TITLE: Genetic analysis across different spatial scales reveals multiple dispersal mechanisms for the invasive hydrozoan *Cordylophora* in the Great Lakes

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ABSTRACT

Discerning patterns of post-establishment spread by invasive species is critically important for the design of effective management strategies and the development of appropriate theoretical models predicting spatial expansion of introduced populations. The globally invasive colonial hydrozoan *Cordylophora* produces propagules both sexually and vegetatively and is associated with multiple potential dispersal mechanisms, making it a promising system to investigate complex patterns of population structure generated throughout the course of rapid range expansion. Here we explore genetic patterns associated with the spread of this taxon within the North American Great Lakes basin. We collected intensively from 8 harbors in the Chicago area in order to conduct detailed investigation of local population expansion. In addition, we collected from Lakes Michigan, Erie, and Ontario, as well as Lake Cayuga in the Finger Lakes of upstate New York in order to assess genetic structure on a regional scale. Based on data from 8 highly polymorphic microsatellite loci we examined the spatial extent of clonal genotypes, assessed levels of neutral genetic diversity, and explored patterns of migration and dispersal at multiple spatial scales through assessment of population level genetic differentiation (pairwise F_{ST} and factorial correspondence analysis), Bayesian inference of population structure, and assignment tests on individual genotypes. Results of these analyses indicate that *Cordylophora* populations in this region spread predominantly through sexually produced propagules, and that while limited natural larval dispersal can drive expansion locally, regional expansion likely relies on anthropogenic dispersal vectors.

INTRODUCTION

Understanding the dynamics of range expansion by introduced populations is a crucial task of invasion biology. Such expansions can be mediated by natural dispersal mechanisms, transport by anthropogenic vectors within the recipient region, or subsequent independent introductions beyond previously existing range limits (Roman 2006; Brown & Stepien 2009). Evaluating the contributions of these mechanisms to the spread of invasive species is critical for accurate risk assessment and design of appropriate management strategies (Hampton *et al.* 2004; Stepien *et al.* 2005) and enables construction of accurate theoretical models aimed at predicting invasion rates (Suarez *et al.* 2001; Hastings *et al.* 2005). In addition, detailed knowledge of post-establishment spread may allow researchers to leverage species invasions as models to test general hypotheses describing the properties of range expansions (Byers & Pringle 2006).

A large number of recent studies have underscored the utility of molecular genetic methods for reconstructing biological invasion histories (Le Roux & Wieczorek 2009). While much attention has understandably been paid to determining sources of introductions (Muirhead *et al.* 2008), the availability of high variability multilocus genetic datasets and analytical methods allowing exploration of complex demographic scenarios also has enabled detailed investigation of post-establishment expansion patterns (Estoup *et al.* 2004). It is now widely recognized that the mechanisms driving these patterns may be strongly influenced by spatial scale, with different dispersal vectors operative locally, regionally, and globally (Pauchard & Shea 2006). Empirical studies confirm theoretical expectations that invasive populations often spread by a combination of local "diffusive" spread mediated by natural dispersal mechanisms and long-distance "jump" dispersal mediated either by rare natural events or by anthropogenic vectors (Hastings *et al.*

2005). These scalar effects have been shown to have strong influences on population structure of invasive plant and animal taxa in both terrestrial and aquatic systems (e.g. Wilson *et al.* 1999; Williams *et al.* 2007; Roura-Pascual *et al.* 2009). Explicit incorporation of multiple spatial scales into genetic analyses of invasive populations should thus considerably aid understanding of the stratified dispersal patterns driving population expansion (Havel *et al.* 2006; Ward 2006).

Further contributing to the dynamics of population expansion is the capacity of many invasive taxa to generate both sexually and asexually produced dispersive propagules. The availability of both reproductive modes can have important implications for the structure of invasive populations and the risks associated with their spread (Sakai *et al.* 2001). The dispersal of vegetatively produced fragments has proven to be a particularly effective mechanism of population expansion for a wide variety of invasive taxa, including marine algae (Husa & Sjotun 2006; Scheibling & Melady 2008), terrestrial plants (Decruyenaere & Holt 2005; Kowarik & Samuels 2008), and marine invertebrates (Ting & Geller 2000; Bullard *et al.* 2007). The combination of multiple dispersal mechanisms operating over different spatial scales and the availability of both sexual and asexual modes of reproduction can generate complex patterns of population genetic structure, including departures from expectations based on normally distributed dispersal distances and wide geographic ranges of clonal genotypes (Darling *et al.* 2009).

The colonial euryhaline hydrozoan *Cordylophora* (Family Oceaniidae) is a globally invasive taxon that offers a promising system for examining these dynamics. *Cordylophora* colonies are polymorphic and dioecious, possessing feeding polyps (hydranths) as well as male and female

reproductive polyps (sporosacs; Smith 2001). Eggs are fertilized while still contained within the female sporosac, and free-swimming planulae subsequently emerge and are active for only a short period of time (~24 hours) before settling directly on appropriate substrate to form new colonies; there is no intermediate medusa stage (Gili & Hughes 1995; Smith 2001). In addition to sexual reproduction, *Cordylophora* is capable of rapid vegetative proliferation by asexual budding, resulting in the formation of dense branching colonies. Vegetative growth not only facilitates local fouling, but may also provide an important mechanism for population expansion. Fragments of colonies containing even very small amounts of living tissue ("menonts") within the protective outer perisarc are capable of establishing new colonies under favorable conditions (Roos 1979). Mechanical disruption may cause these fragments to break away from established colonies and subsequently serve as current-driven dispersive propagules (Koetsier & Bryan 1995). Menonts are also highly resistant to various stressors including changes in salinity and temperature as well as a number of biofouling control efforts (Folino-Rorem & Indelicato 2005), and some have suggested that they may serve as effective propagules for long distance anthropogenic dispersal (Folino 2000; Pienimäki and Leppäkoski, 2004). *Cordylophora* is thus capable of spread by a number of different mechanisms, including local colony expansion through vegetative growth, sexual population expansion by either natural (current-mediated) or anthropogenic dispersal of planulae, and asexual expansion by dispersal of drifting or fouling menonts.

Here we explore the genetic structure of invasive *Cordylophora* populations in the North American Great Lakes basin. *Cordylophora* is one of nearly 200 introduced taxa known to be established in the Great Lakes (Ricciardi 2006). The taxon was first reported in Lake Erie in 1957

(Davis 1957) and later shown to be a common resident of the western Lake Erie basin (Hubschman & Kishler 1972). A more recent study reported *Cordylophora* from throughout the Great Lakes and associated waters, including the Finger Lakes in upstate New York (Folino-Rorem *et al.* 2009). The increased prevalence of *Cordylophora* has led to its recognition as a nuisance in the Great Lakes and other non-native regions, particularly in the United States and Europe, where it has been found colonizing and obstructing intake passages of power plant cooling systems (Folino-Rorem & Indelicato 2005; Escot *et al.* 2007). Although the ecological impacts of *Cordylophora* are largely unknown, at high densities it likely modifies aquatic trophic structures by competing with larval fish for prey (Olenin & Leppäkoski 1999), and its filamentous structure may act to enhance the settlement and recruitment of invasive dreissenid mussel larvae (Folino-Rorem *et al.* 2006).

