

Population Structure of the Dragonfly *Sympetrum obtrusum*

at Pierce Cedar Creek Institute

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ABSTRACT

We examined dragonfly population structure using amplified fragment length polymorphism (AFLP) markers for the White-faced Meadowhawk (*Sympetrum obtrusum*). We used one AFLP marker producing 114 alleles to determine the relatedness between adult and nymphal populations located at Pierce Cedar Creek Institute near Hastings, MI. Adults comprised one genetic population regardless of sample site, but juveniles comprised separate genetic populations at their two locations and two unique populations were found within a single location. Each breeding pond comprised distinct genetic populations, and each year of juveniles comprised a unique genetic population. Dragonflies appear to constitute distinct populations, despite occupying the same area. Our results suggest that either a temporal factor or dispersal controls *S. obtrusum* population structure. Further research is needed to determine what causes population structure in *Sympetrum obtrusum*. Understanding the reason(s) for population structure in dragonflies could aid in developing management strategies for environments where dragonflies are found.

INTRODUCTION

Over 19 million km² of Earth's land surface have been altered by humans through agricultural, industrial, and recreational activities (Reich et al. 2001). As a result, substantial environmental changes are occurring rapidly. Biogeochemical processes are being altered; climate change is accelerating; and biological diversity is declining (Vitousek et al. 1997). Dragonflies utilize both terrestrial and aquatic ecosystems, and habitat loss could lead to a decline in species density and diversity (Corbet 1991). It is necessary to recognize how dragonflies disperse and how populations are structured in order to understand how habitat degradation would affect them.

Dragonflies are often the dominant predaceous insects in aquatic ecosystems (Benke and Benke 1975); however, fish predation has a powerful effect on the spatial distribution of nymphal populations (Pierce et al. 1985). Nymphs have different survival strategies depending on the presence of predators; when fish are present nymphs are motionless to avoid detection, and in areas without fish they swim to escape from other predators (Pierce et al. 1985). Nymphal density is inversely related to the growth and survival of nymphs, indicating density is likely the regulating factor of dragonfly populations (Buskirk 1987a). Adult dragonflies, unlike nymphs, are not restricted by density; their increased mobility allows movement to areas of reduced competition (Buskirk 1987b). After emerging from their natal pond, dragonflies move up to 1.5 km away from the water to forage (Levett and Walls 2011), but later return to areas near nymphal habitats to breed before dying (Conrad et al. 1991). Dragonfly nymphs can overwinter for multiple years, which create a pattern of overlapping generations (Kormondy and Gower 1965).

Dragonflies serve as indicators of both aquatic and terrestrial habitat degradation (IUCN 2004) because of their relatively short generation period, prominent trophic status, amphibious life history, and diversity (Reece and McIntyre 2009). These characteristics make them sensitive to changes in their environment, and dragonfly species richness is positively related to water quality (Oertli et al. 2002). Additionally, dragonfly abundance is a predictor of species richness across other taxa (Clark and Samways 1996).

Despite their status as an indicator species, and that 14% of European dragonfly species are being threatened with extinction, dragonflies are an understudied taxa; little even basic information such as distributions and ranges are known for most dragonfly species worldwide (IUCN 2010). For example, while several studies 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), there is a lack of information about how adult and nymphal populations are connected. While the role of both nymphal and adult dragonflies in the ecosystem is well understood, and studies have been conducted on the relatedness of adult dragonflies, there has been no significant research into the genetic relatedness between the terrestrial adult population and the aquatic nymphal population.

The main focus of our research was to determine how dragonfly populations are structured and whether or not nymphal dragonflies are genetically similar to the adults in the same area. Specifically, we tested the null hypothesis that there is no genetic difference between nymph and adult populations of *Sympetrum obtrusum* at Pierce Cedar Creek Institute.

METHODS

Sampling

We sampled two sites for nymphal dragonflies and three sites were sampled for adults. Twenty-nine *Sympetrum obtrusum* nymph samples were collected from Aurohn Lake, north of the Pierce Cedar Creek Institute (PCCI) property line, and 31 nymph samples were collected from an ephemeral shrub swamp located south of the PCCI visitor center along the tree line of the Southeast Prairie (aka Tallgrass) (Figure 1). Nymphs were sampled from the shoreline out to a depth of approximately 1 m using D-nets (Benke and Benke 1975). We sampled nymphs between May 21 and June 5, 2012. Whole nymphs were preserved in separate containers containing 70% ethyl alcohol. We collected 30 adults from the Southeast Prairie near the ephemeral swamp, 30 from the North Prairie (aka Brewster), and 30 from the Aurohn Prairie (Figure 1). Adults were sampled using aerial nets (Buskirk 1987). The middle, right leg was taken from each adult to monitor re-sampling. Adult sampling began when adults started emerging on June 19, 2012 and was completed June 27, 2012. We identified nymphs to the species level using dichotomous keys obtained from the Michigan Odonata survey online database (O'Brien 2008), and adults were identified to the species level in the field using a species field guide (Mead 2003).

