2008 on public property in Cook, DuPage and Will Counties in Illinois and Door and Ozaukee Counties in Wisconsin from June to November 2008. This proposed work will address priority 1 recovery task 3.1.
3. Determine genetic variability: Genetic analyses of HED will be useful in obtaining much needed information on the population structure, the amount of genetic diversity between and among populations, and the potential movement of individuals between sites in Cook, DuPage and Will Counties in Illinois and Door and Ozaukee Counties in Wisconsin. This proposed work will address priority 2 recovery task 2.4.
4. Conduct searches for new populations statewide in Wisconsin. Surveys will be

conducted at highest priority sites identified by Vogt, 2003. Surveys will begin in late June, 2008 and continue into July, 2008. The proposed work will address priority 1 recovery task 3.5.

EXPECTED RESULTS AND/OR BENEFITS:

This project will provide data critical to the conservation of the HED. Habitat complexes currently occupied by the dragonfly will be resurveyed to learn more about their current use and breeding status of these sites. This information is needed to implement the recovery plan for the species. In addition, it will enable public and private land managers to better protect and manage for this species. This project also will identify potential sites for future ecological research. At the end of the project, the information base will expand knowledge of HED occurrences, estimates of population size, genetic variability, and threats to existing populations. While sampling for HED, the habitat quality and any threats will be assessed to identify potential management and protection actions required for the long-term viability of the dragonfly. Recommendations identifying potential areas for restoration activities and for habitat protection strategies, which could include voluntary registry, conservation agreements or easements, or acquisition from willing sellers, will be provided. Landowners and managers will be contacted in the process of obtaining permission to conduct the surveys, and later when informing them of survey results. Both contacts allow for the unique opportunity to talk about conservation needs of the species. Working relationships or partnerships with local conservation organizations will be developed whenever possible and enable the implementation of long-term monitoring and conservation work at the local level.

The population genetic structure and genetic connectivity among HED locations on local and range-wide scales is a major gap in our knowledge of this species. Microsatellite frequency data can be used to estimate gene flow on the local level, while mtDNA haplotype frequencies can be used to infer historical relationships among sites. Because the population dynamics of HED were unknown when the recovery plan was completed, the status of the species and the recovery criteria for reclassification and delisting were based on theoretical knowledge of metapopulations (USFWS 2001). In other words, the spatial scale and structure of HED populations is essentially unknown at this point. Effective recovery and management is simply not possible without knowing the spatial scale and structure of populations. Once genetic data are combined with ecological data on the dispersal and movement of HED in IL, and WI, it will be possible to plan effectively for habitat preservation, restoration, and creation. In addition, completing this work will assist in gaining better understanding of the species status and identifying appropriate and attainable recovery criteria.

*Federal Recovery Actions to be Implemented

Priority 1 Recovery Tasks (Hine's Emerald Dragonfly Recovery Plan (USFWS 2001)):

3.1 Search for *S. hineana* larval habitat within existing adult sites

3.5 Search for *S. hineana* populations in Wisconsin

3.10 Search for *S. hineana* populations in Illinois

5.1 Encourage private landowners to conserve the Hine's emerald dragonfly

Priority 2 Recovery Tasks (Hine's Emerald Dragonfly Recovery Plan (USFWS 2001)):

1.2.1.1 Conduct presence/absence surveys

1.2.1.2 Conduct census surveys

2.4 Conduct studies: Genetics

APPROACH/METHODS:

This grant proposal addresses high priority recovery actions including surveys to document the presence and determine HED's breeding status in two states. In Illinois and Wisconsin sampling is needed to confirm presence and potential breeding at multiple sites. Several sites where adults have been documented have not been studied for 3-4 years. Techniques developed by Dr. Soluk have lead to an improved understanding of the larval habitat and thus sampling for larvae and exuviae is much more efficient than before.

Initial site surveys will be conducted between May and June. The wetlands will be mapped, identifying areas of potential HED larval habitat. These surveys will be conducted mid-June through July. Transects will be divided into 5, 1m x 1m, plots. Exuviae will be collected, preserved in ethanol, and identified within transects. The number of transects surveyed will likely vary among sites. Each transect will be monitored once per week during the emergence period. Adult activity will also be observed to obtain a better idea of where female oviposition sites are located. Adult surveys will continue into August depending on the location of the survey. The locations of exuviae collected and adult oviposition sites will be recorded using GPS coordinates and placed within a database.