The rapid expansion of *Cordylophora* throughout the Great Lakes despite limits to natural current-driven dispersal across the region suggests that both local diffusive spread and human-mediated long-distance dispersal have likely contributed to contemporary population structure. To investigate population genetic patterns associated with the spread of *Cordylophora* at both local and regional scales we have adopted a stratified sampling approach, collecting intensively from one locale (the Chicago area harbors in western Lake Michigan, encompassing less than 25 kilometers) as well as a number of sites distributed across the region (a scale of approximately 920 kilometers). Analyses were based on data from 8 highly polymorphic microsatellite loci, and were aimed at assessing several aspects of *Cordylophora* population structure relevant to range expansion in the region. In particular, we investigated the extent to which repeated multi-locus genotypes (clones) contribute to population structure locally and regionally, and we utilized both

population- and individual-level assessments of genetic connectivity between collection sites to infer patterns of gene flow associated with multiple potential dispersal mechanisms acting on different spatial scales.

METHODS

Tissue collection

Cordylophora colonies were sampled from 15 sites in the Great Lakes basin, including 13 sites in the Great Lakes and 2 in the Finger Lakes in upstate New York (Table 1, Figure 1). The focus of our specimen collection was a cluster of 8 sites distributed across approximately 25 kilometers in the Chicago area of southwest Lake Michigan (Figure 1). In addition, we sampled opportunistically from additional sites in the Great Lakes basin, with the intent of assessing patterns of population structure across the region. Hereafter, we refer to the 15 individual collection sites as "samples." Multiple samples were collected from 4 "locales," including the Chicago harbors in southwest Lake Michigan (8 samples) and locales in eastern Lake Michigan, Lake Ontario, and Cayuga Lake (2 samples each); only one sample was taken from Lake Erie. Patterns observed within these locales (particularly among the Chicago harbors) are referred to as "local" patterns; in contrast, patterns distributed across multiple locales are referred to as "regional" patterns.

In almost all cases, *Cordylophora* colonies were found secondarily fouling dreissenid mussel shells attached to solid substrates. Specimens were obtained by scraping approximately 30 cm^2 patches of dreissenids from pilings and/or floats under marina docks. Each specimen was taken from a different float or piling in order to prevent re-sampling of single colonies. The single

exception to this protocol was at Maranatha Bridge, where *Cordylophora* was found fouling a steel pipe and wood pilings. Since fouled surfaces at this site were spatially continuous, colonies were collected from substrate every 15 to 20 centimeters in an attempt to avoid re-sampling. At three sites, we collected *Cordylophora* tissue found fouling multiple mussels within single scrapes. Such sampling was conducted for 15 scrapes at 59th Street Harbor, 22 scrapes at Burnham Harbor, and 18 scrapes at Muskegan. Two or three mussels were sampled per scrape. All specimens were preserved in 100% ethanol for genetic analysis.

Molecular methods

Three to ten hydranths (feeding polyps) were removed from each colony and pooled for whole genomic DNA extraction using DNeasy columns (Qiagen). Hydranths were removed from single uprights to avoid pooling tissue from multiple colonies. In some cases, hydranths were unavailable and tissue was freed from within the perisarc by isolating single stolons and crushing them manually with sterile plastic pestles prior to DNA extraction.

Eight microsatellite loci (CC02, CC08, CC11, CC16, CC22, CC29, CC31, and CC32) were amplified as previously described (Schable *et al.* 2008). Recent phylogenetic reconstruction has revealed multiple highly diverged cryptic evolutionary lineages among invasive populations of *Cordylophora*, two of which have been observed in the Great Lakes basin (Folino-Rorem *et al.* 2009). The fresh water lineage, studied here, is the dominant one in the region, although some sites excluded from the current study are known also to harbor a second lineage with greater apparent tolerance for brackish habitat. The microsatellite loci used in the current study were found to amplify consistently for the dominant Great Lakes *Cordylophora* lineage; however, with

the exception of CC08 and CC22 they fail to amplify for any other *Cordylophora* lineages, including the one other lineage observed previously in the region (Schable *et al.* 2008). Colonies deriving from other lineages, if present at any of our collection sites, would be thus effectively screened out of the current study; in addition, a subset of specimens were sequenced at a single diagnostic nuclear locus (the 28S large subunit rRNA; Folino-Rorem *et al.* 2009) to confirm lineage identity (data not shown).

For CC02, CC22, CC31 and CC32 amplification was conducted using the following touchdown PCR cycling parameters: 95º C for 150s; 20 cycles of 95º C for 20s, 64º C (-0.5º C per cycle) for 20s, 72º C for 30s; 15 cycles of 95º C for 20s, 50º C for 20s, 72º C for 30s; 72º C for 10m. Loci CC08, CC16 and CC29 were amplified using a similar touchdown program with annealing temperatures starting at 59º C, and locus CC11 was amplified using a single annealing temperature with the following cycling parameters: 94º C for 5m; 35 cycles of 95º C for 30s, 56º C for 60s, 72º C for 60s; 72º C for 15m. Reactions were conducted in 15 μL total volume containing 0.5 U Taq DNA polymerase (Qiagen), 1 x PCR buffer, 1 μM each forward and reverse primer, 1mM dNTPs, 1.6 mM $MgCl_2$, and 10-100 ng of template DNA. Amplified products were sized on an ABI 3730xl DNA Analyzer using GeneScan-500 LIZ size standard (ABI) and raw data were analyzed using GENEMARKER v. 1.60 (Softgenetics).

Genetic data analysis

Repeated multilocus genotypes were detected using GENALEX v. 6 (Peakall & Smouse 2006). For all clones we estimated the probability of the genotype arising (P_{gen}) as well as the probability of obtaining the observed number of repeats of that genotype (P_{sex}) assuming random

sexual reproduction and the observed frequency of alleles in the population within which the clone was identified (Parks & Werth 1993). Given the likelihood that repeated genotypes represent multiple tissue specimens drawn from the same colonies (see Results and Discussion), these genotypes were removed from the dataset for all subsequent analyses.

Allelic richness and gene diversity for each sample were calculated using FSTAT v. 2.9.3.2 (Goudet 2001). In the case of allelic richness, estimates were corrected for sample size by rarefaction to 14 individuals. Estimates of pairwise genetic differentiation (F_{ST}) between all samples and between locales were obtained using MSANALYZER v. 4.0 (Dieringer & Schlötterer 2002), with 10,000 permutations to assess significance and Bonferroni correction for multiple tests.

To assess population structure, we conducted three dimensional factorial correspondence analysis using GENETIX v. 4.05 (Belkhir *et al.* 2004). Analyses were performed both on the entire dataset and on a subset of the data including only samples from the Chicago area. In addition, we conducted Bayesian inference of population structure using the software STRUCTURE v. 2.1 (Falush *et al.* 2003). We assessed likelihoods for models with the number of clusters ranging from $K = 1$ to $K = 12$. For each value of K, we carried out five independent Markov Chain Monte Carlo (MCMC) runs with 100,000 generations discarded as burn-in followed by an additional 1,000,000 generations. We chose the model with the highest posterior probability as the one best representing the true underlying genetic structure, and determined the value of K for that model to be the best estimate of the number of populations in the full dataset. Subsequent to this analysis, we also explored a dataset comprising Chicago harbors only, conditioned on $K = 2$, in

order to further examine population structure within this region. We also assessed correlation between the geographic distribution of harbors (in decimal degrees latitude) and mean assignment ratios in cluster 1 for each of the 8 Chicago samples.

