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ORIGINAL PAPER

Conservation in Hine's sight: the conservation genetics of the federally endangered Hine's emerald dragonfly, *Somatochlora hineana*

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Abstract Hine's emerald dragonfly (*Somatochlora hineana*) is distributed in discrete fen and wet meadow habitats over its range from Ontario, Canada, to Missouri, USA. Habitat destruction in the vicinity of Chicago, IL, and other areas lead to its designation as an US federal endangered species in 1995. Our main goal was to delineate the population genetic structure of the species within the northern recovery unit centered on the Door Peninsula in Wisconsin and the southern recovery unit in the Des Plaines River Valley near Chicago, IL. Sites on the Door Peninsula, WI, are in a matrix of agricultural development and second-growth forest and were used as a best available approximation of a pristine system for the dragonfly. We nondestructively sampled 557 adults and larvae from 16 sites in Illinois, Michigan, and Wisconsin from 2008 through 2011 and used ten microsatellite markers to estimate levels of genetic variability, and genetic structure. Mean allelic richness across all sites and years was 5.03 (±0.64) and expected heterozygosity was 0.52 (±0.032). Northern and southern recovery units as designated in the original recovery plan were genetically distinct. We delineated two genetic populations in the northern unit and three within the southern including two disjunct sites.

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Introduction

The Hine's emerald dragonfly (*Somatochlora hineana*) is currently distributed in discrete sites in the Upper Peninsula of Michigan and south-central Ontario, Canada, in the north of its range through the Door Peninsula south along the western shore of Lake Michigan in Wisconsin to the Des Plaines River Valley near Chicago, Illinois (Fig. 1). It has recently been found in disjunct locations in southwestern Wisconsin along the Wisconsin River. It also exists in the Ozark Mountains of Missouri, but because of potential hybridization (Meredith Mahoney, personal communication) with other *Somatochlora* species in the area and resulting difficulties with positive identification (Tim Cashatt, personal communication), dragonflies from these sites were not included in the current study. Hine's emerald dragonfly has been extirpated from its type locality and other sites in Ohio and from sites in Indiana and was listed as an endangered species under the US Endangered Species Act in 1995 due to habitat destruction and population declines (USFWS 2001). It is the only dragonfly among the 52 insect species on the US endangered species list. The species recovery plan for *S. hineana* includes delineation of a northern recovery unit (Door Peninsula in Wisconsin and sites in Michigan) and a southern recovery unit that includes sites along the Des Plaines River in Illinois, Cedarburg Bog, Wisconsin, newly discovered sites in SW Wisconsin, and sites in Missouri. In both units, the species must meet specific criteria in terms of numbers of sites occupied, numbers of adults/site, and persistence at sites over time in order to

