

Single-sample estimation of effective population size in several populations of the endangered Hine's emerald dragonfly

Emy M. Monroe^{1,2} and Hugh B. Britten^{1,3}

¹Department of Biology, University of South Dakota, 414 East Clark Avenue, Vermillion, South Dakota 57069 USA

Abstract: Hine's emerald dragonfly (*Somatochlora hineana*) is the only odonate on the US Endangered Species list. It prefers discrete fen-and-wet-meadow habitat from Ontario, Canada, to Missouri, USA. This habitat has been destroyed across much of *S. hineana*'s range. Its conservation genetics were assessed by microsatellite analysis in a previous study. We applied 2 common single-sample estimators to the same data set to estimate effective population size (N_e), or effective number of breeders, in 5 populations (separated into adult and naiad stage classes) across the species' range in 2008 and 2010–2011. Populations of the species in the Upper Peninsula of Michigan, the Door Peninsula of Wisconsin, and along the Des Plaines River Valley in Illinois are made up of individuals collected from multiple sites, but the other 2 populations, at Cedarburg Bog, Wisconsin, and along the Lower Wisconsin River, consist of samples from single habitats disjunct from other known sites. N_e for *S. hineana* were similar to those for other endangered insects and ranged from 22 adults in the Des Plaines River Valley population in 2010 to 200 adults in the Door Peninsula population in 2010 based on approximate Bayesian estimation in ONeSAMP and from 8 naiads in the Door Peninsula population to 419 adults in the Des Plaines River Valley population based on the linkage disequilibrium method in NeEstimator. These N_e values confirm the endangered status of this species and indicate that efforts to maintain current habitats and connectivity to suitable habitat are essential to maintaining genetic diversity.

Key words: N_e , ONeSAMP, LDNe, *Somatochlora hineana*

Hine's emerald dragonfly (*Somatochlora hineana*) was listed as endangered under the US Endangered Species Act in 1995 because of habitat destruction and population decline (USFWS 2001) and is the only dragonfly among the 52 insect species on the US endangered species list. *Somatochlora hineana* is currently distributed in discrete sites in the Upper Peninsula of Michigan and south-central Ontario, Canada, at the northern end of its range and 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 also occurs in disjunct locations in southwestern Wisconsin, along the Wisconsin River, in Cedarburg, Wisconsin, and in the Ozark Mountains of Missouri. *Somatochlora hineana* is extirpated from its type locality and other sites in Ohio and from sites in Indiana.

The ecology of the species has been described elsewhere (Soluk et al. 2000), but the patchy distribution of suitable habitat for the *S. hineana* naiad and its 2- to 5-y life-span (D. Soluk, University of South Dakota, unpub-

lished data) are important features related to its conservation. The naiads inhabit relatively isolated fen habitats that have seasonal sheet flow or somewhat channelized flow in small rivulets that are susceptible to natural and anthropogenic changes in flow regimes and other disturbances (USFWS 2001, Soluk et al. 2000). Naiads of all age classes occupy the burrows of the crayfish *Cambarus diogenes* as refugia against drought and winter conditions (USFWS 2001, Soluk et al. 2000).

Somatochlora hineana habitat has been heavily affected by human development across a significant portion of its range (USFWS 2001). Habitat disturbance is particularly acute in the Des Plaines River Valley near Chicago, Illinois. The species recovery plan for *S. hineana* includes delineation of a northern recovery unit (Door Peninsula and Washington Island, 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 southwestern Wisconsin, and sites in Missouri. Both units must meet specific criteria in

E-mail addresses: ²Present address: Whitney Genetics Lab, US Fish and Wildlife Service, 555 Lester Avenue, Onalaska, Wisconsin 54650 USA, emy_monroe@fws.gov; ³hugh.britten@usd.edu

DOI: 10.1086/682073. Received 16 June 2014; Accepted 25 November 2014; Published online 20 May 2015.
Freshwater Science. 2015. 34(3):1058–1064. © 2015 by The Society for Freshwater Science.