To test for correlation of genetic distance (F_{ST}) with geographic distance we performed Mantel tests using the Isolation by Distance Web Service v. 3.16 (Jensen *et al.* 2005). Pairwise geographic distances were estimated as straightline distances between collection sites using Google Earth v. 4.3 (beta). One thousand randomizations were performed to assess significance of correlation. Mantel tests were conducted for samples within the Chicago area and also for a dataset within which all Chicago area individuals were clustered as a single sample. The latter was done to assess correlation at a regional scale while removing the bias imposed by the large number of samples in Chicago possessing relatively low paired genetic and geographic distances.

As an assessment of migration between collection sites we conducted individual assignment tests using GENECLASS v. 2.0 (Piry *et al.* 2004). We adopted the Bayesian criterion of Rannala & Mountain (1997) to determine genotype assignments and assessed probabilities through Monte-Carlo resampling of 1000 individuals using the algorithm specified by Paetkau *et al.* (2004). The sample with the highest probability of assignment was considered the most likely source for the assigned genotype.

RESULTS

Assessment of clonal reproduction

Only 7 genotypes were found repeated in the dataset (Table 2). All were repeated only once and were confined to single samples; no repeated genotype was shared across multiple collection sites. In all but one case, repeated genotypes were found fouling neighboring dreissenid mussels within the same sampling scrape. The exception was the repeated genotype found at Maranatha Bridge (MB), which was the only site from which collected colonies were not found fouling dreissenid mussel substrate.

For each repeated genotype, the probability of two occurrences of that genotype arising *via* random sexual recombination (P_{sex}) was extremely low, ranging from 5.73×10^{-14} to 2.02×10^{-22}. It is thus highly likely that these represent true clonal genotypes. However, in 6 out of 7 cases the spatial extent of clones was limited to approximately 30 cm^2 (the size of a single sampling scrape), so it is also likely that these repeated genotypes represent multiple tissue specimens drawn from single colonies extending over multiple mussels, as opposed to multiple independent colonies. At Maranatha Bridge, attempts were made to take tissue specimens from spatially separated colonies to avoid such resampling; nevertheless, given the unusual substrate at that site we cannot rule out the possibility that the repeated clonal genotype observed there also derives from a single spatially extended *Cordylophora* colony. For multiply sampled scrapes, only 10.9% (6 out of 55) were found to harbor colonies extended over multiple mussels.

Genetic diversity

Measures of microsatellite diversity were generally high in all samples, with expected heterozygosity (H_E) ranging from 0.6271 to 0.7939 and allelic richness (A_R) ranging from 5.93 to 9.30 alleles after rarefaction to 14 individuals (Table 1). Regionally, there was little geographic

pattern in the distribution of genetic diversity, although samples in Cayuga Lake did exhibit significantly lower diversity than those found in other locales. Within the Chicago harbors there was no significant difference in H_E across samples. However, A_R was found to be significantly lower ($P = 0.036$) in the northern harbors (MO, BE, DV; mean $A_R = 7.50$) than in southern harbors (59, IJ, OJ; mean $A_R = 8.69$).

Genetic structure and gene flow

Pairwise F_{ST} values indicate significant genetic differentiation between almost all samples, even those within the same locale (Table 3). In some cases, differentiation between neighboring samples was extremely high. For instance, F_{ST} was 0.1112 between the two Cayuga Lake sites, despite geographic separation of only 1.5 kilometers. Differentiation was substantial and significant between all locales. Three geographically separated samples did exhibit genetic affinities, with small and non-significant F_{ST} values between the Lake Erie sample and samples BB in Lake Ontario and C1 in Cayuga Lake (Table 3).

Two different approaches to identifying population structure within the Great Lakes basin provide a largely consistent picture of the genetic relationships between samples. Factorial correspondence analysis graphically illustrates the divergence between geographically proximate samples suggested by pairwise F_{ST} values, with the two Cayuga Lake samples and the two Lake Ontario samples (BB and SP) showing clear separation (Figure 2A). In contrast, two samples from eastern Lake Michigan (MB and MSK) cluster tightly in the analysis, as do samples comprising the Chicago harbors. Independent analysis of the Chicago samples reveals an interesting pattern, with the distribution of samples along the axis representing factor 1

(explaining 21.9% of genetic variance) perfectly paralleling the distribution of samples from north to south along the coast of Lake Michigan (Figure 2B).

Bayesian inference of population structure reveals similar patterns. The plot of model likelihood [Ln(L)] versus number of clusters (K) reveals a single peak likelihood value at $K = 8$, with models at higher K reaching an apparent plateau at slightly lower likelihood values (Figure 3A). At $K = 8$, neighboring samples within Cayuga Lake and Lake Ontario show clear assignment to different clusters (Figure 3B), whereas the two samples from eastern Lake Michigan belong to a single cluster and substantial affinities are indicated between three geographically separated sites in Lake Erie, Lake Ontaria (BB) and Cayuga Lake (C1), all consistent with FCA results. Individuals in the Chicago samples do not appear to assign consistently to a single cluster. Instead, individuals from northern harbors (MO, BE, DV) assign predominantly to a different cluster than those from southern harbors (IJ, OJ, 59). When clustering analysis was run independently on Chicago samples with $K = 2$, mean assignment of individuals to cluster 1 declined regularly and significantly from north to south (Figure 4; $r^2 = 0.7850$, $P = 0034$). This pattern was reflected in significant correlation between genetic distance (F_{ST}) and geographic distance observed among Chicago samples as determined by Mantel test (Figure 5A). Similar tests with the Chicago harbors collapsed into a single sample revealed no such correlation at a regional scale (Figure 5B).

Assessment of gene flow among samples using assignment of individual genotypes showed little indication of migration between samples outside of the Chicago area (Table 4). However, substantial migration was suggested between Chicago harbors by the large proportion of

individuals assigned to samples other than those from which they had been collected (23%). This inferred gene flow was noticeably directional, with 95% of misassigned individuals being attributed to samples located to the south of those from which they were actually collected.

DISCUSSION

Contribution of clonality to genetic diversity and spread of *Cordylophora*

Theory suggests that the capacity to reproduce vegetatively may substantially increase likelihood of invasion success (Sakai *et al.* 2001). Studies of invasive plant taxa have provided particularly strong empirical support for this hypothesis. For instance, a recent review of introduced terrestrial plants revealed that clonality accurately predicts the likelihood of transition from established to naturalized (Milbau & Stout 2008), and demographic models suggest that the availability of both sexual and asexual reproductive modes results in higher performance of invasive taxa within certain families, particularly when nutrients are readily available (Burns 2008). Widespread distributions of clonal invertebrate lineages suggest that similar mechanisms may underly the success of some invasive animal taxa, as well (Mergeay *et al.* 2006).

Vegetative reproduction combined with mechanisms facilitating the generation and dispersal of tissue fragments appears to be especially conducive to rapid spread of invasive populations. Both modeling (Ruesink & Collado-Vides 2006) and experimental studies (Ceccherelli & Cinelli 1999; Wright & Davis 2006) of the marine alga *Caulerpa taxifolia* revealed that recruitment by fragmentation and post-recruitment vegetative growth together contribute to that species' extreme invasiveness. Similar studies have reported the capacity for population expansion *via* vegetative propagules in a number of widely invasive marine algae, including *Caulerpa racemosa*

(Ceccherelli & Cinelli 2001), *Heterosiphonia japonica* (Husa & Sjotun 2006) and *Codium fragile* ssp tomentosoides (Scheibling & Melady 2008). In terrestrial plant systems, population expansion by the riparian invasives *Arundo donax* (Khudamrongsawat *et al.* 2004; Decruyenaere & Holt 2005), *Ailanthus altissima* (Kowarik & Saumel 2008) and *Mimulus guttatus* (Truscott *et al.* 2006) appears in all cases to be dependent in large part on flood-mediated dispersal of clonal fragments. Although such phenomena have been observed most frequently among plant taxa, some invasive aquatic invertebrates have been shown to disperse by similar mechanisms. Bullard *et al.* (2007), for instance, determined that fragmentation and reattachment by colonial ascidians (including the invasive species *Botrylloides violaceus*, *Botryllus schlosseri*, and *Didemnum vexillum*) likely contributes substantially to population expansion.