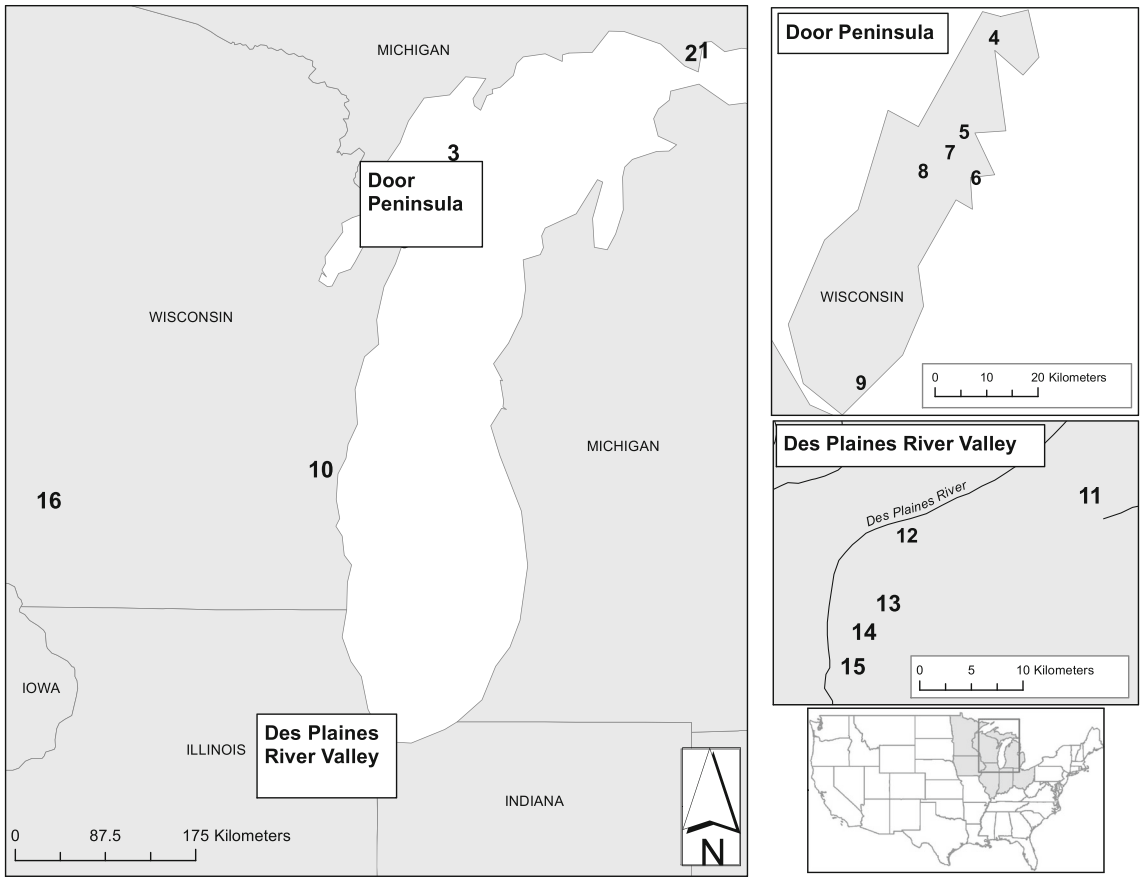

Fig. 1 Map of locations where *S. hineana* were sampled for this study. *Map insets* place the general study area within the context of the continental United States and provide relatively accurate sampling locations within the Door Peninsula, WI and along the Des Plaines River, IL. Numbered sites are as in Table 1: *1* Ackland Road Fen, *2* Sumberby Swamp, *3* Washington Island, *4* Mink River Estuary, *5* Mud Lake North, *6* Toft Point, *7* Bailey's Harbor Swamp, *8* Peil Creek, *9* Kelner Fen, *10* Cedarburg Bog, *11* MacMahon Woods, *12* Keepataw Preserve, *13* Long Run Seep, *14* River South Parcel, *15* Lockport Prairie Preserve, *16* Lower Wisconsin River

be removed from the US Endangered Species List (USFWS 2001).

Like most Odonates, the ecology of the aquatic Hine's emerald dragonfly larvae differs considerably from that of its volant adult stage. Larvae require open fens over dolomitic bedrock which supports slow-flowing rivulets or areas of slow sheet-flow (Soluk et al. 2000). *S. hineana* larvae occupy burrows of crayfish (primarily *Cambarus diogenes* Giard) that apparently provide habitat during dry periods in the late summer and early fall and during winter even though *C. diogenes* will eat *S. hineana* larvae (Pintor and Soluk 2006). Larvae are insectivorous and may take up to 5 years to develop from egg to adult (Soluk et al. 2000, Pintor and Soluk 2006). Adults eclose in mid-summer and live for 6–8 weeks (Soluk et al. 1998, 2000). Adult *S. hineana* are capable of strong sustained flight and like the larvae, adults are insectivorous and breeding takes place in or near larval habitat (Foster and Soluk 2006).

Although there is considerable overlap in habitat use between adult and larval *S. hineana*, larval habitat requirements appear to be the limiting factor for the species' distribution and population size (USFWS 2001). Larval habitat has been particularly affected by human activity in the Des Plaines River Valley near Chicago, IL, where fen habitats are linearly distributed along or near the Des Plaines River within a heavy industrial/urban matrix (Cashatt 1991). In addition to concerns for outright habitat loss (e.g., a six-lane highway bridge was recently completed that crosses prime larval habitat), wetland habitats are subject to altered hydrological regimes, water pollution due to urban run-off, and invasion by weedy exotic plants such as phragmites (*Phragmites australis*) and buckthorn (*Rhamnus* spp.; USFWS 2001). Additionally, *S. hineana* habitat in the Des Plaines River Valley or adjacent to it is owned and/or managed by a diverse array of governmental (i.e., municipal, county, state) and private (e.g., railroads, electrical generation plants, mining operations) entities each with its own economic and conservation priorities. This situation greatly complicates efforts to preserve and restore larval habitat. *S. hineana* habitat has been much less

affected by human activity in other parts of its current range. On the Door Peninsula (and associated Washington Island) in Wisconsin and the Upper Peninsula of Michigan, for example, it exists in a largely agricultural, second-growth forest, and low-density urban matrix. These more pristine areas are actively preserved for their value as vacation destinations.

The main aims of this study were to estimate region-wide population genetic structure in the majority of Hine's emerald dragonfly's range (Missouri and Ontario sites were not included) and to delineate population segments of *S. hineana* along the Des Plaines River and on the Door Peninsula. The first goal is to determine if the current division into northern and southern recovery units in the USFWS recovery plan is biologically defensible. The second goal was to assess the effect of the construction of the US Interstate 355 (I355) extension, a six-lane highway bridge completed in 2007 that crosses the Des Plaines River through prime *S. hineana* larval habitat and to inform habitat management for the species in the Chicago, IL, area. Additionally, in some cases, we were able to determine trends in genetic variability for sites from which dragonflies were sampled in multiple years. We used ten species-specific microsatellite markers (Monroe et al. 2010) to compare population genetic structure and variability for the species in the heavily urbanized Des Plaines River Valley against *S. hineana* found in the more pristine Door Peninsula and in other disjunct habitats in Wisconsin and Michigan.

Methods

Non-lethal sampling

Federal, state, and local permits were obtained for handling adult and larval *S. hineana* and all tissues collected for genetic analyses were taken by non-lethal methods. Monroe et al. (2010) describe the methods used to obtain DNA samples from adult and larval dragonflies. Adults were carefully netted either in flight or as they perched, wing clips were removed and stored in vials dry. Larvae were sampled in the field either by dip-netting along streamlets or by pumping from crayfish burrows (Pintor and Soluk 2006) and returned to the lab in individual sample cups. Once larvae were identified as *S. hineana*, a single tarsus (distal segment of the leg) was removed with forceps by pulling. Most of the adult specimens were collected during the 2010 and 2011 flight seasons, but some adults were collected in 2008 from Cedarburg Bog, Wisconsin, all larvae were collected during the spring, summer, and fall of 2010 and 2011 (Table 1).

