As the Dragon Flies: Population Structure in *Sympetrum obtrusum* Dragonflies

JOCELYN L. FAYDENKO, LENA M. BUTLER, AND BRADLEY J. SWANSON

Central Michigan University, 1200 S. Franklin Street, Mount Pleasant, MI, 48859

October 2013

Abstract: *We analyzed population structure between adults and nymphs for the White-faced Meadowhawk (Sympetrum obtrusum). We used one amplified fragment length polymorphism (AFLP) marker producing 135 alleles to determine how related the populations of adults and nymphs are at Pierce Cedar Creek Institute near Hastings, MI. We examined data collected from 2012 and 2013 and found that the majority of adults comprised separate genetic populations from nymph populations, and each year of adults comprised a unique genetic population. Despite occupying the same location, dragonfly adults and nymphs appear to constitute distinct populations. Our results suggest adults disperse from the natal area to breed.*

Keywords: population structure, dispersal, AFLP, *Sympetrum obtrusum*

Introduction

Dragonflies are an understudied taxa, with little known about basic information such as ranges and distributions for most species worldwide (IUCN 2010), despite being indicators of habitat quality and water quality (IUCN 2004, Reece and McIntyre 2009, Oertli et al. 2002). Further, previous studies have examined the movement and spatial distribution of either dragonfly nymphs (Benke and Benke 1975, Pierce et al. 1985, Buskirk 1987a) or adults (Conrad et al. 1991, Remsburg et al. 2008), but there is little information about how populations of nymphs and adults are connected. Understanding this connection is especially important given the amount of the Earth's surface that has been modified by anthropogenic activities (Reich et al. 2001) and the dragonfly's dependence upon both terrestrial and aquatic habitats (Corbet 1991).

Within aquatic ecosystems, dragonfly nymphs are usually the dominant predaceous insects (Benke and Benke 1975). However, fish predation has a strong effect on the spatial distribution of nymph populations (Pierce et al. 1985). Nymphs have two survival strategies when predators are present; nymphs remain motionless to avoid detection from fish, while in areas without fish they swim to escape from other predators (Pierce et al. 1985). The survival and growth of nymphs is inversely related to nymph density, and this relationship regulates the number of juveniles present within a habitat (Buskirk 1987a). If nymph population density was high, then the respective adult population density would be expected to be high. However, if nymph population density was low, then adult population density would be low, resulting in adults from other areas moving into that area to utilize its resources and find mates.

As a result, unlike nymphs, adult dragonflies are not restricted by density. They are able to move to lower-density populations due to their increased mobility (Buskirk 1987b). Adult dragonfly reproduction occurs near the aquatic habitats where nymphs are found (Conrad et al. 1991). After emerging from their natal ponds, sexually immature dragonflies will move away

from the water to forage, using light as a key factor in orientation (Remsburg et al. 2008). In a mark-recapture study, 15% of the marked individuals disappeared from the population at some point in their lifecycle, but dispersal was not specifically studied (Conrad et al. 1991).

The patchy nature of dragonfly habitat suggests that they exist as either isolated populations or as metapopulations (Suhonen et al. 2010). Dispersal to other metapopulations affects population size and persistence, spatial distribution, gene flow, and adaptation to local environments (Kleinhans and Jonsson 2011). Each subpopulation in a metapopulation faces extirpation, making dispersal key for regional survival (Nee and May 1992), although recurring extinctions and recolonization reduces genetic diversity, increasing the likelihood of the metapopulation going extinct (Austin et al. 2011). A low rate of dispersal combined with habitat degradation can lead to local extirpation, with an extinction rate as high as 30% in areas of low quality habitat. Dispersal is biased towards higher quality habitats, so extinction increases in areas of low quality (Nee and May 2011).