Cordylophora would appear to be an ideal candidate for population expansion by fragmentation and subsequent dispersal of vegetatively produced propagules. Rapid clonal proliferation of *Cordylophora* by asexual budding combined with the stress-resistant characteristics of colony fragments (menonts) under unfavorable conditions have together been cited as an effective means for *Cordylophora* to spread rapidly in fouling communities associated with anthropogenic dispersal vectors (Folino 2000). In addition, dispersing fragments of non-native *Cordylophora* colonies have been detected at high frequency in currents of the some river systesms (Koetsier & Bryan 1995).

Our genetic analysis, however, suggests that the dispersal of vegetatively produced *Cordylophora* propagules is extremely limited in the Great Lakes basin. In 6 out of 7 cases, clonal genotypes were collected from neighboring dreissenid mussels within single 30 cm^2 scrapes, indicating that

these genotypes very likely derive from multiple tissue specimens drawn from single spatially extended colonies. Given the size of sampling scrapes and the relatively infrequent appearance of clonal genotypes even within scrapes (approximately 11% of scrapes tested), clonal spatial subrange for *Cordylophora* appears typically limited to the centimeter scale. This suggests that, contrary to expectation, colony fragmentation is unlikely to be a major contributor to the spatial spread of *Cordylophora* populations in this region. It is probable that long distance anthropogenic dispersal of *Cordylophora* instead proceeds through the transport of sexually produced propagules that have settled into fouling communities on human dispersal vectors.

Although *Cordylophora* would appear to gain little from its potential for vegetative propagation, the primary benefits of asexuality to colonizing populations—namely, avoidance of negative demographic and genetic effects associated with founding events (Roman & Darling 2007)—may be relatively unimportant to populations in the Great Lakes basin. Genetic diversity measures suggest that these populations may have averted substantial bottlenecks during colonization. Even in the most genetically depauperate samples (those from Cayuga Lake), H_E was over 0.6 and A_R was approximately 6 alleles after rarefaction to 14 individuals, and most samples possessed H_E values over 0.7 and A_R over 7 (Table 1). Without direct knowledge of source populations, it is impossible to assess whether or not observed diversity in the Great Lakes region represents a substantial reduction associated with colonization. However, the observed diversity levels suggest that *Cordylophora* populations are unlikely to suffer from negative effects of genetic bottlenecks. The reduction in diversity observed in Cayuga Lake mirrors previous studies showing lowered diversity of introduced Great Lakes populations in peripheral lakes (Colautti *et*

al. 2005; Lewis *et al.* 2000), consistent with the hypothesis of secondary invasion of these lakes *via* anthropogenic vectors associated with lower propagule pressure (MacIsaac *et al.* 2004).

Regional genetic structure of *Cordylophora* in the Great Lakes

Recent empirical studies have revealed numerous examples of introduced populations escaping dramatic losses of genetic diversity (Roman & Darling 2007; Wares *et al.* 2005). For many introduced taxa, multiple introductions from genetically divergent sources appear to facilitate the transfer of diverse invasive populations (Dlugosch & Parker 2008). Multiple introductions have been cited in the transfer of highly diverse populations for a number of invasive taxa in the Great Lakes, including *Dreissena polymorpha* (Stepien *et al.* 2005), *Neogobius melanostomus (=Apollonia melanostoma)* (Brown & Stepien 2009), *Proterorhinus semilunaruis (=marmoratus)* (Stepien *et al.* 2005), and *Bythotrephes longimanus* (Colautti *et al.* 2005). In addition, multiple introductions clearly have played a role in the global spread of *Cordylophora*: a previous study reported several non-native regions, including the Great Lakes, harboring multiple highly diverged evolutionary lineages of the genus (Folino-Rorem *et al.* 2009). Although the current analysis is restricted to sites known to harbor only the lineage more common to the Great Lakes basin, the fact that multiple incursions have contributed to the invasion history of *Cordylophora* in the region suggests the possibility that high within-population diversity may result from repeated introductions of genotypes from source populations.

The observed distribution of genetic variation within the study region does suggest that some locales may have received multiple *Cordylophora* introductions from genetically divergent sources. Independent introduction events with limited subsequent gene flow could account for the

dramatic genetic differentiation between geographically proximate samples collected from multiple sites in both Cayuga Lake and Lake Ontario (Table 3; Figure 3). Again, in the absence of data from the native range it is impossible to determine if this observed differentiation predates the invasion of the Great Lakes basin. Limited dispersal capacity for *Cordylophora* (see below) provides ample opportunity for *in situ* differentiation of populations, so the Cayuga Lake and Lake Ontario samples could derive secondarily from populations within the Great Lakes basin that differentiated subsequent to initial introduction. Alternatively, it is possible that the observed population structure at these two locales is driven by drift following single introductions. This hypothesis seems less likely, however, as gene flow sufficient to drive population expansion to neighboring sites without obvious founder effects should also be sufficient to limit drift.

On a regional scale, the genetic structure observed between *Cordylophora* samples paints a complex picture of gene flow across the Great Lakes basin. All collection locales (Chicago, eastern Lake Michigan, Lake Erie, Lake Ontario, and Cayuga Lake) were significantly differentiated from each other (Table 3). However, there were indications of greater genetic connectivity between the Lake Erie sample and samples BB in Lake Ontario and C1 in Cayuga Lake (Table 3; see also Figure 3B). Genetic studies of other invasive species in the Great Lakes have revealed varying patterns of connectivity across the region, with some taxa exhibiting signatures of high gene flow consistent with anthropogenic movement of large propagule pools over long distances while others maintain significant regional scale genetic differentiation (Colautti *et al.* 2005; Stepien *et al.* 2005; Brown & Stepien 2009). The complex patterns of genetic connectivity observed in *Cordylophora* may result from distinct populations within the Great Lakes (derived either from multiple native sources or from *in situ* post-introduction

differentiation) coupled with long-distance dispersal events throughout the region. Given the improbability of natural current-mediated gene flow between sites in Lake Erie, Lake Ontario, and Cayuga Lake, the observed genetic connectivity between samples at those sites likely reflects long-distance anthropogenic dispersal driven by movements of vessels between lakes. Such patterns have now been observed for multiple invasive taxa introduced to the Great Lakes basin (Wilson et al. 1999; Colautti et al. 2005).

Local genetic structure and larval dispersal

On a more restricted spatial scale, the genetic pattern observed in the Chicago harbors provides an unusually compelling illustration of local post-establishment expansion. Measures of pairwise genetic differentiation (Table 3), factorial correspondence analysis (Figure 2B), and Bayesian inference of population structure (Figure 3B) all indicate that dispersal between these harbors is insufficient to prevent formation of significant population structure, despite a maximum distance of only 21 km between samples. Particularly interesting is the fact that this structure appears to correlate strongly with the geographic distribution of samples. Factorial correspondence analysis clearly reveals a pattern of increasing genetic differentiation as separation between samples increases (Figure 2B), and STRUCTURE analysis indicates that individual genotypes assign to different clusters in the northern and southern parts of the Chicago range, with a gradual transition in assignment ratio along the north/south axis (Figure 4).