DNA extraction and microsatellite amplification

Tissue samples were placed into individual 0.2-µl PCR tubes and 95 % ethanol was allowed to evaporate from the tarsal samples. Both wing and tarsal tissues were soaked in 35 µl of sterile deionized and demineralized water overnight at 4 °C. The next day, genomic DNA was isolated with the Zygem (Hamilton, New Zealand) PrepGEM insect extraction kit following the manufacturer's instructions, except we doubled the incubation time. After extraction, the samples were spun down, and isolates were removed from remaining wing and tarsal bits and placed into a 96-well plate. Ten microsatellite markers from Monroe et al. (2010) were used to genotype 557 *S. hineana* from 16 sampling sites in Michigan, Wisconsin, and Illinois (Table 1). Microsatellites were amplified as in Monroe et al. (2010) and PCR products were multiplexed and separated with high-resolution gel capillary electrophoresis on an Applied Biosystems (Foster City, CA) 3500 genetic analyzer and scored in GeneMapper (Applied Biosystems). The resulting dataset was purged of individuals failing to amplify at more than four loci after attempts to re-amplify any missing loci. Finally, both adult and larval museum specimens from 1996 ($n = 3$), 2002 ($n = 7$), 2003 ($n = 4$), and 2006 ($n = 9$) were extracted and amplified at the same ten loci with the same protocols as for the contemporary samples.

Data analyses

The presence of null alleles was determined with MICRO-CHECKER (van Oosterhout et al. 2006) in combined samples from each location (mixed adults and larvae from all years) as proxy for populations prior to further analyses. Range-wide (for this study) genetic structure was evaluated with ten independent runs of 100,000 iterations after a 20,000 burn-in period in STRUCTURE 2.3.3 (Pritchard et al. 2000). We used the admixture model and correlated alleles with putative collection location information (Francois and Durand 2010). Based on those results, for each sub-population (see results section) we then ran ten independent runs of 100,000 iterations after a 20,000 burn-in period with the admixture model and correlated allele frequencies in STRUCTURE. The ΔK value (Evanno et al. 2005) and STRUCTURE bar plots were used to infer the most biologically relevant number of populations from all three STRUCTURE analyses. Results from STRUCTURE were used to determine population assignment and further analyses were conducted on these inferred population designations. Linkage disequilibrium was tested with the log-likelihood of the G-statistic in FSTAT (Goudet 1995) and significance was determined after sequential Bonferonni corrections (Rice 1989), Hardy–Weinberg equilibrium (HWE; Guo and Thompson 1992) was tested with Arlequin

Table 1 Summary of field sampled *S. hineana* adult (A) and larval (L) age classes collected over each sampling year that were genotyped at ten microsatellite loci

# On Fig. 1 and sample site name	2008	2009	2010	2011	Total sample size	H_E (±SE) for total sample
1. Ackland Road Fen, MI	na	na	na	12 A na L	12	0.37 (0.10)
2. Sumerby Swamp, MI	na	na	7 A na L	22 A 24 L	53	0.39 (0.08)
3. Washington Island, WI	na	na	na	22 A	22	0.44 (0.07)
4. Mink River Estuary, WI	na	na	19 A 21 L	3 A 2 L	45	0.40 (0.08)
5. Mud Lake North, WI	na	na	20 A 25 L	1 A 52 L	98	0.47 (0.08)
6. Toft Point, WI	na	na	18 A na L	na	18	0.49 (0.08)
7. Bailey's Harbor Swamp, WI	na	na	na	5 A na L	5	0.54 (0.08)
8. Peil Creek, WI	na	na	4 A 25 L	na	29	0.47 (0.08)
9. Kelner Fen, WI	na	na	na	24 A na L	24	0.47 (0.09)
10. Cedarburg Bog, WI	24 A na L	1 A na L	10 A na L	25 A na L	60	0.57 (0.06)
11. MacMahon Woods, IL	na	na	0	na A 4 L	4	0.49 (0.11)
12. Keepataw Preserve, IL	na	0 A 19 L	1 A 9 L	0 A 9 L	38	0.57 (0.08)
13. Long Run Seep, IL	na	na	0 A 4 L	0 A 2 L	6	0.49 (0.08)
14. River South Parcel, IL	na	na	0 A 16 L	0 A 40 L	56	0.59 (0.07)
15. Lockport Prairie Preserve, IL	na	na	19 A 27 L	6 A 16 L	68	0.59 (0.07)
16. Lower Wisconsin River, WI	na	na	na	19 A na L	19	0.57 (0.06)

Location names reference numbered locations in Fig. 1. Total sample size was used to generate H_E, which is unbiased heterozygosity averaged over all loci. Sites where no sampling efforts were made in a year are designated "na"

3.11 (Excoffier and Lischer 2010). Observed and expected heterozygosity values were calculated in GenAlEx (Peakall and Smouse 2006) and alleleic richness was calculated by rarefaction (Petit et al. 1998) in FSTAT. Neighbor-joining trees (Saitou and Nei, 1987) were constructed with genetic distance values in POPTREE2 (Takezaki et al. 2010) for comparison to the STRUCTURE results. In POPTREE2, five distance values (D_a, corrected and uncorrected F_{ST}, and corrected and uncorrected D_{ST}; Takezaki et al. 2010) were analyzed with 1,000 bootstrap iterations. A hierarchical pattern of genetic structure for populations was quantified with 1,000 permutations of analysis of molecular variance (AMOVA; Excoffier et al. 1992) using Arlequin 3.11. To determine within and among population variation with AMOVA, the samples were grouped by sampling site within the populations designated by the STRUCTURE results.