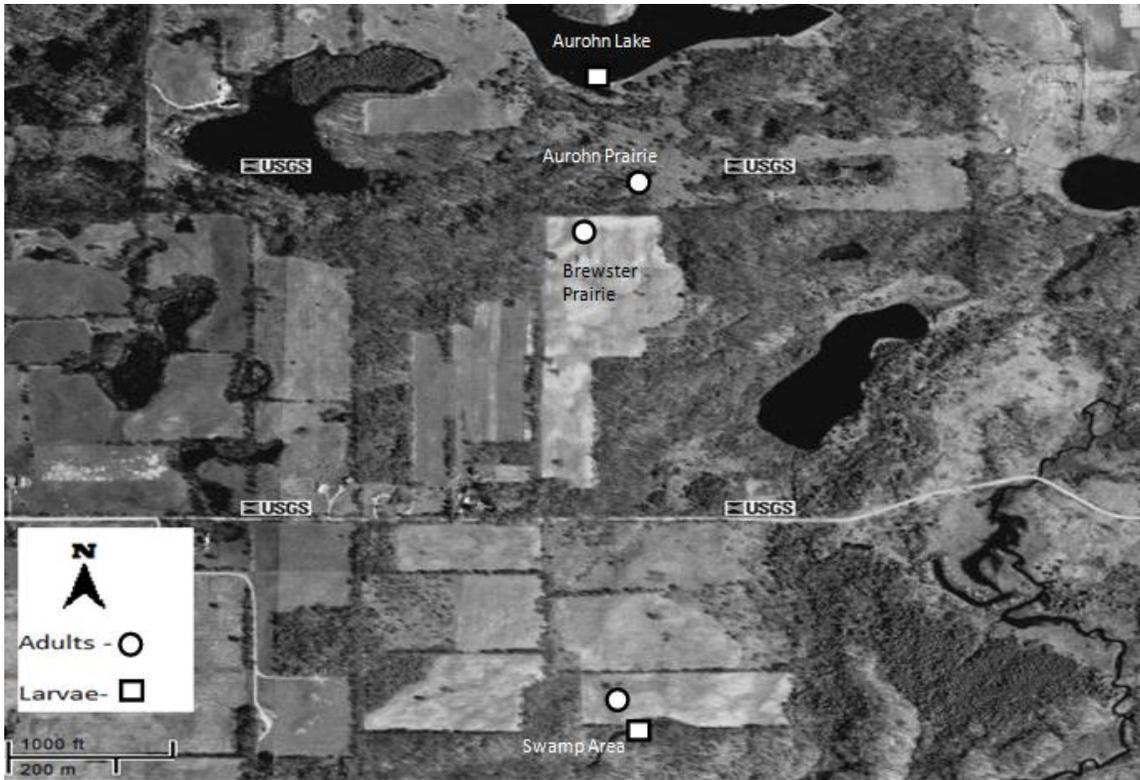


Figure 1. Map of the areas where *Sympetrum obtrusum* was sampled. The circles represent the areas where adults were collected, and squares indicate nymphal sampling sites. The adults were collected in the Aurohn Prairie, the North (Brewster) Prairie, and the Southeast (Tallgrass) Prairie and nymph were sampled in the swamp area and from the south end of Aurohn Lake.

Genetic Analysis

We extracted DNA from the abdomen from each nymph and the legs from the adults (Hadrys et al. 2005) using DNeasy tissue kits (QIAGEN Inc., Valencia, CA). We used the AFLP Plant Mapping Protocol (Life Technologies, Corp., Carlsbad, CA) for our analysis, although we used 30 cycles instead of 20 cycles in the selective amplification polymerase chain reaction (PCR). AFLP profiles can exhibit a large number of bands. Because of this, we used only one primer-pair combination. DNA was amplified using the selective AFLP marker pair; E-TAG with M-CGC (Eurofins MWG Operon, Huntsville, AL), with the selective *EcoRI* primer tagged with a fluorescent dye (5~HEX). We analyzed the products on an automated DNA sequencer (ABI Prism 3130 Genetic

Analyzer, Applied Biosystems, Inc., Foster City, CA), and scored bands using GeneMapper 4.0 software. Peaks between 50-400 bp were accepted if the peak height was above 70 for fluorescent intensity. Band presence was scored for each individual as either present (1) or absent (0), and the resulting data file was entered into the program STRUCTURE v.2.2.3 (Pritchard et al. 2000) to estimate the most likely number of populations.