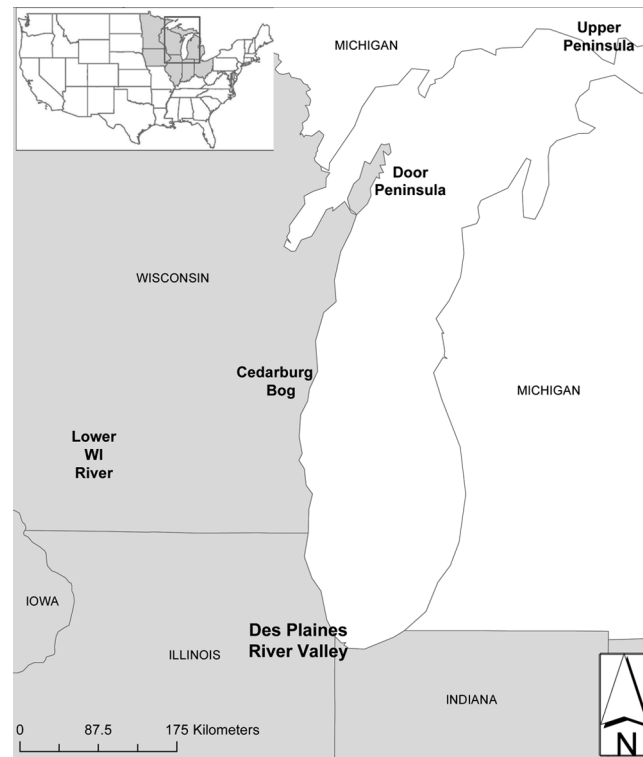


Figure 1. Map of general locations for the 5 main populations of *Somatochlora hineana* delineated in Monroe and Britten (2014), for which effective population size was estimated. Map inset places the general study area within the context of the continental USA.

terms of number of sites occupied, number of adults/site, and persistence at sites over time for the species to be removed from the endangered species list (USFWS 2001). Monroe and Britten (2014) used 10 nuclear microsatellite markers to test for genetic delineation of the recovery units and to elucidate the finer-scale population genetic structure of *S. hineana* within them. Both recovery units were clearly delineated using a Bayesian assignment test and Nei's genetic distances (Nei 1972, Monroe and Britten 2014). The northern recovery unit consists of 2 populations: one at Door Peninsula/Washington Island and the other at sites in the Upper Peninsula of Michigan (Monroe and Britten 2014). The southern recovery unit consists of 3 populations: one at Des Plaines River Valley near Chicago, one at Cedarburg Bog that had a strong temporal structure (2008 vs 2011), and one at sites along the Lower Wisconsin River in southwestern Wisconsin (Missouri sites were not included) (Monroe and Britten 2014).

The microsatellite data allowed us to estimate genetically effective population sizes (N_e) for *S. hineana* over 1 or 2 y in several populations. N_e is the size of an ideal population that has the same rate of genetic drift and inbreeding (loss of allelic diversity and heterozygosity) as the real population being studied (Crow and Kimura 1970). Ideal populations are abstractions that have constant sizes, equal sex ratios (or are hermaphrodites), equal family sizes, nonoverlapping generations, and are closed to immigra-

tion and emigration. N_e estimates are usually much smaller (i.e., ~10% for vertebrates) than census population sizes (N) because of deviations from the behavior of ideal populations in terms of reproductive ecology (Frankham et al. 2010). Estimates of N_e have become increasingly important in conservation planning because they measure the susceptibility of small populations to short-term inbreeding depression and the loss of genetic potential needed for long-term persistence (e.g., Palstra and Ruzzante 2008, Frankham et al. 2010, Luikart et al. 2010, Traill et al. 2010, Holleley et al. 2014). Thus, N_e estimates provide an important vital statistic in conservation and act as an index of the strength of genetic stochasticity likely to impinge on populations of conservation concern and the strength of inbreeding and genetic drift driving a species' extinction vortex (Gilpin and Soulé 1986). N_e estimates are important in minimum viable population (MVP) modeling aimed at estimating the probability that a given species will persist for a predetermined amount of time (Traill et al. 2010). The connection between a genetic abstraction (N_e) and real demographic parameters for populations (e.g., sex ratio, population fluctuation) means that N_e estimates often are interpreted loosely as the number of breeding adults in the focal population.