While dispersal of adults can occur, it is unclear whether adults return to their natal water source to breed or if they breed elsewhere. Furthermore, due to nymphs' ability to overwinter for multiple years, dragonfly populations may rotate year after year, with a similar genetic population emerging every other year (Pintor and Soluk 2006). If dragonflies are subject to this temporal life cycle rather than dispersal, loss of either the aquatic or terrestrial habitat is likely to result in the localized extirpation of the local dragonfly populations. The main focus of our research was to determine if dragonflies are philopatric to their natal aquatic habitats. Last year's research found three distinct genetic clusters (putative populations) of *S. obtrusum* nymphs—one in the Tallgrass Swamp and two in Aurohn Lake (41% of individuals in one cluster and 51% of individuals in the other) (Faydenko et al. 2012). Two populations of adults

were found. The majority of the adults (85%) clustered in a group unrelated to any of the nymphal populations while the second set of adults (15%) was associated with the Aurohn Lake nymphal cluster containing 59% of the samples. This suggests population structure could be affected by a cyclic generation period or a dispersal factor.

Specifically, the null hypothesis we test is that the nymphal and adult dragonflies are a single genetic population with little emigration. This could occur with individuals spending a single year as nymphs or with temporal-based population structure due to overlapping generations (Kormondy and Gower 1965). If individuals spend a single year as nymphs, the adults will be genetically similar to the nymphs of the previous year. In contrast, if there is temporal-based population structure we expect to see a repetitive cycling of populations year after year. One year would produce a unique population of adults dissimilar to adults that emerged the year prior. In this study, we evaluate our null hypothesis by examining the genetic make-up of adult dragonflies over two years in relation to the nymphal population. If nymphal dragonflies remain in their natal habitat for either one or multiple years, supporting the null hypothesis, we would expect to see a distinct adult population emerge each year that resembles a former nymph population. In contrast, if dispersal controls population structure we would find little to no genetic overlap between nymphs and the surrounding adults.

Methods

Sampling

We sampled from 8 total sites across 2 years, the summer of 2012 and the summer of 2013, with 3 sites for nymphal sampling and 5 sites for adult sampling (Fig. 1). For the 2012 nymphal sites, we sampled 31 *Sympetrum obtrusum* nymphs from Aurohn Lake, a man-made

lake just north of the Pierce Cedar Creek Institute (PCCI) property. We also sampled 29 nymphs from the Tallgrass Swamp in 2012, which is located south of the Visitor's Center along the tree line of the Tallgrass Prairie. The nymphs were sampled from May 21 and June 5, 2012. The