Results

Adult and larval sampling

Adult and larval *S. hineana* were sampled from a total of 16 discrete wetland or fen locations (nine in the northern recovery unit and seven in the southern recovery unit; Fig. 1) from 2008 through 2011 with the most successful

Table 2 Summary genetic data for larval and adult samples of *S. hineana* collected at four sites during a single sampling year

Sample Site (year)	sample size	Mean Allelic Richness (±SE)	H_E (±SE)	H_O (±SE)	Nei's Genetic identity	Pairwise F_{ST} between age classes
Sumerby Swamp, MI						
Larvae (2011)	22	3.4 (0.7)	0.36 (0.09)	0.27 (0.08)	0.983	0.016
Adults (2011)	24	3.5 (0.6)	0.41 (0.07)	0.28 (0.06)		
Mink River Estuary, WI						
Larvae (2010)	21	3.5 (0.7)	0.40 (0.08)	0.35 (0.09)	0.990	0.010
Adults (2010)	19	3.5 (0.7)	0.37 (0.07)	0.32 (0.08)		
Mud Lake North, WI						
Larvae (2010)	20	3.6 (0.6)	0.44 (0.09)	0.35 (0.10)	0.973	0.022
Adults (2010)	25	4.8 (0.8)	0.51 (0.07)	0.33 (0.07)		
Lockport Prairie, IL						
Larvae (2010)	27	4.7 (0.7)	0.54 (0.08)	0.50 (0.07)	0.964	0.013
Adults (2010)	19	5.0 (0.9)	0.61 (0.07)	0.45 (0.09)		

Sample sizes reflect successful amplification at ten microsatellite loci. H_E is unbiased heterozygosity averaged over all loci and H_O is observed heterozygosity, Nei's genetic identity and pairwise F_{st} values between age classes at each site

sampling efforts in 2010 and 2011 (Table 1). A total of 557 dragonflies was included in the genetic analyses, only samples that amplified at six or more of our microsatellite loci were included. We sampled 306 individuals (149 larvae and 157 adults) from the northern recovery unit and 251 individuals (146 larvae and 105 adults) from the southern recovery unit (Table 1). Sample sizes for larvae and adults were small in a few locations, but adequate for analyses if adult and larval samples could be pooled from each location (Table 1). We selected four sites from which we had our largest sample sizes of both larvae and adults (N = 19–27) in a given year in order to investigate levels of genetic similarity between age classes (Table 2). Nei's genetic identity (Hedrick 2000) ranged from 0.964 to 0.990 and F_{ST} values ranged from 0.022 to 0.010 and allelic richness and heterozygosity estimates were very similar (Table 2), so we pooled larval and adult samples by location each year for all subsequent analyses.

Genetic results

Ten microsatellite loci, with 5–18 alleles (Table 3), amplified in 479 of the 557 animals genotyped, 55 individuals failed at one locus, 18 at two loci, four at three loci and only one failed at four loci, so all of these were left in the analyses. Most of the loci (35 of 40 locus-by-population combinations) were diagnosed to have null alleles due to homozygous excess (with the Bonferroni setting) in MICROCHECKER. There was no evidence for large allele drop-out but 23 of the 35 loci with null alleles may have had issues with stutter due to a shortage of heterozygotes. However, it is likely that our small and endangered populations could have homozygous excess due to inbreeding, and loci with a high incidence of null alleles have been shown to be effective in Bayesian analyses of genetic structure (Carlsson 2008). Although estimates of population differentiation may be over-estimated due to null alleles, it is not as strongly affected when populations are not highly divergent (Chapuis and Estoup 2007), which we do not expect since adult dragonflies are strong dispersers. Therefore we used all ten loci in further analyses.

Overall genetic structure for the entire dataset resulted in the most likely number of genetic clusters for K = 2 (Fig. 2a) with the Evanno et al. (2005) method and the bar plot of probability of population membership shows clear delineation of two clusters (Fig. 2b). We also present the bar plot of probability of population membership for K = 3, 4, and 5 (Fig. 2c, d, e), since these have increasing support in the traditional method of Pritchard et al. (2000; data not shown). Note that the next most likely number of clusters (i.e., K = 3) using the Evanno et al. (2005) method separates Cedarburg Bog from the northern and southern recovery units (Fig. 2c). The second set of STRUCTURE analyses on the northern and southern clusters resulted in K = 2 in the north corresponding to: (1) the seven Door Peninsula sites (including Washington Island) and (2) the two Upper Peninsula sites (Fig. 3). In the southern recovery unit, K = 3, corresponding to: (1) the six sites along the Des Plaines River Valley with the Lower Wisconsin River, (2) the 2008 Cedarburg Bog sample, and (3) the 2010 and 2011 Cedarburg Bog samples (Fig. 3). We will refer to the five discrete locations (Upper Peninsula, Door Peninsula, Cedarburg Bog, Lower Wisconsin River, and Des Plaines River Valley) as populations below.