The resulting correlation between genetic and geographic distance (Figure 5A) reflects a strong pattern of isolation by distance (IBD). Such patterns are typically interpreted to reflect migration-drift equilibrium (Hutchison & Templeton 1999). However, historical evidence suggests that

Cordylophora populations in the Chicago harbors are unlikely to have achieved such equilibrium. The first observational records of *Cordylophora* in the area date to 1990 (Terrence Marsh, personal communication), and the presence of colonies throughout the Chicago harbors was not recognized until approximately 10 years later (NCF-R, personal observations). In light of this evidence, the likelihood is that the Chicago area *Cordylophora* populations represent a very recent introduction, and the assumption of migration-drift equilibrium seems unreasonable. The observed pattern of IBD is thus more likely the consequence of serial founder effects accompanying range expansion from a single initial introduction, and may in fact be temporally unstable as the population approaches equilibrium.

Two additional lines of evidence support the hypothesis of local expansion following a single recent introduction to the Chicago area. First, microsatellite diversity decreases along this axis (Table 1), with allelic richness dropping significantly in northern harbors (MO, BE, and DV) relative to those in the south (59, IJ, OJ). This is consistent with the expectation of decreasing genetic diversity—driven in particular by the loss of rare alleles—at the periphery of expanding populations (Ibrahim *et al.* 1996). Second, individual assignment tests suggest moderate levels of recent migration between Chicago harbors, with the vast majority of this gene flow (95%) occurring from south to north. Overall, genetic evidence indicates regular expansion of the *Cordylophora* population from south to north between Chicago harbors, and suggests that limitations to dispersal relative to the geographic scale of the metapopulation have resulted in serial founder effects driving the emergence of an IBD pattern.

These observations are all consistent with local range expansion mediated by natural dispersal in the Chicago area, and generally conform to the expectation of normally distributed dispersal distances associated with short-lived planulae in a relatively low current system. The striking contrast between local and regional genetic structure in Great Lakes *Cordylophora* populations thus provides empirical support for a general model of stratified dispersal in invasive populations consisting of local diffusive spread driven by natural dispersal mechanisms combined with regional spread driven by long-distance anthropogenic vectors (Suarez *et al.* 2001). A growing number of studies have described similar patterns associated with a variety of invasive taxa. Within the Great Lakes, for instance, genetic study of *D. bugensis* has revealed that jump dispersal mediated by recreational boats can result in considerable deviation from patterns expected in populations expanding by larval dispersal (Wilson *et al.* 1999). Similarly, in a recent study of the invasive Brazilian peppertree *Schinus terebinthifolius* Williams *et al.* (2007) described genetic spatial autocorrelation at local scales along with genetic clines extending around recently introduced populations, suggesting diffusive dispersal associated with local population expansion. On larger spatial scales, however, genetic connectivity between geographically separated sites indicated long-distance dispersal likely driven by anthropogenic vectors. Additional genetic analyses have implicated both local diffusion and long-distance jump dispersal in the invasive spread of the earthworm *Dendrobaena octaedra* (Cameron *et al.* 2008), the fire ant *Solenopsis invicta* (Shoemaker *et al.* 2006), and the riparian weed *Impatiens glandulifera* (Walker *et al.* 2009). These studies are supported by modeling approaches revealing the importance of stratified dispersal to invasive spread (Muirhead *et al.* 2006; Roura-Pascual *et al.* 2009), as well as empirical reconstructions of invasion histories based on historical and contemporary observational records (Suarez *et al.* 2001; Locey & Stone 2006). Thus, although

the invasion history of *Cordylophora* in the Great Lakes basin appears to be somewhat unusual in terms of the role of vegetative reproduction in population expansion, the overall effect of spatial scale on population structure instantiates a general pattern exhibited by invasions of both plant and animal taxa in a wide range of recipient environments.

We should note that the discontinuity between observed genetic connectivity patterns at local and regional scales may be influenced by certain aspects of our study design. First, it is possible that the Chicago area is atypical with respect to local dispersal dynamics. Additional investigation of local genetic patterns was limited to several locales with only two samples, precluding thorough comparison with Chicago. The patterns we did observe in those other systems suggest that there may be substantial variation in dispersal dynamics at different locales: in eastern Lake Michigan low differentiation indicates the possibility of substantial larval dispersal between samples, while in Lake Ontario and Cuyuga Lake very high differentiation suggests limited genetic exchange. Second, our limited ability to explore genetic patterns at intermediate spatial scales (e.g. within lakes) prevents us from excluding the possibility of larval dispersal operating over scales significantly larger than those we observed, or of mixed patterns of genetic connectivity shaped by combinations of larval dispersal and anthropogenic spread. Despite these caveats, the pronounced overall effect of spatial scale on genetic structure strongly implicates both local diffusive spread and regional jump dispersal in the expansion of invasive *Cordylophora* in our study region.

Further, although inference of limited larval dispersal within our study system is consistent with low dispersal capacity reported for other aquatic invertebrate taxa, particularly cnidarians

(Darling *et al.* 2004; Ayre & Hughes 2000), realized dispersal will reflect both larval behavior and the hydrodynamic properties of aquatic habitat (Bilton *et al.* 2002) and thus may vary considerably depending on the recipient environment. This is particularly relevant for a taxon known to invade lotic, lentic, and estuarine habitats (Folino-Rorem *et al.* 2009), and it is important to note that the observed limitations to dispersal in the Great Lakes may not be predictive of dispersal capacity in other regions. Similarly, population expansion by fragmentation may be more pronounced in systems with current regimes more conducive to both colony disruption and dispersal of fragments. In fact, the observation of drifting fragments in the Mississippi River (Koetsier & Bryan 1995) suggests that invasive populations in such systems may exhibit much broader spatial extent of clonal genotypes. Dramatically different patterns of genetic connectivity thus may be expected in systems other than the Great Lakes, particularly rivers and estuaries where *Cordylophora* commonly establishes.

Conclusions

Cordylophora in the Great Lakes appears to be unusual among invasive taxa capable of reproducing both sexually and asexually. Dispersal by fragmentation was negligible even on a local scale, and unlike a number of other systems we found no evidence of widespread local or regional distribution of clonal genotypes. In addition, introduced *Cordylophora* populations showed no signs of reduced neutral genetic diversity, despite evidence that many invasive taxa capable of asexual reproduction succeed in the face of dramatic genetic bottlenecks (Roman & Darling 2008). Although we did observe an effect of spatial scale on population structure, this appears to be mediated by differences between limited local larval dispersal and regional jump dispersal assisted by anthropogenic vectors, and not by differences in efficacy of clonal dispersal

over different scales. It remains to be seen whether systems more conducive to the generation and dispersal of clonal fragments (e.g. high flow lotic systems) might contribute to substantially different structure among invasive *Cordylophora* populations. The analysis conducted here, along with studies exploring the ecological and economic impacts of *Cordylophora*, should provide a valuable resource for understanding risks posed by this invasive taxon in the Great Lakes. Additionally, our results underscore the ability of genetic methods to reveal dynamics of invasiveness that are unexpected given the known biology of introduced taxa, and further emphasize the importance of investigating invasion dynamics at multiple spatial scales to capture the multiplicity of dispersal mechanisms driving range expansions.