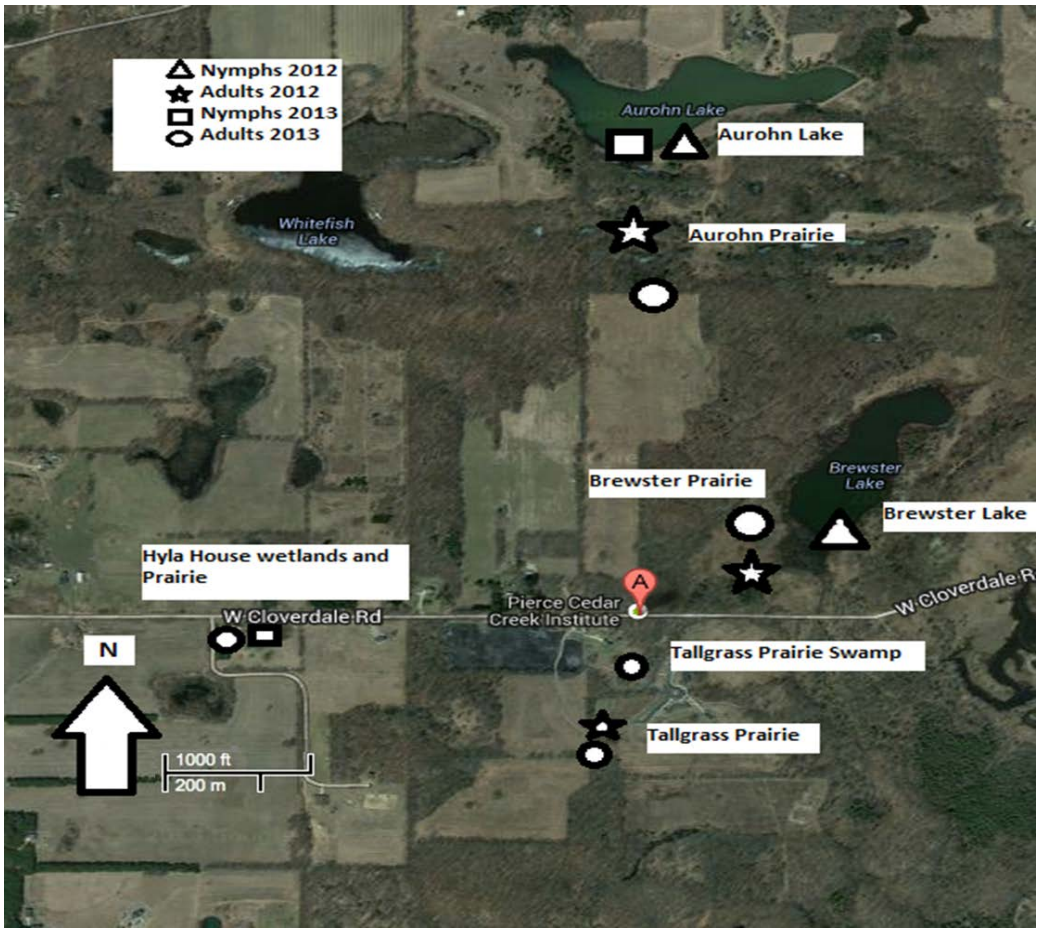

Figure 1. The map of the sites where *Sympetrum obtrusum* was sampled. The triangles are the sites were the 2012 nymphs were collected. The stars are the sites where the 2012 adults were collected. The circles are the various sites where the 2013 adults were sampled, and the squares were the 2012 sites for the nymphs. The nymphs for 2012 were collected at Aurohn Lake and Tallgrass Swamp. The adults for 2012 were collected at Brewster Prairie, Aurohn Prairie, and Tallgrass Prairie. The adults for 2013 were collected at Hyla House, Aurohn Prairie, Brewster Prairie, Tallgrass Prairie, and the Visitor's Center Retention Pond. The nymphs for 2013 were collected at Aurohn Lake and Hyla House.

adults were not collected until they started to appear, which for the summer 2012 adults began June 19, 2012 and was completed June 27, 2012. We collected 30 adults from Aurohn Prairie, 30 from Brewster Trail Prairie, and 30 from Tallgrass Prairie. For the summer of 2013 we sampled 39 *Sympetrum obtrusum* nymphs from Aurohn Lake, and 38 nymphs from a wetland

area near Hyla House. Thirty adults were collected at the Aurohn Prairie, 30 from Brewster Prairie, 30 from Tallgrass Prairie, 30 from Hyla House wetlands and field, and 13 from the Visitor Center Retention Pond. Nymphs were collected between May 13 and May 29, 2013, and adults were sampled between June 24 and July 11, 2013. We attempted to sample nymphs from the Tallgrass Swamp in 2013, but no dragonfly nymphs were found. In addition, we did not sample nymphs and adults from Hyla House wetlands in 2012 because we were unaware of the site. Adults were not sampled near the Visitor Center Retention Pond in 2012 because there were no adults present in the area during that year.

For both 2012 and 2013 we used D-nets to turn over the substrate at the bottom of the wetland sites; we then stored the nymphs in 70% ethyl alcohol. In both years of sampling we collected the middle right leg from each adult sample, in order to avoid resampling in the field. The nymphs were identified to the species level with a dichotomous key obtained from the Michigan Odonata survey online database (O'Brien 2008); the adults were classified to the species level using a field guide (Nikula, Sones and Stokes 2002) in the field for both the summer of 2012 and 2013.

Genetic Analysis

The DNA was extracted from the abdomen of each nymph, and the leg from each adult sample (Hadrys et al. 2005) using DNeasy tissue kits (QIAGEN Inc., Valencia, CA). For the analysis of the samples, the AFLP Plant Mapping Protocol (Life Technologies, Corp., Carlsbad, CA) was used, although we increased the number of cycles to 30 for the selective amplification polymerase chain reaction (PCR) to increase the number of amplicons. We only used one primer-pair combination, E-TAG with M-CGC (Eurofins MWG Operon, Huntsville, AL) with the selective EcoR1 primer tagged with a fluorescent dye (5~HEX), to amplify DNA for PCR

since AFLP profiles tend to display a large number of bands. The products were analyzed with an automated DNA sequencer (3130 Genetic Analyzer, Life Technologies, Inc., Foster City, CA), and the bands were scored using GeneMapper 4.0 software (Life Technologies, Inc., Foster City, CA). We only accepted peaks that were between 50-400 base pairs in size and had a height that was above 70 fluorescent units. For each individual, the band presence was scored as either present (1) or absent (0).