When grouped into these five populations, none of the 225 locus-by-locus-by-population combinations were found to have significant linkage of loci, but only 12 of the 50 locus-by-population combinations were in HWE. All of the loci in the Door Peninsula population were out of HWE, and in all 39 cases, HWE failed to be met because of

Table 3 Summary genetic data from 557 individual *S. hineana* genotyped at ten microsatellite loci

Population	Locus (# of alleles)	N	AR	Ho	H_E	F
Upper Peninsula, MI	A7 (6)	64	2.434	0.172	0.259	0.331
	B106 (18)	62	2.840	0.210	0.389	0.456
	B110 (16)	64	4.102	0.484	0.578	0.155
	C119 (16)	65	8.239	0.415	0.830	0.495
	C7 (13)	64	3.515	0.359	0.324	−0.119
	223 (7)	65	2.254	0.062	0.090	0.311
	A108 (10)	65	2.364	0.000	0.118	1.000
	B103 (5)	64	2.981	0.453	0.523	0.126
	B8 (11)	63	4.033	0.587	0.644	0.080
	D10 (7)	65	2.150	0.062	0.090	0.309
	Mean (±SE)	64.1 (0.3)	3.491 (0.574)	0.280 (0.065)	0.384 (0.081)	0.315 (0.096)
Door Peninsula, WI	A7 (6)	240	2.457	0.058	0.134	0.565
	B106 (18)	240	4.745	0.283	0.405	0.298
	B110 (16)	235	7.470	0.634	0.765	0.170
	C119 (16)	237	5.207	0.283	0.614	0.538
	C7 (13)	239	5.558	0.703	0.735	0.042
	223 (7)	238	3.248	0.185	0.210	0.120
	A108 (10)	236	2.941	0.068	0.236	0.712
	B103 (5)	233	3.661	0.476	0.597	0.200
	B8 (11)	234	5.012	0.641	0.718	0.105
	D10 (7)	230	2.396	0.161	0.292	0.448
	Mean (±SE)	236.2 (1.0)	4.270 (0.511)	0.349 (0.078)	0.471 (0.076)	0.320 (0.073)
Cedarburg Bog, WI	A7 (6)	60	4.762	0.217	0.652	0.665
	B106 (18)	54	4.818	0.241	0.330	0.265
	B110 (16)	57	7.993	0.614	0.760	0.185
	C119 (16)	59	3.869	0.237	0.593	0.596
	C7 (13)	60	8.487	0.783	0.852	0.073
	223 (7)	53	4.674	0.358	0.488	0.258
	A108 (10)	58	2.276	0.017	0.373	0.953
	B103 (5)	58	3.932	0.552	0.633	0.121
	B8 (11)	60	4.728	0.483	0.730	0.332
	D10 (7)	59	2.271	0.102	0.309	0.668
	Mean (±SE)	57.8 (0.8)	4.781 (0.651)	0.360 (0.077)	0.572 (0.060)	0.412 (0.092)
Des Plaines River Valley, IL	A7 (6)	172	3.707	0.308	0.467	0.338
	B106 (18)	169	6.306	0.473	0.658	0.278
	B110 (16)	169	7.664	0.615	0.779	0.208
	C119 (16)	170	8.163	0.459	0.843	0.454
	C7 (13)	171	7.906	0.813	0.841	0.031
	223 (7)	172	4.440	0.424	0.432	0.015
	A108 (10)	170	2.259	0.071	0.138	0.488
	B103 (5)	167	3.000	0.569	0.647	0.119
	B8 (11)	172	5.176	0.587	0.700	0.159
	D10 (7)	169	3.129	0.243	0.374	0.349
	Mean (±SE)	170.1 (0.5)	5.175 (0.699)	0.456 (0.067)	0.588 (0.073)	0.244 (0.052)

Table 3 continued

Population	Locus (# of alleles)	N	AR	Ho	H_E	F
Lower Wisconsin River, WI	A7 (6)	19	3.842	0.263	0.471	0.426
	B106 (18)	18	2.990	0.167	0.256	0.329
	B110 (16)	19	6.681	0.632	0.745	0.130
	C119 (16)	19	4.976	0.053	0.647	0.916
	C7 (13)	19	5.842	0.737	0.822	0.080
	223 (7)	16	3.000	0.125	0.331	0.610
	A108 (10)	18	6.546	0.222	0.640	0.643
	B103 (5)	17	3.000	0.588	0.533	−0.137
	B8 (11)	19	4.684	0.474	0.637	0.237
	D10 (7)	17	3.998	0.471	0.622	0.221
	Mean (±SE)	18.1 (0.4)	4.556 (0.453)	0.373 (0.075)	0.570 (0.056)	0.345 (0.098)
Grand mean (±SE) over all loci and populations	All loci	109.3 (11.6)	5.029 (0.639)	0.364 (0.032)	0.517 (0.032)	0.327 (0.037)

Individuals were collected at 16 sampling sites which were grouped according to STRUCTURE results which combine age class (larva and adult), and years into five populations. Sample size (N), AR is alleleic richness as calculated in FSTAT with a minimum sample size of 16, H_O is observed heterozygosity, H_E is unbiased heterozygosity, F is the fixation index ($1 - H_O/H_E$)

homozygous excess (Table 3). All but two locus-by-population combinations had positive fixation indices, indicating inbreeding (Table 3). Average fixation indexes ranged from 0.24 in the Des Plaines River Valley, IL to 0.35 in the small isolated Cedarburg Bog, WI, population. The Illinois population had the highest allelic richness with a mean value of 5.2 and the two lowest richness values were in populations in the northern group, the UP of Michigan with 3.5 and the Door Peninsula, WI, with 4.3 (Table 3).