N_e can be estimated in 2 general ways. Demographic methods rely on field-based approaches that measure ≥ 1 of the deviations from the ideal for the population under

study. Demographic data, such as fluctuations in N over ≥ 2 generations, differences in reproductive output (family size) among adults in the population, and sex ratio, can be used to estimate long-term average N_e (Frankham et al. 2010, Luikart et al. 2010). These types of demographic data can be difficult to collect for wild populations, so conservation biologists have come to rely more on genetic or indirect methods of estimating N_e (Luikart et al. 2010). A considerable body of theory exists regarding genetic estimators of N_e (see Luikart et al. 2010). This body of theory mostly addresses whether the estimator captures inbreeding N_e (loss of heterozygosity), which reflects the N_e of the parental and earlier generations, or variance N_e , which reflects more recent changes in allele frequencies or loss of alleles (Luikart et al. 2010). Temporal methods of estimating N_e require genotypic data from ≥ 2 generations, whereas 1-sample methods do not (Luikart et al. 2010, Holleley et al. 2014).

Our goal was to estimate N_e with 2 single-sample estimators from 5 *S. hineana* populations derived from microsatellite frequency data over 2 y, a period that is too short for use of temporal methods that require samples >1 generation apart. We used the method of Waples and Do (2008) based on linkage disequilibrium with bias correction (Waples 2006) and the ability to handle missing data (Peel et al. 2013), and the approximate Bayesian method of Tallmon et al. (2004). We concluded that intensive management of *S. hineana* populations should continue because of very small (typically < 100) estimates of N_e (Traill et al. 2010).

METHODS

We obtained all specimens under required federal, state, and local permits, and took all tissues collected for genetic analyses by nonlethal methods as described by Monroe et al. (2010). We removed wing clips from carefully netted flying or perched adults and stored them dry. We used methods published by Pintor and Soluk (2006) to collect naiads and returned them to the laboratory for identification before removing 1 tarsus (distal segment of the leg). We collected most naiads and adults during spring, summer, and autumn 2010 and 2011. However, we collected adults from Cedarburg Bog, Wisconsin, in 2008 and 2011. Collections were attempted in 2010 at Cedarburg Bog, but very few adults were observed over the entire flight season and only 8 were captured. This sample size was too small to analyze. We extracted whole genomic DNA from wing clips and tarsi as described by Monroe and Britten (2014) and genotyped extracts at 10 microsatellite loci (Monroe et al. 2010, Monroe and Britten 2014; Table 1).

Monroe and Britten (2014) delineated 5 populations across the range of *S. hineana*. We estimated effective population size (N_e) separately for each population to avoid

biasing our estimates because of population structure (Holleley et al. 2014). Most N_e estimation methods assume non-overlapping generations, so when applicable, we divided each population into stage classes: naiads only, adults only, and naiads and adults combined. For the adult stage, this assumption is reasonable, although generations can overlap if naiads grow at different rates, leading to mixed emergence classes. We combined naiads of different lengths (ages) to achieve adequate sample sizes for N_e estimates. We also obtained N_e estimates for mixed adult and naiad groups in each population, even though this approach violated the assumption of nonoverlapping generations, so that we could assess the effects of a clear violation of the assumption against the naiads- and adults-only groups.

We made estimates for all 3 groups in each population for which samples were available with the aid of the online program ONeSAMP (Tallmon et al. 2008), which implements an approximated Bayesian computation and is a summary of several statistics (Tallmon et al. 2004). In all cases, we used populations with ≥ 19 individuals with prior effective population sizes set from 2 to 100. Sample sizes and the number of loci used in ONeSAMP differed in some cases for some populations because loci that did not vary within an individual population were eliminated as were individuals with >1 missing locus. We also used LDNe (Waples and Do 2008) (implemented in NeEstimator version 2.01; Do et al. 2013, Peel et al. 2013) to estimate N_e for each population by stage class, and compared these estimates to the ONeSAMP estimates (Gomez-Uchida et al. 2013, Hoehn et al. 2012, Jansson et al. 2012, Johnstone et al. 2012, Skrbinek et al. 2012). We excluded the adult plus naiad group in the LDNe estimation because this grouping constituted a gross violation of the assumption of nonoverlapping generations and no summary statistics were involved (as with the ONeSAMP estimator). LDNe estimates used the sample-size-bias correction (Waples 2006), excluded low-frequency alleles at the 0.01 level, and calculated 95% confidence intervals (CIs) by jackknifing over loci.