The Bayesian analysis program STRUCTURE (Pritchard et al. 2000) was used to group individuals together based on genotype similarities, and to also construct populations based on the genotype similarities. STRUCTURE was run three times in a hierarchical fashion to explore population structure. Our first analysis compared summer 2013 adults with summer 2012 nymphs with the Tallgrass Swamp, then with summer 2013 adults and summer 2012 nymphs without Tallgrass Prairie, and then once to compare summer 2013 adults with the nymphs and adults from summer 2012. We performed the subsampling to ensure that the four populations from STRUCTURE did not lump together or diverge from each other as we compared the different sets of populations with each other. We wanted to be consistent with our data; when the results were run they showed that the populations did not change to a different combination, which means that there are in fact distinct populations. STRUCTURE (Pritchard et al. 2000) was used with a burn-in period of 10,000 iterations and data collection of 10,000 iterations following the burn-in. Evanno's K (ΔK) (Evanno et al. 2005) was used to measure the difference in the 2^{nd} order rate of change with the most likely population number being indicated by the greatest ΔK. The STRUCTURE Q-plot was used to display the proportion of an individual's genetic make-up deriving from each genetic cluster identified by STRUCTURE. We compared the number of bands among our genetic clusters using an ANOVA followed by

pairwise t-tests on each genetic cluster. Similarly, the F_{st} values and the pair wise values for Nei's Coefficient of Genetic Diversity were calculated using the program AFLP Surv 1.0 (Vekemans 2002) between the genetics clusters identified by STRUCTURE.

Results

Thirty-one *Sympetrum obtrusum* nymphs from Aurohn Lake and 38 nymphs from Tallgrass Swamp were successfully analyzed from summer 2012. We also successfully analyzed 30 adults from Aurohn Prairie, 30 from Brewster Trail Prairie, and 30 from Tallgrass Prairie from summer 2012. From the summer of 2013, 39 *Sympetrum obtrusum* nymphs from Aurohn Lake and 38 nymphs from Hyla House were successfully analyzed. For adults, we were able to successfully analyze 30 from Aurohn Prairie, 30 from Hyla House Field, 30 from Tallgrass Prairie, 30 from Brewster Prairie, and 13 from the Visitor's Center Retention Pond (133 total). There were a total of 135 polymorphic bands that were produced for summer 2013, 21 more bands than from summer 2012 (Faydenko et al. 2012).

Evanno's K indicated that there were 4 (K=4) populations of *Sympetrum obtrusum* located on PCCI's property (Fig. 2). The four clusters were: Cluster 1, including the majority of the adults captured in 2013 from Aurohn Prairie, Brewster Prairie, Hyla House, and Tallgrass Prairie; Cluster 2, including the majority of the adults captured in 2012 from Aurohn Prairie, Brewster Prairie, and Tallgrass Prairie; Cluster 3, including all of the Aurohn Lake nymphs captured in 2012 as well as a small number of adults captured at Aurohn Prairie in 2012 (n = 2), Aurohn Prairie in 2013 (n = 5), Brewster Prairie in 2012 (n = 3), Brewster Prairie in 2013 (n = 3), Hyla House 2013 (n = 1), and Tallgrass Prairie 2012 (n = 1); and Cluster 4, comprised of all of the Tallgrass Swamp nymphs captured in 2012. Excluding the nymphs from the Tallgrass

Swamp with the 2012 samples, there were a total of 3 populations representing the same 3