Neighbor-Joining trees grouped sample sites into recovery units and locations similar to the STRUCTURE analyses but with no temporal structure at Cedarburg Bog, using five distance estimators Nei's D_a (Nei et al. 1983), F_{ST} (corrected and uncorrected; Latter 1972), D_{ST} (corrected and uncorrected; Nei 1972). In all cases, bootstrap support was very low (Fig. 4). AMOVA was highly significant ($P < 0.000$; Table 4), but 91 % of variation was contained within sampling sites, only 3 % was among populations within the northern or southern populations, and 6 % was among the five populations, and F_{ST} was 0.086.

Discussion

Delineation of northern and southern recovery units

The northern and southern recovery units were delineated in the species recovery plan based on Bailey's (1995) ecoregions with the northern recovery unit corresponding to the Laurentian mixed forest province and the southern to the eastern broadleaf forest province. As with many species recovery plans, this delineation was made in the absence of detailed information on the ecology or landscape genetics of *S. hineana*. A survey of the *ND3* mtDNA locus suggested that the Illinois populations were more diverse than the Wisconsin ones and that the Wisconsin populations were segregating a single haplotype not found in Illinois (Purdue et al. 1996). Our analysis of ten microsatellite markers supports the original delineation of recovery units. While our AMOVA and F-statistics suggest weak genetic structure across all our sample locations, Bayesian assignments carried out in STRUCTURE clearly divide our samples into two clusters corresponding to the two recovery units.

Genetic structure within recovery units

Because several tens of kilometers separate some Hine's emerald dragonfly sites from their nearest neighbor (e.g., Upper Peninsula, MI, and Door Peninsula, WI) within each recovery unit, it is important to investigate the possibility that genetic structure exists at spatial scales smaller than recovery units. *S. hineana* exists in discrete habitat patches throughout its range and it is tempting to consider the dragonflies in each patch as constituting a "population" that can be managed as a relatively separate entity. However, it is essential that connectivity between habitat patches be estimated in order to determine the appropriate spatial scale of management actions such as clearing of invasive plants or manipulation of site hydrology. Our

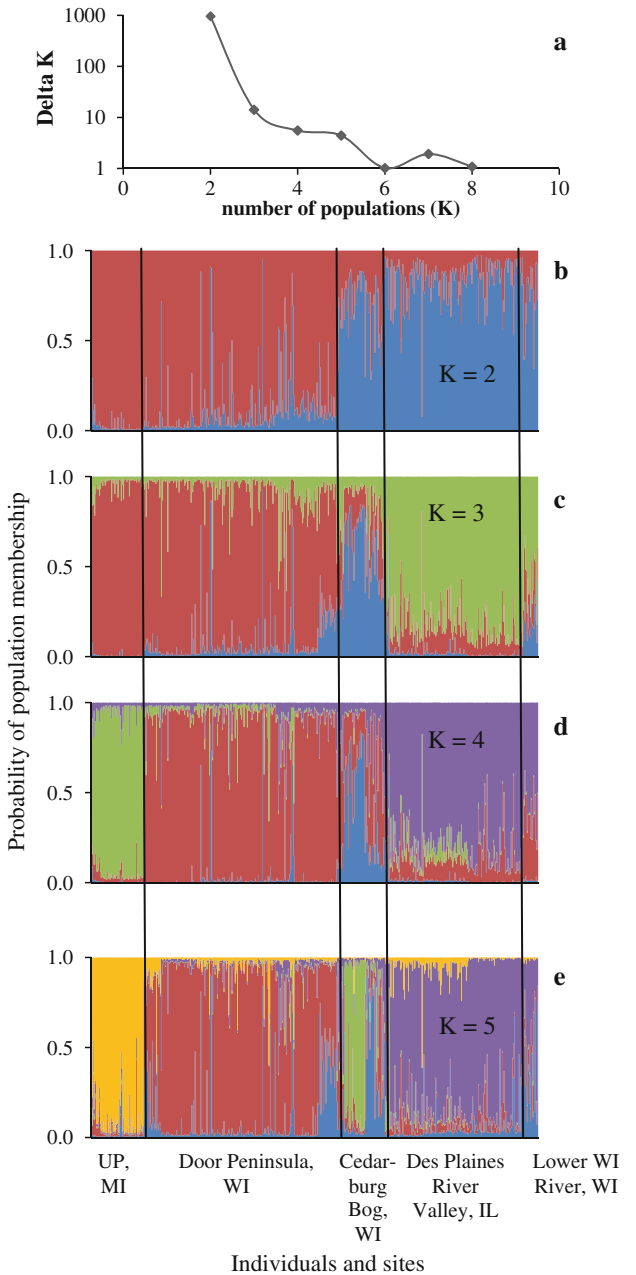

Fig. 2 STRUCTURE results from ten replicate analyses testing K = 1–8. *Line graph* **a** the ΔK results of the Evanno et al. (2005) method. *Bar plots* **b–e** for K = 2–5 for the same data set, *black vertical bars* delineate sampling sites as in Fig. 1