RESULTS

ONeSAMP and LDNe estimates of effective population size (or effective number of breeders) were similar in 4 of the 8 comparisons between groups: adults only and naiads only from the Upper Peninsula in 2011, adults only from the Door Peninsula in 2011, and naiads only from the Des Plaines River Valley in 2010. ONeSAMP provided an estimate with 95% CIs for all groups, but LDNe was unable to provide an estimate for 4 groups and had an infinite upper bound for another 4 groups (Table 1). Both methods yielded very low estimates of N_e for an insect. Estimates ranged from 8 to 419 (Table 1). CIs were much smaller for the ONeSAMP than for the LDNe estimates (Table 1). Values of the 2 estimators differed most for populations

Table 1. Estimated effective population (N_e) sizes and 95% confidence intervals (CIs) of *Somatochlora hineana* grouped into 5 populations and separated into adults only, naiads only, or combined adults and naiads for each sampling year. Sample sizes are the number of genotyped individuals used in the ONeSAMP or NeEstimator (LDN_e) programs. NA = not available.

Population and year	Sample size	Number of loci	Age class	ONeSAMP N_e estimate (95% CI)	LDN _e estimate (95% CI)
Upper Peninsula, Michigan					
2011	22	10	Adult	26 (20–37)	25 (9–∞)
2011	23	9	Naiad	29 (23–43)	46 (12–∞)
2011	45	10	All	65 (47–112)	NA
Door Peninsula, Wisconsin					
2010	55	9	Adult	200 (127–414)	17 (7–43)
2010	70	8	Naiad	53 (38–93)	100 (35–∞)
2010	125	9	All	320 (148–2177)	NA
2011	37	10	Adult	37 (28–57)	46 (26–120)
2011	52	10	Naiad	31 (24–44)	8 (4–13)
2011	89	10	All	120 (83–217)	NA
Cedarburg Bog, Wisconsin					
2008	24	9	Adult	25 (19–38)	No estimate given
2011	23	10	Adult	29 (22–44)	No estimate given
Des Plaines River Valley, Illinois					
2010	19	9	Adult	22 (17–31)	419 (33–∞)
2010	27	9	Naiad	44 (34–74)	63 (81–7743)
2010	46	9	All	78 (60–132)	NA
2011	64	10	Naiad	81 (64–123)	No estimate given
Lower Wisconsin River, Wisconsin					
2011	19	10	Adult	55 (39–88)	No estimate given

from the Door Peninsula in 2010 (Table 1). LDN_e and ONeSAMP estimates differed by a factor of 10 for adults and by a factor of 2 for naiads (Table 1). ONeSAMP estimates for naiads differed from estimates for adults by a factor of ~4, and LDN_e estimates for naiads differed from estimates for adults by a factor of 5. However, ONeSAMP estimates for adults were similar to those for naiads for the Upper Peninsula and Door Peninsula populations in 2011 (Table 1). The adult plus naiad groups had the highest estimates and the widest CIs in both years (Table 1).

DISCUSSION

We estimated genetically effective population sizes (N_e) for *Somatochlora hineana* from 5 populations that were delineated by analyses of 10 microsatellite markers (Monroe and Britten 2014). Estimation of N_e may be complicated for the Hine's emerald dragonfly because its naiads take several years to develop and different cohorts could potentially develop at different rates, which introduces the potential for sampling overlapping generations (Luikart et al. 2010). Adult samples from any given sampling location also could represent multiple generations, although as