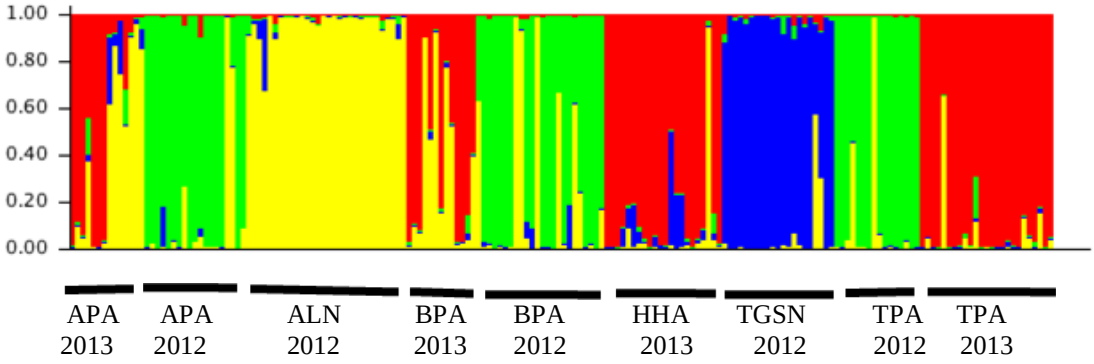

Figure 2. Q-plot of *Sympetrum obstrusum* population clusters assigned by STRUCTURE. Each individual is represented by a vertical bar with the percentage of the bar represenenting the proportion of the individual's genotype deriving from the respective genetic cluster.
APA 2013 = Aurohn Prairie adults captured in 2013,
APA 2012 = Aurohn Prairie adults captured in 2012,
ALN 2012 = Aurohnn Lake nymphs captured in 2012
BPA 2013 = Brewster Prairie adults captured in 2013,
BPA 2012 = Brewster Prairie adults captured in 2012,
HHA 2013 = Hyla House adults captured in 2013,
TGSN 2012 = Tallgrass Swamp nymphs captured in 2012,
TPA 2012 = Tallgrass Prairie adults captured in 2012,
TPA 2013 = Tallgrass Prairie adults captured in 2013

clusters identified in the full analysis. This indicated that the inclusion of the Tallgrass Swamp nymphs had no impact on the composition of the genetic clusters.

We found a significant difference ($F_{3, 180}$=213.3; $p < 0.01$) in the number of bands among the four genetic clusters identified by STRUCTURE. All pairwise comparisons were significantly different ($p < 0.01$); the greatest number of bands per individual was found in Tallgrass Swamp, and the fewest were found in Cluster 3 (Fig. 3).