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FIGURE LEGENDS

Figure 1. Distribution of collection sites within the Great Lakes basin. Site IDs are as in Table 2. Approximate west longitude and north latitude are shown on x and y axes, respectively. Sites within the Chicago area are shown as an inset; scale of inset is approximately 15 by 30 kilometers.

Figure 2. Three dimensional factorial correspondence analysis. A) all samples; B) samples in Chicago area only (populations enclosed by the oval in A).

Figure 3. Bayesian inference of population structure. A) Plot of model likelihood score [Ln(L)] versus the number of clusters specified for the model (*K*). Results are for the mean plus or minus standard deviation of 5 independent runs. Arrow indicates the most likely model at $K = 8$. B) Plot of individual genotype assignments when $K = 8$. Each genotype is represented by a thin vertical line, with proportional membership in each of $K = 8$ clusters indicated by color. Black vertical lines separate collection sites, with site IDs indicated below the plot and locality membership indicated above.

Figure 4. Correlation of STRUCTURE assignments with geographic distribution of Chicago harbors. For all individuals within each Chicago harbor, mean assignment into the first of two clusters (y axis) is plotted against each harbor's north latitude (x axis). Coefficient of determination (R^2) and significance of correlation (*P*) are shown.

Figure 5. Results of Mantel tests. A) populations within Chicago area only; B) all populations, with Chicago samples collapsed into a single population (see Methods for details). Values for Mantel's *Z* statistic, coefficient of determination (R^2), and significance of correlation (*P*) are indicated for both tests.

TABLES AND FIGURES

Table 1. Genetic diversity of *Cordylophora* samples from the Great Lakes basin. *N*, number of individuals; N_a, number of alleles; A_r, allelic richness (with rarefaction to 14 individuals); H_E, gene diversity. For locales with multiple samples, mean values of A_r and H_E are shown in italics; means bearing the same superscript letters fall into the same significance groups.

Site	ID	Latitude	Longitude	*N*	N_a	A_r	H_E
CHICAGO							
Montrose	MO	41°57'40.25"N	87°38'22.08"W	33	10	7.42	0.7023
Belmont	BE	41°56'36.72"N	87°38'15.54"W	25	9.625	7.98	0.7699
Diversity	DV	41°55'56.05"N	87°37'59.17"W	32	8.625	7.09	0.7290
DuSable	DS	41°53'6.72"N	87°36'39.24"W	16	7.25	7.00	0.7414
Burnham Harbor	BH	41°51'12.37"N	87°36'37.35"W	73	12.5	7.77	0.7298
59th Street Harbor	59	41°47'18.69"N	87°34'29.86"W	57	11.875	8.03	0.7551
Inner Jackson Harbor	IJ	41°46'39.25"N	87°34'38.70"W	37	12.25	9.02	0.7353
Outer Jackson Harbor	OJ	41°46'40.25"N	87°34'26.01"W	39	12.5	9.02	0.7939
						7.92^{A}	*0.7440*^{A}
E. LAKE MICHIGAN							
Maranatha Bridge	MB	43°10'6.18"N	86°17'28.80"W	20	9.375	8.42	0.7141
Muskegan	MSK	43°13'51.06"N	86°15'58.98"W	48	14.125	9.30	0.7570
						8.86^{A}	*0.7450*^{A}
LAKE ONTARIO							
Bradock Bay	BB	43°18'29.46"N	77°42'29.16"W	25	8	7.12	0.6783
Southpoint Marina	SP	43°10'37.92"N	77°31'8.40"W	27	9.875	8.36	0.6761
						7.74^{A}	*0.6770*^{A}
LAKE ERIE	E	42°07'49.38"N	80°06'33.54"W	24	8.5	7.56^{A}	0.6993^{A}
CAYUGA LAKE							
Cayuga 1	C1	42°28'10.26"N	76°30'11.34"W	24	8.875	7.58	0.6271
Cayuga 2	C2	42°27'30.48"N	76°30'52.20"W	20	6.875	5.93	0.6304
						6.76^{B}	*0.6290*^{B}

Table 2. Clonal genotypes observed in dataset. Genotypes are named according to their population of origin. *N*, number of times the genotype appears in the dataset; N_{gen}, total number of genotypes in the sample from which the genotype was collected; P_{gen}, probability of incidence of genotype; P_{sex}, probability of observing *N* copies of the genotype in the sample, assuming sexual reproduction and the allele frequencies observed in the sample.

Genotype	***N***	N_{gen}	P_{gen}	P_{sex}
59-12	2	42	2.08×10^{-19}	4.49×10^{-15}
59-33	2	42	7.25×10^{-12}	4.97×10^{-20}
BH-26	2	47	1.17×10^{-11}	1.55×10^{-19}
MSK-9	2	31	6.01×10^{-13}	2.02×10^{-22}
MSK-17	2	31	1.83×10^{-13}	1.87×10^{-19}
MSK-25	2	31	9.87×10^{-15}	5.46×10^{-18}
MB-6	2	12	2.69×10^{-8}	5.73×10^{-14}

Table 3. F_{ST} values. All pairwise comparisons are shown below the diagonal. Pairwise comparisons between regional populations are shown above the diagonal. Values that are NOT significant after Bonferroni correction for multiple tests are indicated in bold italics.

	Chicago								Cayuga		Erie	E. Michigan		Ontario	
	IJ	**OJ**	**59**	**BH**	**DS**	**DV**	**BE**	**MO**	**C1**	**C2**	**E**	**MB**	**MSK**	**BB**	**SP**
IJ	-														
OJ	***0.0134***	-													
59	0.0218	0.0151	-												
BH	0.0484	0.0232	0.0426	-											
DS	0.0540	0.0327	0.0505	0.0322	-				0.0785		0.0458	0.0475		0.0492	
DV	0.0603	0.0347	0.0472	0.0258	***0.0216***	-									
BE	0.0261	***0.0103***	0.0173	0.0197	***0.0266***	0.0321	-								
MO	0.0602	0.0474	0.0636	0.0340	0.0316	0.0350	***0.0189***	-							
C1	0.0533	0.0744	0.0681	0.0858	0.1208	0.1106	0.0803	0.1083	-		0.0410	0.0598		0.0578	
C2	0.1373	0.1277	0.1193	0.1572	0.1909	0.1619	0.1401	0.1825	0.1112	-					
E	0.0336	0.0452	0.0437	0.0674	0.0929	0.0828	0.0553	0.0976	***0.0185***	0.1054	-	0.0273		0.0284	
MB	0.0510	0.0505	0.0589	0.1049	0.1222	0.1340	0.0751	0.1253	0.0768	0.1491	0.0436	-		0.0295	
MSK	0.0326	0.0339	0.0398	0.0699	0.0901	0.0917	0.0452	0.0887	0.0443	0.1058	0.0278	0.0246	-		
BB	0.0462	0.0546	0.0572	0.0909	0.1184	0.1230	0.0779	0.1325	0.0626	0.1653	***0.0347***	0.0570	0.0448	-	
SP	0.0631	0.0591	0.0693	0.0855	0.1183	0.1046	0.0779	0.1135	0.0681	0.1071	0.0592	0.0946	0.0441	0.0761	

Table 4. Results of assignment tests in GENECLASS. Source populations are listed by column, recipient populations by row. Populations in Chicago are set off from other populations by a box in the upper left corner. Individuals assigned to the sampling site from which they were collected are indicated in bold along the diagonal. Inferred migrations from south to north in the Chicago area are indicated with gray shading.