the emergent class, they are the only contributors to the next generation. Given these considerations, we opted to estimate N_e separately for adults and naiads and for the 2 groups combined. Although potential overlap in generations is not ideal, ONeSAMP apparently performed well despite some overlap in generations at the sample sizes we had available (Luikart et al. 2010). The LD method implemented in NeEstimator (LDN_e) also performed fairly well despite somewhat lower than recommended sample sizes and only 10 loci. The estimates the LDN_e method was able to provide were similar to the ONeSAMP estimates in 4 of 8 comparisons. Authors of other studies using LDN_e commonly have reported estimates of infinite upper bounds, but the method has been considered the best estimate when N_e was small (Beebe 2009, Saarinen et al. 2009). Others have reported that LD was not as precise as ONeSAMP for several natural populations of fruit flies (Barker 2011). Our estimates based on the 2 methods are within 2 orders of magnitude, and the 95% CIs of the ONeSAMP method (Table 1) corroborate the endangered status of this species. Nevertheless, we recommend using our LDN_e estimates only for relative comparisons among populations and across seasons. Estimates for the mixed age classes,

which violate the nonoverlapping assumption were all biased overestimates compared to estimates calculated for separated age classes. This bias could be a consequence of the larger sample size used with mixed age classes or the violation of the assumption of nonoverlapping generations. In the first case, investigators have shown that ONeSAMP estimates are correlated with sample size (Gomez-Uchida et al. 2013). Despite these issues with our data and the estimators, differences between methods or between years are only 1 order of magnitude for the Door Peninsula 2010 population where the range of estimates is 17 to 320. The other instance where an order of magnitude difference exists is in the Des Plaines River Valley where the 2010 adult N_e estimate is 22 with ONeSAMP and 419 with LDNe.

In the 2 isolated populations (Cedarburg Bog and the Lower Wisconsin River), N_e estimates from ONeSAMP ranged from 25 to 55 reproductive individuals/generation. These estimates are consistent with our observations while collecting adults. For example, sampling efforts were attempted over $\frac{1}{2}$ of the 2011 adult flight season at both locations. We observed very few specimens and captured none. No N_e estimates were obtained with LDNe for Cedarburg Bog or the Lower Wisconsin River.

N_e estimates are not much more encouraging for the population in the Upper Peninsula of Michigan. The 2011 estimates are <100 for the adults and naiads, and ONeSAMP delivered an estimate of only 65 with naiads and adults combined. The N_e estimates for the population in the species' stronghold on the Door Peninsula appears to be fluctuating wildly, and estimates range from a few tens to a few hundred between years. Thus, congruence between years is low for this population. Barker (2011) attributed a similar result in fruit flies to extreme environmental variation. Hine's emerald dragonfly may experience similar variation because of irregular flooding or drought. The habitat of *S. hineana* is highly variable, and water levels fluctuate greatly among seasons and over years, which may drastically affect the winter burrowing sites for the naiad stages. Even though the Door Peninsula is the stronghold

for this species, many of the fens on the Peninsula are affected by water levels in Lake Michigan, which have been low since the late 1990s (Assel et al. 2003). With lake levels low, water level in the fens can be greatly affected by seasonal rainfall, creating very different conditions during the short adult flight and breeding seasons between 2 years. N_e estimates for the Des Plaines River Valley population are similar to those in the Upper Peninsula and are low and variable enough to elicit concern for this population's persistence. Soluk and Mierzwa (2012) estimated the census population size of naiads for the Des Plaines River Valley at ~2000 individuals in 2011 and 2012. These estimates yield $N_{e(ONeSAMP)}/N$ ratios of 0.022 and 0.041 in 2010 and 2011, respectively. These ratios are within the range reported for other endangered insects (e.g., Saarinen et al. 2009).

Perhaps the most striking result is that the 2011 Door Peninsula adult N_e estimate is similar in magnitude to estimates from isolated sites at Cedarburg Bog and the Lower Wisconsin River. The sites on the Door Peninsula are considered to be the species' stronghold where many hundreds of dragonflies may be in flight in a given season (EM, personal observation), yet this population has an N_e indicative of a population with a much smaller census population size. Table 2 provides estimates of N_e for other insects of conservation concern. The studies cited were selected because their authors used microsatellite data from a comparable number of loci and populations. Care should be taken when comparing N_e estimates across insect taxa for a number of reasons, including vastly differing life histories, but the estimates for the Hine's emerald dragonfly are within the range of N_e estimates reported for other insects with small populations and restricted distributions.