We used the Bayesian analysis program STRUCTURE (Pritchard et al. 2000) to group individuals based on genotype similarities and construct populations based on those similarities. We ran STRUCTURE five times for each potential population size (K=1 to 7) with a burn-in period of 1,000 and 1,000 iterations following burn-in. We used a statistical analysis known as Evanno's K (ΔK) to determine the most probable population size (Evanno et al. 2005). Evanno's K compares the second order rate of the change in log likelihood between population estimates relative to the variation in the log likelihoods, where the population number with the highest ΔK is the most probable number of populations. After STRUCTURE was run, the log probabilities for each output were used to calculate ΔK for each population size. The STRUCTURE Q-plot was determined to show the most probable distribution of individuals within each population. F_{st} values were calculated using the program AFLP Surv (Vekemans 2002), and we used GenAlEx (Peakall and Smouse 2006) to determine the pair wise values for Nei's Coefficient of Genetic Diversity. We performed a G-test to determine if the proportion of adults that were dissimilar from the majority of the population was significantly different among any of our three sites.

RESULTS

A total of 114 polymorphic bands were produced. Twenty-one nymphs from the swamp, 29 nymphs from Aurohn Lake, 24 adults from the prairie west of Brewster Lake, 16 adults from the swamp, and 20 adults from the prairie near Aurohn Lake were successfully analyzed, with a median value of 12 bands per individual. Evanno's K showed that the most likely number of populations was $K=4$ (Figure 2). The nymphs had three distinct populations, which we refer to based on the color assigned by the STRUCTURE plot (Figure 3): one population in the swamp area (blue) and two populations in Aurohn Lake (yellow and green). The adults were assigned to one population (red) regardless of sample site, although there were a few green individuals at all sites. The overall F_{st} value for the population was 0.29, and pair wise F_{st} values ranged from 0.00 to 0.51 (Vekemans 2002). Pairwise values for Nei's Coefficient of Genetic Identity (Peakall and Smouse 2006) ranged between 0.941 and 0.998 (Table 1). We found no significant difference in the proportion of adults from the red and green populations in Brewster Prairie (79.17%:20.83%), Aurohn Prairie (85.00%:15.00%), and Tallgrass Prairie (93.75%:6.25%; $G=1.98$; $p=0.36$).

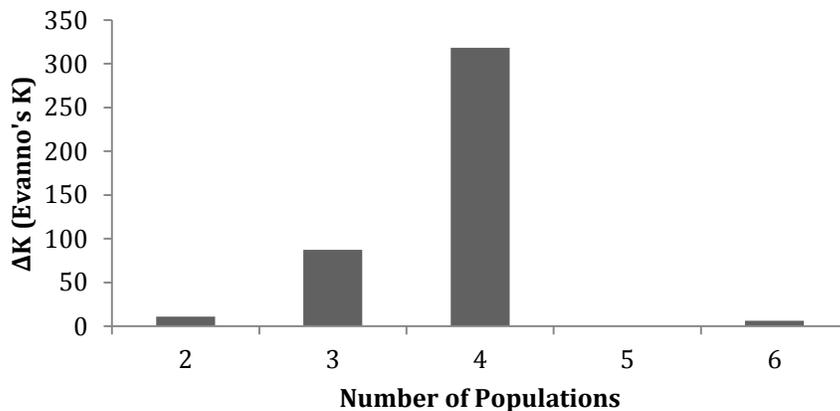


Figure 2. Graph of the Evanno's K value for *Sympetrum obstrusum* collected on the PCCI property across five different sampling locations. Evanno's K is a measurement of the rate of change in log probability data of successive K values.

Table 1. Pairwise comparisons of Nei’s Coefficient of Genetic Identity (above the diagonal) and F_{st} values (below the diagonal) for *Sympetrum obstrusum*, according to the population the dragonflies were assigned to by use of the STRUCTURE software.

	Adults	Aurohn Lake Nymphs (Yellow)	Aurohn Lake Nymphs (Green)	Swamp Nymphs
Adults	----	0.967	0.971	0.941
Aurohn Lake Nymphs (Yellow)	0.029	----	0.998	0.949
Aurohn Lake Nymphs (Green)	0.028	0.000	----	0.947
Swamp Nymphs	0.051	0.042	0.044	----

DISCUSSION

We found three distinct nymphal populations of *S. obstrusum* nymphs—one in the swamp area and two in Aurohn Lake. The adults mostly comprised one population, regardless of sampling site, although there were some adult individuals clustered with one of the Aurohn Lake nymphal populations. Given these results we believe that there is either a temporal factor in population structure, or that significant dispersal is occurring.