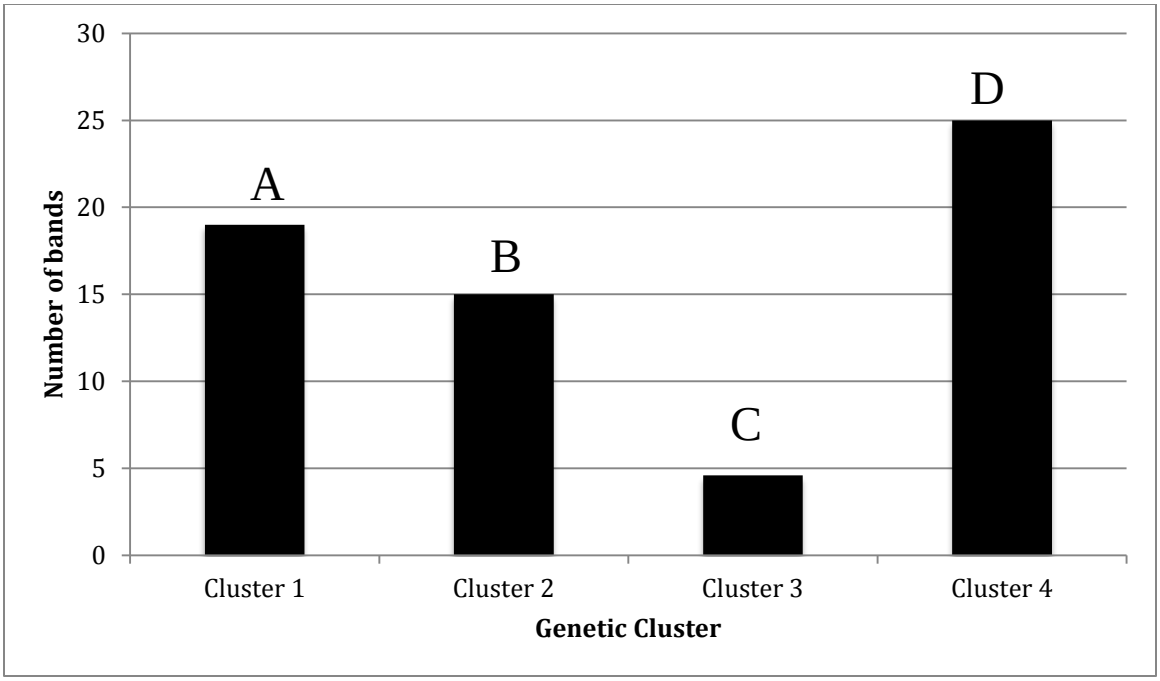

Figure 3. Average number of AFLP bands per individual from four genetic clusters of *Sympetrum obtrusum* at Pierce Cedar Creek Institute in Michigan. Bars with different letters indicate a significantly different average number of bands ($p < 0.05$). Clusters aredescribed in text and Figure 2.

The overall F_{st} value was 0.112. The pairwise values for Nei's Coefficient of Genetic Identity (Vekemans 2002) ranged from 0.970 to 0.993 (Table 1).

Table 1. The F_{st} values (below the diagonal) and the pairwise comparisons of Nei's Coefficient of Genetic Identity (above the diagonal) for *Sympetrum obstrusum*, according to the populations the dragonflies were assigned to by using STRUCTURE.

	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Cluster 1	--	0.978	0.993	0.970
Cluster 2	0.134	--	0.987	0.971
Cluster 3	0.070	0.216	--	0.97
Cluster 4	0.231	0.166	0.110	--

Discussion

In order to produce a more accurate genetic characterization of the samples, in 2013 we increased the number of iterations used to analyze our data and increased the number of samples. These changes showed that the Aurohn Lake nymph samples from 2012 consisted of one unique

genetic population instead of two (Cluster 4) as indicated in the previous study (Faydenko et al. 2012). This reanalysis argues against the hypothesis that multiple generations of nymphs, from different populations, exist in Aurohn Lake at the same time as previously suggested (Faydenko et al. 2012).

The adults we sampled in summer 2013 produced results similar to those seen in the 2012 samples. Both years showed the adults existed as two unique genetic clusters; a larger population that was unrelated to any of the nymphal populations (Cluster 1) and a smaller group, which was part of the Aurohn Lake nymph population (Cluster 2). All of the adults that were members of Cluster 2 were sampled from two areas relatively close to Aurohn Lake (Aurohn Prairie Border and Brewster Prairie). The sample locations farther away from Aurohn Lake (Hyla House and Tallgrass Prairie) were dominated by individuals from Cluster 1 with only two individuals belonging to the Aurohn Lake nymph population. Analysis of the samples resulted in two distinct genetic clusters from 2012 and 2013, which suggests that the adults were neither produced from Aurohn Lake nor Tallgrass Swamp. While a three-year cycle is possible, there is not a fixed number of years nymphs must spend in the water (Pintor and Soluk 2006). This observation was supported by our data as we saw adults associated with the Aurohn Lake genetic cluster both years, suggesting differential maturation rates of nymphs.

Given that no other *S. obtrusum* nymphs were found around Pierce Cedar Creek Institute, our data suggests that adult dispersal is occurring. We did not see any nymphs from Aurohn Lake that belonged to the large adult genetic clusters from 2012 and 2013, therefore, those adults originated elsewhere and dispersed into the area. In addition, while we expected to see more 2013 adults belonging to the Aurohn Lake nymph genetic cluster, it is possible many of those nymphs have yet to fully mature before emerging as adults, many of them may have dispersed

elsewhere, or the population size is relatively small (Pintor and Soluk 2006). The low number of bands found in Cluster 3, which contained the Aurohn Lake nymphs, relative to the other clusters further supports that it is a smaller population.