Conservation implications

Our microsatellite results show that Hine's emerald dragonfly populations exist in 5 presumably genetically isolated populations in the bulk of its distribution (Monroe and Britten 2014). Each of these populations persists with

Table 2. Estimated effective population (N_e) sizes and 95% confidence intervals (CIs) for insects of conservation concern.

Species	N_e (95% CI)	Number of loci	Analytic method	Number of populations	Citation
<i>Coenagrion mercuriale</i>	81 (39–2971) 372 (168–∞)	14	Maximum likelihood	6	Watts et al. 2007
<i>Cyclargus thomasi Bethune-bakeri</i>	14 (9–20) 51 (34–81)	12	Approximate Bayesian	2	Saarinen et al. 2009
<i>Carabus violaceus</i>	190 (90–850) 530 (120–?)	13	Maximum likelihood	2	Keller et al. 2005
<i>Hydrophilus piceus</i>	4 (3–11)	3	Maximum likelihood	1	Beebe 2007
<i>Lasioglossum oenotherae</i>	336 (83–?) 223 (86–?) 961 (124–?)	6	Maximum likelihood	3	Zayed and Packer 2007

low N_e s that are consistent with N_e estimates for other endangered insects. Furthermore, our multiyear estimates indicate that the dragonfly's N_e s can vary over ~ 1 order of magnitude year to year in the species' stronghold on the Door Peninsula. Our $N_{e(\text{ONE SAMP})}/N$ ratios also are low and consistent with those of other endangered insects, and although we have only 2 of these ratios, they corroborate our field observations that Hine's emerald dragonfly adults may number 1 or 2 orders of magnitude more than their N_e s. These N_e estimates confirm the endangered status of the dragonfly and raise concerns for its susceptibility to short-term inbreeding depression and the loss of genetic potential needed for long-term persistence (e.g., Palstra and Ruzzante 2008, Frankham et al. 2010, Luikart et al. 2010, Traill et al. 2010, Holleley et al. 2014). Conservation genetics theory suggests that Hine's emerald dragonfly is exposed to genetic stochasticity that could increase the strength of inbreeding and genetic drift in its populations and drive the species' extinction vortex (Gilpin and Soulé 1986, Frankham et al. 2010). The only conservation actions that can buffer the species from these effects are those that will increase N_e for each Hine's emerald dragonfly population.

Preservation and restoration of Hine's emerald dragonfly habitat is an ongoing effort that should eventually increase both the census and genetically effective population sizes for the species across its range. Connectivity between sites that contain several discrete habitat areas that support subpopulations (e.g., Door Peninsula and Des Plaines River Valley) of the dragonfly should be maintained because subpopulation isolation will decrease N_e (Frankham et al. 2010). Sites on the Door Peninsula and along the Des Plaines River that once supported the dragonfly, but no longer do, should be maintained as parts of habitat corridors allowing gene flow between habitat areas. These currently unoccupied sites may also be considered for re-introduction of the species if they can be restored and maintained as suitable habitat. The McMahon Wood Fen in the Des Plaines River Valley is an example of such a site.

ACKNOWLEDGEMENTS

We thank the many Soluk lab summer research technicians, L. Heintzman, L. Brotkowski, A. Monroe, and M. Lefdal for help obtaining field specimens. Funding was provided by the Illinois State Toll Highway Authority, and we thank A. La Porte for assistance as our point of contact with that agency. Additional funding was provided by the Illinois Department of Natural Resources and US Fish and Wildlife Service. None of our funding sources had any role in study design, execution of the work, or writing of the manuscript. In-kind support was provided by The Nature Conservancy Wisconsin Chapter, the DuPage County Forest Preserve District, and the University of Wisconsin-Milwaukee Field Station. Funding for the genetic analyzer was provided by the National Science Foundation grant #MRI-0923419. We thank L. Riley and J. Filler for assistance in the laboratory, J. Brown for creating the map, K. Lah (US Fish and Wildlife Service Chicago

Field Office) for his continuing support, and 2 anonymous referees for helpful comments on earlier versions of this manuscript. All specimens were obtained under permits TE805269-11 and TE805269-13 issued by the US Fish and Wildlife Service as well as all required state and local permits.

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