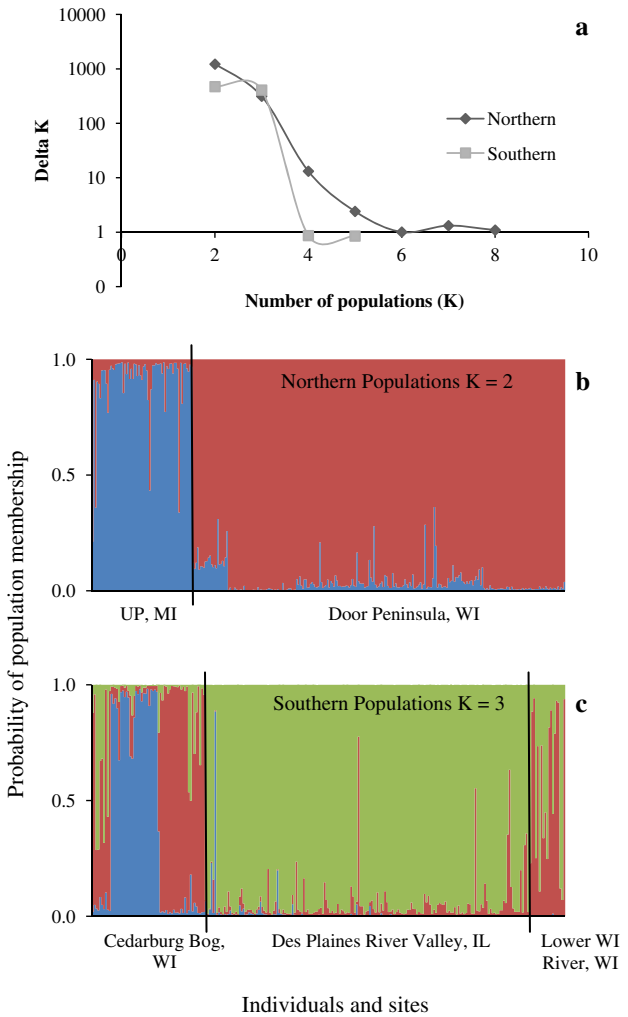

Fig. 3 STRUCTURE results from five replicate analyses testing K = 1–8 for each of two populations as designated by an overall STRUCTURE analysis. The *top chart* presents the ΔK results of the Evanno et al. (2005) method for the northern and southern populations and *bar plots* are for K = 2 in the north and K = 3 in the south. *Black vertical lines* delineate sampling sites as in Fig. 1

results clearly show further sub-structuring within each recovery unit, with some of the more distantly spread sampling sites separated into discrete populations. In the northern recovery unit, there are clearly two populations: the Upper Peninsula, MI, and Door Peninsula, WI, which are separated by 214 km following the contour of Lake Michigan's shoreline with its associated wetland habitats. Dragonflies sampled from the most northern site in the Door Peninsula population on Washington Island are 69 km (straight line distances) from Kelner Fen at the base of the Door Peninsula but are considered one genetic population that includes all of the intervening sites (Fig. 1).

The picture is more complex in the southern recovery unit. Specifically, Cedarburg Bog, which is equidistant between the southern Door Peninsula and the Des Plaines River Valley (Fig. 1), is genetically distinct from the other southern recovery unit populations (see Fig. 2c, K = 3). These results are driven by apparent temporal structuring within Cedarburg Bog in which the 2008 sample is distinct from the 2010 and 2011 samples combined (Fig. 3c). Sample sizes (Table 1) from this site were small making it possible that genetic drift alone could have driven the observed temporal differences. It is also possible that gene flow from an undetected nearby site could have had a large

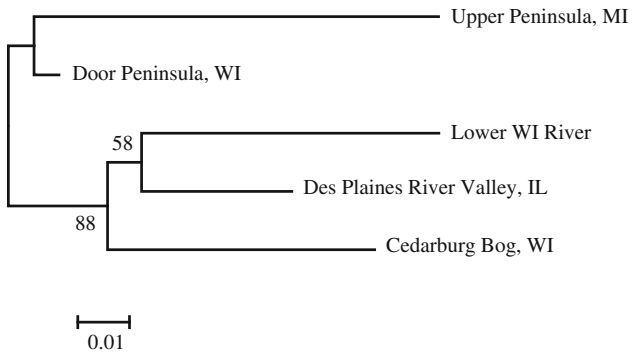

Fig. 4 Neighbor-joining tree constructed with Nei's genetic distance in POPTREE2, *numbers* at *nodes* are support from 1,000 bootstrap iterations. NJ trees with Fst (corrected and uncorrected), Dst (corrected and uncorrected) were identical except for exact support values which ranged from 49 to 86 for the LWR-IL node and from 88 to 92 for the CBB-node

Table 4 Results from analysis of molecular variance (AMOVA) for *S. hineana* grouped by sampling site into five populations based on STRUCTURE results