A temporal-based population structure is possible because nymphs can overwinter for multiple years (Pintor and Soluk 2006) prior to transitioning into the adult dragonfly population. Overlapping generations have been observed in various genera, including *Sympetrum* (Kormondy and Gower 1965). If the nymphal dragonflies remain in their natal habitat for multiple years, we could see different generations of nymphs emerging in the same year. In this scenario, the nymphs belonging to the green and yellow populations are from 2011, the red population of adults (Figure 3) represents the nymphs from August 2010, and the green adults are nymphs from August 2011 that emerged after one year. If

there is no dispersal, we would expect the majority of next year's adult population to be comprised of green and yellow individuals. If the population structure we found is temporally based, and if dispersal does not occur, degradation of either aquatic or terrestrial habitat could result in local extinction of dragonfly populations. Given this, management strategies for dragonflies would extend to both aquatic and terrestrial areas. This statement is supported by the high degree of genetic similarity seen within the adults and each of the nymphal populations. Similarly, the low F_{ST} values suggest that genetic exchange is occurring between the adults and the nymphal population.

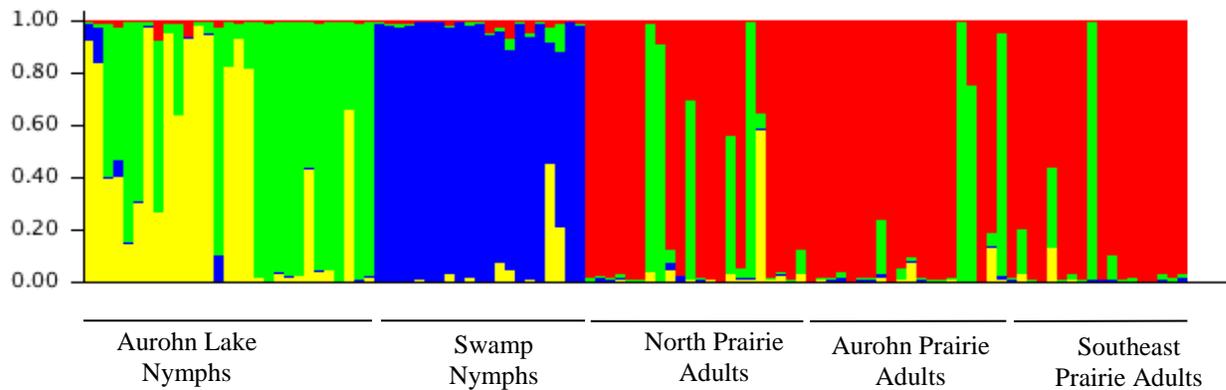


Figure 3. Q-plot of *Sympetrum obstrusum* population clusters assigned by STRUCTURE. Each individual is represented by a vertical bar partitioned into $K=4$ segments. The area with primarily yellow bars represents one Aurohn Lake nymphal population and the primarily green bars represent a second nymphal population in the lake. The blue area represents the swamp nymph, and the area that consists of mostly red bars represents all of the adults sampled.

Alternatively, our results could indicate dispersal of adults. If dispersal was responsible for population structure, little to no overlap genetic would be observed between nymph and the surrounding adult population. In our study, the majority of adults belonged to the red population, which had no nymphal representatives in our study. There were eight green individuals, but the proportion of green adults was not

significantly different among the three sites. This suggests adults can disperse across the entirety of our sampling area. This result is expected in the context of a dispersal driven system, as the red adults would belong to one or more off-site populations we did not sample. If dispersal was occurring, we would expect entirely different nymphal and adult population in 2013, and the loss of either aquatic or terrestrial habitat would not lead to local extirpation of dragonflies.

We found only one population of nymphs in the swamp area; this result could be due to its ephemeral nature. Since the swamp area dries up every year, the nymphs may not survive, meaning the swamp area acts as a population sink. *Sympetrum* species lay their eggs in low areas that are likely to flood, and often times this behavior can result in a population sink (Wiggins et al. 1980). However, *Sympetrum* nymphs have been known to survive drought for up to eight months (Wiggins et al. 1980). If we see dragonflies from the blue population in future studies, this would indicate that the nymphs survive the ephemeral nature of the swamp; if not, it could be an indication of a population sink or the newly emerged adults disperse to surrounding habitat.

Our study shows that there is spatial structuring in the nymphal populations of White-faced Meadowhawk dragonflies. However, further study is needed to better determine what is responsible for the population structure we observed in the White-faced Meadowhawk dragonflies. Without another year's worth of data, we will not be able to determine if dispersal is occurring, or if a time lag exists between the nymphal and adult populations due to overlapping nymphal generations. Further investigation on the population structure of dragonflies will allow more effective management strategies to be implemented in the future.

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