Each population exhibited a significantly different number of allelic bands. Pairwise comparisons for Nei's Coefficient and the F_{ST} values also indicated that the populations were significantly different from each other. This supports our STRUCTURE results that there are, in fact, four unique *S. obtrusum* populations. The F_{ST} estimates also indicate moderate differentiation between all clusters (Wright 1965), further indicating the genetic distinctiveness among every genetic cluster. Several of the estimates are close to or exceed $F_{ST} = 0.2$ which allows for fixation of different alleles, suggesting very limited genetic exchange.

Dispersal of adults must be occurring in our system given that few adults are associated with our nymphal cluster, but there does not appear to be much genetic exchange between adults born in different areas given that none of the Aurohn Lake nymphs were part of the major adult clusters in 2012 or 2013. This may be unique to our system if the Aurohn Lake population is small; however, Aurohn Lake adults were present in 2012 and 2013, so we would expect to see some individuals exhibiting alleles from both populations, which did show in our results. An alternative explanation is that the majority of 2012 and 2013 adults formed distinct genetic populations, because they dispersed from elsewhere. While large-scale dispersal of both sexes is uncommon, it is not unheard of (Pusey 1987), with the majority of both sexes dispersing away from their natal area to avoid inbreeding (Conrad et al. 1999). If there is a high rate of dispersal, mixing of populations would occur, causing all of the individuals to look genetically similar to each other. If this were the case, then adults would be genetically similar to nymphs within the area because they would belong to the same genetic cluster. However, if adults were produced

from different ponds exhibiting exclusive breeding with individuals from the same area, then those adults may not resemble genetic populations of nymphs from other ponds. Analysis of our nymph samples from 2013 will produce a more definite conclusion.

Adults' dispersal alone does not mean populations are immune from extirpation for if the aquatic environment is altered or destroyed, distinct populations, such as Aurohn Lake, may be eliminated. Furthermore, dispersal may not promote interbreeding among populations. It has been suggested that the main dispersal period occurs during the sexually immature phase of a dragonfly's life (Conrad et al. 1999), but our data suggests that natal dispersal by the adults is just as likely to be important. Despite the ability of adults and nymphs to disperse, long-term conservation of dragonflies will require the maintenance of habitat suitable for adults near the aquatic habitat necessary for breeding. The adult life span of *S. obtrusum* is relatively short (approximately three months) with females dying shortly after mating. Isolated patches of habitat are thus unlikely to experience much interpopulation dispersal and will experience the increased likelihood of extirpation associated with limited dispersal (Schreiber 2010). The beneficial effects of dispersal are constrained by the dispersal distance of the organism (Johst et al. 2002) suggesting that population persistence will be maximized in *S. obtrusum* when multiple habitat patches are within median adult dispersal distances.

Acknowledgements

We would like to thank Pierce Cedar Creek Institute for their financial support and use of their facilities and property. We would also like to thank Ken and Alice Jones for allowing us to use Aurohn Lake for one of our research locations.

Literature Cited

Austin, A., O. Ovaskainen, and I. Hanski. 2011. Size and genetic composition of the colonizing propagules in a butterfly metapopulation. Oikos **120**: 1357-1365.

Benke, C., and S. S. Benke. 1975. Dynamics and life histories of coexisting dragonfly populations. Ecology **56**: 302-317.

Buskirk, J. V. 1987a. Density-dependent population dynamics in larvae of the dragonfly *Pachydiplax longpennis*: a field experiment. Oecologia **72**: 221-225.

Buskirk, J. V. 1987b. Influence of size and date of emergence on male survival and mating success in a dragonfly *Sympetrum rubicundulum*. American Midland Naturalist **118**: 169-176.

Conrad, K. F., K. H. Wilson, I. F. Harvey, C. J. Thomas, and T. N. Sherratt. 1991. Dispersal characteristics of seven odonate species in an agricultural landscape. Ecography **22**:524-531.

Corbet, P. S. 1991. Dragonflies: behavior and ecology of Odonata. Pages 564-580. Comstock Publishing Associates, Ithaca, New York.

Evanno G., S.Regnaut., and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Pages 2611-2620. Department of Ecology and Evolution, University of Lausanne, Switzerland.