Statewide surveys proposed for WI will begin in late June and continue into July. Sites to be surveyed will be those identified by Vogt, 2003 as highest priority based on vegetation analysis. Surveys will primarily be for larvae by pumping of crayfish burrows, although adults and exuviae surveys will be utilized as well. All sites surveyed will be identified in a spreadsheet with Latitude and Longitude given in decimal degrees, date, and odonata species and number of individuals found. Final report summarizing finds will be prepared and sent to WDNR for approval and redistribution.

Exuvial and adult surveys will be followed by larval searches. Burrow pumping will be used to search for larvae within crayfish burrows at sites where exuviae were not found. Additional sites may also be chosen for sampling if other high quality habitat areas are identified. Larval location will be marked using GPS coordinates and placed within a database. An estimate of population size will be made for each site based on larval surveys.

All pertinent data will be provided to each state's Heritage database managers. In addition, newly discovered sites will be added to the Hine's emerald dragonfly database maintained at the Illinois Museum. Finally, reports summarizing the findings will be prepared and sent to state and federal agencies.

Cook, DuPage and Will Counties in Illinois

- 1) Searches for larvae and exuviae to establish whether sites where the adult HED have been observed are currently breeding areas.
- 2) Establish within sites, where successful breeding is occurring.
- 3) Develop population estimates for sites based on larval surveys.

Door and Ozaukee Counties in Wisconsin

- 1) Searches for larvae and exuviae to establish whether sites where the HED has been observed are currently breeding areas.
- 2) Establish within sites, where successful breeding is occurring.
- 3) Develop population estimates for sites based on larval surveys.

Genetic Research:

Several sources of non-destructive DNA samples are potentially available. These include: 1) a single leg can be removed from two year old, or older, HED larvae collected from the field by standard techniques. Larvae will be released and will regenerate the leg; 2) exuviae can be collected from the field during the course of ecological field work or from molting captive larvae; 3) small wing clips can be taken from adults. Field collections should be undertaken for several years because of the small number of samples likely to be collected from each study location each year and to track changes in marker frequencies over time. Statistical analyses require sample sizes that are unlikely to be met over a single (one year) sample period.

Dr. Hugh Britten (University of South Dakota) currently has funding to develop DNA extraction protocols and genetic markers to be used in the proposed study. DNA extraction procedures from non-destructively collected samples are largely developed. It is anticipated that a full suite of microsatellite and mtDNA markers will be developed and in use by late summer 2007. Extracted DNA will be amplified using an MJ Research PTC-200 thermalcycler and fragment analyses and sequencing will be carried out on an ABI 3100-*Avant* genetic analyzer. All necessary equipment is available in the USD Department of Biology. At least 20 microsatellite primers pairs will be available for use in the population genetic analyses. Previously published mtDNA primers are currently being optimized for sequencing of at least several hundred base pairs of mtDNA sequence per sample.

POPGENE (Yeh et al. 1999) will be used to estimate and compare levels of population genetic variability from the sampled locations. These will include number of alleles per locus, number of effective alleles per locus, observed and expected heterozygosity, and tests for conformance to Hardy-Weinberg equilibrium. Estimates of population genetic variability for extant HED populations will be compared to those from the historic samples, given that enough historic samples are available for analysis. TFPGA (Miller 1997) will be used to calculate genetic distances and to test for isolation-by distance using a Mantel test. Arlequin (Schneider 2000) will be used to perform an analysis of molecular variance (AMOVA) that will partition the total variance in allele frequencies within and among sample locations. MIGRATE (Beerli and Felsenstein 2001) will be used to estimate genetically effective population sizes and genetically effective migration rates among the sample locations. An assignment test (GeneClass, Cornuet et al. 1999) will be used to assign probabilities of membership to each sample location for each individual. This procedure, along with the test for isolation-by-distance, estimates of gene flow

rates, and AMOVA will clarify the population genetic structure for HED based on the microsatellite data. These results will be compared across all sampled locations.