	IJ	**OJ**	**59**	**BH**	**DS**	**DV**	**BE**	**MO**	**C1**	**C2**	**E**	**MB**	**MSK**	**BB**	**SP**
IJ	**33**	4													
OJ	1	**38**													
59	1	18	**36**	1			1								
BH	3	13	1	**56**											
DS		1		1	**14**										
DV	1	5		3		**22**	1								
BE		2		2			**21**								
MO	5	5		1			2	**20**							
C1	1								**19**		1				
C2			1							**23**					
E											**24**				
MB		1										**17**	2		
MSK													**48**		
BB		1												**24**	
SP	1														**26**

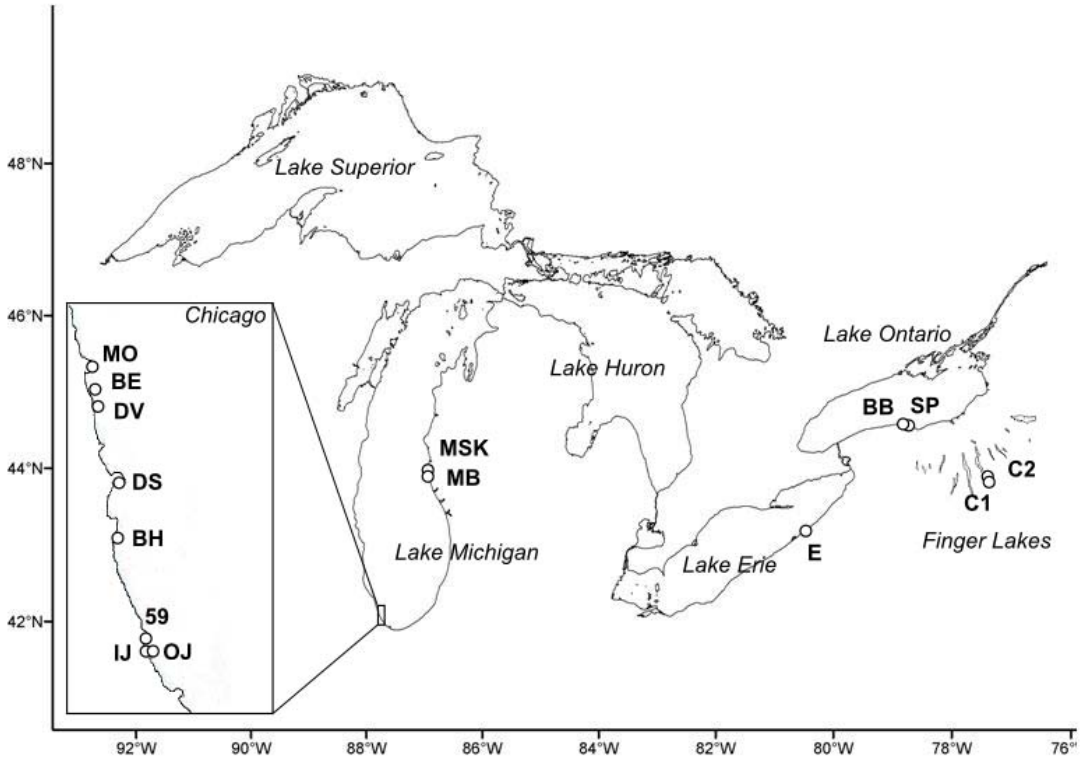
Lake Superior
Lake Huron
Lake Michigan
Lake Erie
Lake Ontario
Finger Lakes
Chicago
MO
BE
DV
DS
BH
59
IJ
OJ
MSK
MB
BB
SP
C1
C2
E
48°N
46°N
44°N
42°N
92°W
90°W
88°W
86°W
84°W
82°W
80°W
78°W