Faydenko, J., T. McVay, and B. J. Swanson. 2012. Population structure of the dragonfly *Sympetrum obtrusum* at Pierce Cedar Creek Institute. Pierce Cedar Creek Institute, Hastings, MI. http://www.cedarcreekinstitute.org/documents/McVayFaydenkoFinal12-21-12_000.pdf

Hadrys, H., W. Schroth, B. Schierwater, B. Streit, and O. M. Fincke. 2005. Tree hole odonates as environmental monitors: non-invasive isolation of polymorphic microsatellites from the neotropical damselfly *Megaloprepus caerulatus*. Conservation Genetics **6**: 481-483.

IUCN. 2004. Guardians of the Watershed: dragonflies and damselflies as reliable indicators of the quality of wetland habitats. http://www.iucn.org. Accessed 1/15/2012.

IUCN. 2010. Habitat loss blamed for more species decline. http://www.iucn.org. Accessed 1/15/2012.

Johst, K., R. Brandl, and S. Eber. 2002. Metapopulation persistence in dynamic landscapes: the role of dispersal distance. Oikos 98: 263 – 270.

Kleinhans, D., and P. R. Jonsson. 2011. On the impact of dispersal asymmetry on metapopulation persistence. Journal of Theoretical Biology **290**: 37-45.

Kormondy, E. J., and Gower J. L. 1965. Life history variations in an association of Odonata. Ecology **46**: 882-886.

Nee, S., and R. M. May. 1992. Dynamics of metapopulations: habitat destruction and competitive coexistence. Journal of Animal Ecology **61**: 37-40.

Nikula, B., J. Sones, D. Stokes, and L. Stokes. Beginner's Guide to Dragonflies. Little, Brown and Company, New York City, New York.

O'Brien, M. 2008. Michigan Odonata survey online database. University of Michigan, Ann Arbor, Michigan. Available from: insectdataserver.ummz.lsa.umich.edu/mos/home.php

Oertli, B., D. A. Joye, E. Castella, D. Cambin, and J. B. Lachavanne. 2002. Does size matter? The relationship between pond area and biodiversity. Biological Conservation **104**:59-70.

Pierce, C. L., P. H. Crowely, and D. M. Johnson. 1985. Behavior and ecological interactions of larval Odonata. Ecology **66**: 1504-1512.

Pintor, L.M., and D.A. Soluk. 2006. Evaluating the non-consumptive, positive effects of a predator in the persistence of an endangered species. Biological Conservation **130**: 584-591.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics **155**: 945-959.

Pusey, A. E. 1987. Sex-biased dispersal and inbreeding avoidance in birds and mammals. Trends in Ecology and Evolution 2: 295 – 299.

Reece, B. A., and N. E. McIntyre. 2009. Community assemblage patterns of odonates in a wetland complex influenced by anthropogenic disturbance. Insect Conservation and Diversity **2**: 73-80.

Reich, P. F., S. T. Numbem, R. A. Almaraz, and H. Eswaran. 2001. Land resource stresses and desertification in Africa. In Bridges, E.M., Hannam, I.D., Oldeman L.R., Pening de Vries, F.W.T., and Scherr, S.J. (eds.). Responses to Land Degradation. Oxford Press, New Delhi, India.

Remsburg, A. J., A. C. Olson, and M. J. Samways. 2008. Shade alone reduces adult dragonfly (Odonata: Libellulidae) abundance. Journal of Insect Behavior **21**: 460-468.

Schrieber, S. J. 2011. Interactive effects of temporal correlations, spatial heterogeneity and dispersal on population persistence. Proceedings of the Royal Society B 277:1907-1914.

Suhonen, J., M. Hilli-Lukkarinen, E. Korkeamaki, M. Kuitunen, J. Kullas, J. Penttinen, and J. Salmela. 2010. Local extinction of dragonfly and damselfly populations in low- and high-quality habitat patches. Conservation Biology **24**: 1148-1153.

Vekemans, X. 2002. AFLP-SURV version 1.0. Distributed by the author. Laboratoire de Génétique et Ecologie Végétale, Université Libre de Bruxelles, Belgium.

Wright, S. 1965. The interpretation of population structure by f-statistics with special regard to systems of mating. Evolution, **19**: 395-420.