PROJECT SCHEDULE:

- A. June to November 2008 Surveys will be conducted on public property in Cook, DuPage and Will Counties in Illinois and Door and Ozaukee Counties in Wisconsin. Surveys will consist of searching fens for larvae, exuviae and adult HED and other Odonata. Identify reproducing populations by documenting presence of larvae or exuviae. Identify and characterize larval habitat used at occupied sites. Characterize habitat at landscape level to better understand this species' distribution within sites. Record field and weather conditions at each location. Collect voucher specimens of all Odonata.
- B. June to July, 2008. Statewide surveys will take place in Wisconsin. Surveys will consist primarily of larval pumping at sites identified as highest priority by Vogt, 2003. Other survey methods will be utilized as opportunity arises. All odonata found at sites surveyed will be documented by recording date, precise location, amount of and type of effort, vegetation and overall site suitability assessment, including abundance of crayfish burrows.
- C. August 2008 to January 2009 Conduct genetic analyses of HED to determine population structure, the amount of genetic diversity between and among populations, and the potential movement of individuals between sites in Cook, DuPage and Will Counties in Illinois and Door and Ozaukee Counties in Wisconsin. DNA will be extracted from samples either as they accumulate from the field or by September 2008. Amplification of microsatellite DNA using polymerase chain reaction (PCR) will occur from September 2008 through November 2008. PCR products will be analyzed at the USD (Univ. Of South Dakota) DNA sequencer facility as they are produced into mid-December 2008. The resulting data will be analyzed in December 2008 and January 2009.
- D. February 2009. Prepare final report and distribute to State and Federal agencies.

PERSONNEL: ROLES AND RESPONSIBILITIES

The responsibilities each state expects to undertake with this project are presented in greater detail throughout this proposal. Additional personnel and contact information for those preparing this proposal are listed in this section.

Joseph Kath, Illinois Department of Natural Resources in cooperation with Kristopher Lah, U.S. Fish and Wildlife Service, will coordinate grant actions in Illinois. Joe Kath and Kris Lah will contract services to qualified and permitted individuals and will submit a final report in cooperation with William Smith, Wisconsin Department of Natural Resources by February 28, 2009. Each state will be responsible for providing data to their Heritage program and endangered species coordinators. Contact person: Joe Kath, Illinois Department of Natural Resources, (217) 785-8764, Joe.Kath@illinois.gov

William Smith, Wisconsin Department of Natural Resources in cooperation with Cathy Carnes, U.S. Fish and Wildlife Service, will coordinate grant actions in Wisconsin. William Smith will

contract services to qualified and permitted individuals, and will submit a final report in cooperation with Joseph Kath, Illinois Department of Natural Resources by February 28, 2009. Each state will be responsible for providing data to their Heritage program and endangered species coordinators. Contact person: William Smith, Wisconsin Department of Natural Resources, (608) 266-0924, WilliamA.Smith@Wisconsin.gov

Dr. Daniel Soluk and Dr. Hugh Britten, University of South Dakota will submit a final report summarizing the results of the inventory and genetic analysis for each State agency and submit to the U.S. Fish and Wildlife Service by February 28, 2009.

PROJECT BUDGET:

Total Project Budget

	<u>Federal Share</u>	<u>State Share (IL and WI)</u>
Total costs:	\$42,198	\$4,689

Total Project Cost: \$46,887.00

Budgets by State

Illinois

	<u>Federal Share</u>	<u>State Share</u>
Contractual Services*:	\$24,398	\$2,711
Salary:	---	---
Travel:	---	---
Total costs:	\$24,398	\$2,711

- *Contractual services include \$6,000 for genetic analysis and \$21,109 for population estimates at Lockport Prairie, several “new/unnamed” sites, and Long Run Seep Nature Preserves and habitat mapping at Romeoville Prairie Nature Preserve*

Total costs of Illinois portion: \$27,109.00

Budgets by State

Wisconsin

	<u>Federal Share</u>	<u>State Share</u>
Salary	-	\$1,978
Contractual Services*:	\$17,800	
Total costs:	\$17,800	\$1,978

* *Contractual services include \$4,000 for genetic analysis, \$7,500 for larval surveys in Door*

County sites, to confirm breeding and \$6,300 to conduct statewide searches for new occurrences.

Total for Wisconsin portion: \$19,778.00

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