Source of variation	*df*	Sum of squares	Variance components	Percentage of variation
Among populations	4	155.28	0.15	5.86
Among sub-populations within populations	11	73.03	0.07	2.73
Within sampling sites	1,098	2,655.02	2.42	91.41
Total	1,113	2,883.32	2.65	100.00

influence on allelic frequencies in one or more of the years. Interestingly, all 24 adults sampled at Cedarburg Bog in 2008 were captured on the same day while the 2010 and 2011 samples were taken over the course of 2–3 weeks. This makes it possible that the adults sampled in 2008 were more closely related to one another than the adults sampled over longer periods as in 2010 and 2011. However, inbreeding coefficients (F_{is}) for the three years at Cedarburg Bog are not statistically different (data not shown) suggesting that this may not be the case. Finally, the Lower Wisconsin River population clusters with the Des Plaines River Valley population based on STRUCTURE (Fig. 2) despite the approximately 250 km that separate the two. The neighbor-joining tree (Fig. 4) suggests weak separation between these two sites. The similarity between these two relatively distant populations probably reflects past genetic connections which were likely stronger in the late Pleistocene and early Holocene than they are today. We recommend that the Lower Wisconsin River location be treated as a separate population within the southern recovery unit because of its distance to the nearest known *S. hineana* site and because it contains two unique alleles at low frequencies (0.056 and 0.028) at the A108 locus.

Genetic structure along the Des Plaines River

The original impetus for the study came from concern for Hine's emerald dragonfly persistence near Chicago, IL, in the Des Plaines River Valley. Our study was designed to compare the population genetic structure of *S. hineana* found in the Des Plaines River Valley to the structure of that found on the Door Peninsula where it is much more abundant. Hine's emerald dragonfly habitat on the Door Peninsula is relatively continuous and occurs within a matrix of small farms, wood lots, vacation homes, and small towns which provides robust numbers of the Hine's emerald dragonfly for a genetic analysis with more power relative to the Des Plaines River populations. STRUCTURE results clearly indicate that the Illinois sites contain one genetic population where *S. hineana* occurs in patches of habitat arranged essentially linearly along a 25-km corridor in the Des Plaines River Valley southwest of Chicago, IL. One caveat is that well over half of the Illinois population samples were from Lockport Prairie and River South parcel, which are only 3.5 km apart and until roads separated them were part of the same wetland system. We would expect dragonflies from these two sample sites to be similar genetically based on the previous contiguous habitat and flight abilities of adults. The next largest sample was collected at Keepataw Reserve, only 8.5 km (along the river corridor) from the River South parcel and well within daily flight distances for adult *S. hineana*. Although we were able to obtain good sample sizes in Illinois, they were only from a few sites making our results from the Door Peninsula important for comparison and in assessing the genetic structure of the dragonfly in relatively pristine conditions. Similar to the Illinois population, all of the sampled Door Peninsula sites are clustered into one genetic population even though the most distant sites on the Door Peninsula (Kelner Fen and Washington Island) are nearly nine times further apart than the two most distant sites in Illinois (Lockport Prairie and MacMahon Woods). This similarity in our results gives us confidence that all of the sampled habitat patches in Illinois that support *S. hineana* are part of one genetic population as they are on the Door Peninsula.

Conservation implications

Results of our essentially range-wide genetic survey of Hine's emerald dragonfly populations have several conservation implications. First, the estimated genetic structure of the dragonfly suggests that division of the dragonfly's range into northern and southern units for management

purposes reflects a real division within the species' distribution. Second, the lack of strong structure within the Des Plaines River Valley and Door Peninsula populations suggests that gene flow among habitats within these areas may be high. This implies that connectivity among habitats should be maintained at its current level or improved in order to prevent demographic or genetic processes from extirpating dragonflies from potentially isolated habitats as both areas are further developed for human use in the future. This is particularly important in the Des Plaines River Valley because the new bridge has been shown to stop dragonfly movement up and down the river (Soluk et al., unpublished data) effectively stopping gene flow among habitats on either side of the bridge. Furthermore, dragonfly habitat in this area is already surrounded by a largely inhospitable urban matrix. Third, the Des Plaines River Valley population is the most genetically diverse of the five that we delineated (Table 3). This suggests that any existing genetic connectivity among the sites should be maintained and potentially augmented to preserve the species' genetic diversity. Fourth, levels of genetic variability at Cedarburg Bog, WI, Lower Wisconsin River, WI, and the Upper Peninsula, MI, suggest that these isolated populations are currently able to maintain their genetic variability either through the buffering effect of relatively large effective population sizes and/or from gene flow from nearby undetected/un-sampled locations.

Finally, our results have implications for any future re-introductions and augmentations that may take place within the dragonfly's historic range. Extirpated populations in each of the recovery units could be re-established with dragonflies from populations within each of the respective units. For example, MacMahon Woods in the Des Plaines River Valley has historically been a habitat at which very small numbers ($n < 5$) of adults and larvae have occasionally been found (i.e., not every year of sampling). Any potential augmentation of this fen should come from other populations within the Des Plaines River Valley that have historically supported larger numbers of dragonflies like Lockport Prairie/River South habitats. This suggestion holds for the disjunct Lower Wisconsin River population as well. Re-introductions or augmentation of the other isolated habitats at Cedarburg Bog and the Upper Peninsula are more problematic because populations at these locations are somewhat genetically distinct. However, both are more genetically similar to the Des Plaines River Valley population (Cedarburg Bog) or the Door Peninsula population (Upper Peninsula) suggesting these populations as sources of dragonflies for augmentations and re-introductions. Finally, captive rearing efforts have been successful for this species (Satyshur et al. 2009). We are currently using our microsatellite markers and developing other nuclear and mtDNA markers to investigate the potential genetic effects of this effort on the persistence of this species.

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