FIGURE 1

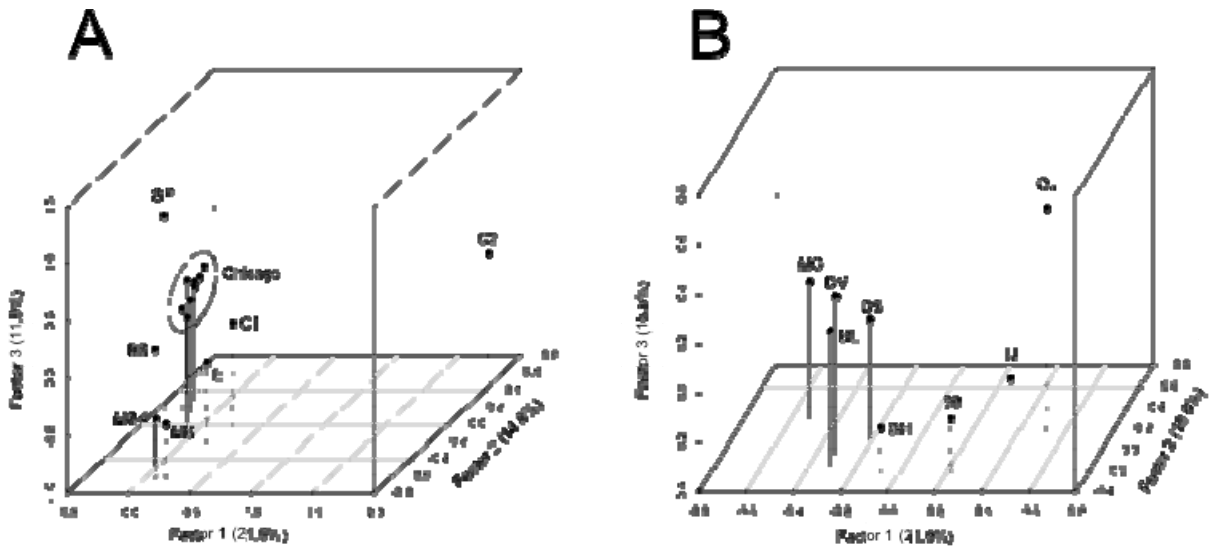

FIGURE 2

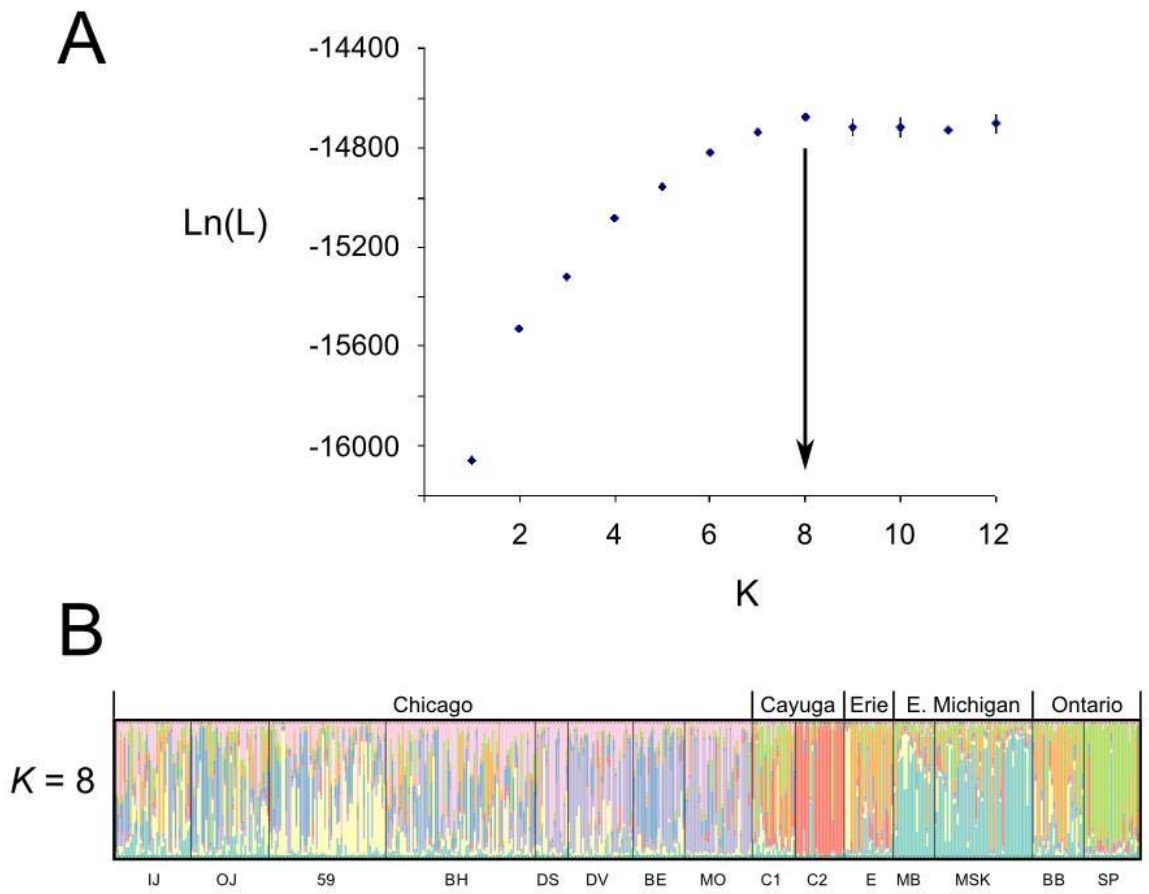
A
-14400
-14800
-15200
-15600
-16000
Ln(L)
2
4
6
8
10
12
K
B
Chicago
Cayuga
Erie
E. Michigan
Ontario
K = 8
IJ
OJ
59
BH
DS
DV
BE
MO
C1
C2
E
MB
MSK
BB
SP

FIGURE 3

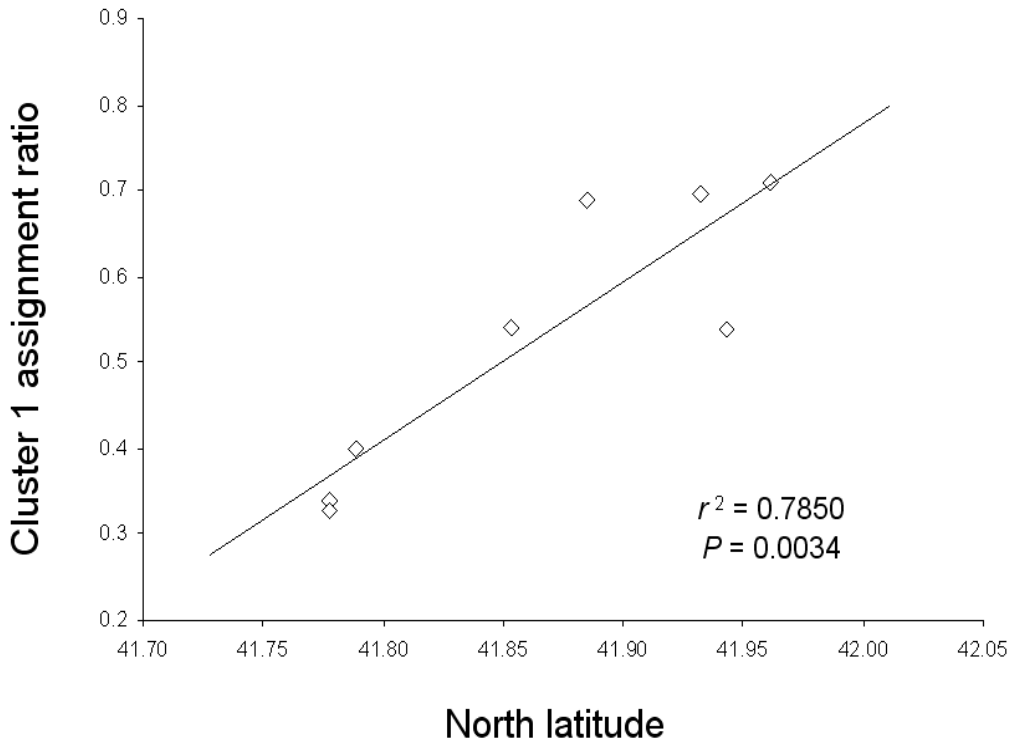
Cluster 1 assignment ratio
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
41.70
41.75
41.80
41.85
41.90
41.95
42.00
42.05
North latitude
r 2 = 0.7850
P = 0.0034

FIGURE 4

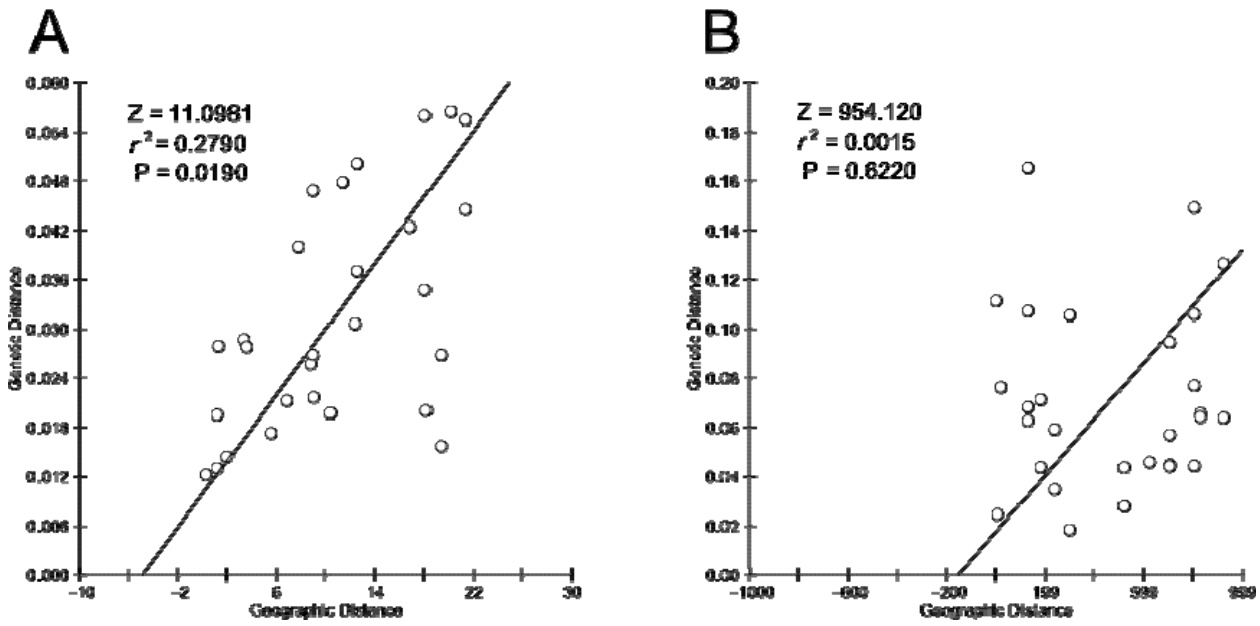
A
Z = 11.0981
r² = 0.2790
P = 0.0190
Genetic Distance
Geographic Distance
B
Z = 954.120
r² = 0.0015
P = 0.6220
Genetic Distance
Geographic Distance

FIGURE 5