Multiple dispersal strategies of the invasive quagga mussel (*Dreissena bugensis*) as revealed by microsatellite analysis

A.B. Wilson, K-A Naish, and E.G. Boulding

Abstract: The recent invasion of the Laurentian Great Lakes by *Dreissena polymorpha* and *Dreissena bugensis* presents a unique opportunity to study the role of high dispersal ability in the colonization success of invading species. While the dispersal of *D. polymorpha* has been characterized by several jump dispersal events, census data suggest a more gradual diffusion of *D. bugensis* from its point of introduction through the lower Great Lakes. In this study, we use six highly polymorphic microsatellite markers to investigate the present-day population genetic structure of *D. bugensis* in North America in an effort to clarify the role of multiple dispersal strategies in its colonization success. In contrast with survey data, which would suggest a gradual spread ultimately resulting in a pattern fitting a model of isolation-by-distance, the significant allelic and F_{ST} differences observed between 18 of 28 population pairs do not correlate with any simple geographic relationship. Deviations from isolation-by-distance are due, in part, to a higher gene flow between disjunct populations than that expected under a normally distributed pattern of dispersal. These results, in combination with recent reports of the quagga mussel outside its established North American range, suggest that boater-mediated jump dispersal of *D. bugensis* may be contributing to its colonization and spread in North America.

Résumé : L'invasion récente des Grands Lacs laurentiens par *Dreissena polymorpha* et *D. bugensis* constitue une occasion unique d'étudier le rôle d'une forte capacité de dispersion pour le succès de colonisation de l'espèce envahissante. Bien que la dispersion de *D. polymorpha* se soit caractérisée par plusieurs sauts, les données d'enquête montrent une diffusion plus graduelle de *D. bugensis* à partir de son point d'introduction dans les Grands Lacs inférieurs. Nous avons utilisé six marqueurs microsatellites fortement polymorphes pour étudier la structure génétique de la population actuelle de *D. bugensis* en Amérique du Nord dans le but de préciser le rôle de diverses stratégies de dispersion pour le succès de la colonisation. Au contraire des données des relevés, qui indiquent une diffusion graduelle finissant par donner une allure conforme à un modèle d'isolement par la distance, les écarts significatifs entre les allèles et les F_{ST} observés entre 18 des 28 paires de populations ne présentaient pas de corrélation avec aucune relation géographique simple. Les écarts au modèle isolement par la distance s'expliquent, du moins en partie, par un flux génétique plus élevé entre les populations isolées que celui que l'on peut prévoir conformément à un régime de répartition à distribution normale. Ces résultats, combinés à des signalements récents de moules quagga à l'extérieur de son aire de répartition établie en Amérique du Nord, portent à croire que les sauts de dispersion de *D. bugensis* favorisés par le déplacement des bateaux pourraient contribuer à sa colonisation et à sa diffusion en Amérique du Nord.

[Traduit par la Rédaction]

Introduction

Biological invasions are characterized by two key phases: the establishment of a species at a location and its subsequent radiation in space (Hastings 1996). Both the establishment of an initial population and successful naturalization of a species in its new habitat are critical to its colonization success. Theoretical ecologists have developed a number of models in an effort to clarify the relationship between demographic parameters and the rate of range expansion of introduced species (Hastings 1996). While empirical verification of these models is incomplete, existing data suggest that rare, long-distance jump dispersal events may play an important role in the spread of recent invaders (Hastings 1996).

Until recently, estimates of species spread following invasion have been limited to ecological and laboratory studies of life history parameters and survey data documenting range expansion (Hastings 1996). With the advent of highly polymorphic molecular markers, a greater understanding of the parameters underlying the spread of a natural population may be obtained, providing data on rates of gene flow between populations and clarifying secondary introductions that may have a significant impact on the population structure of an invasive species.

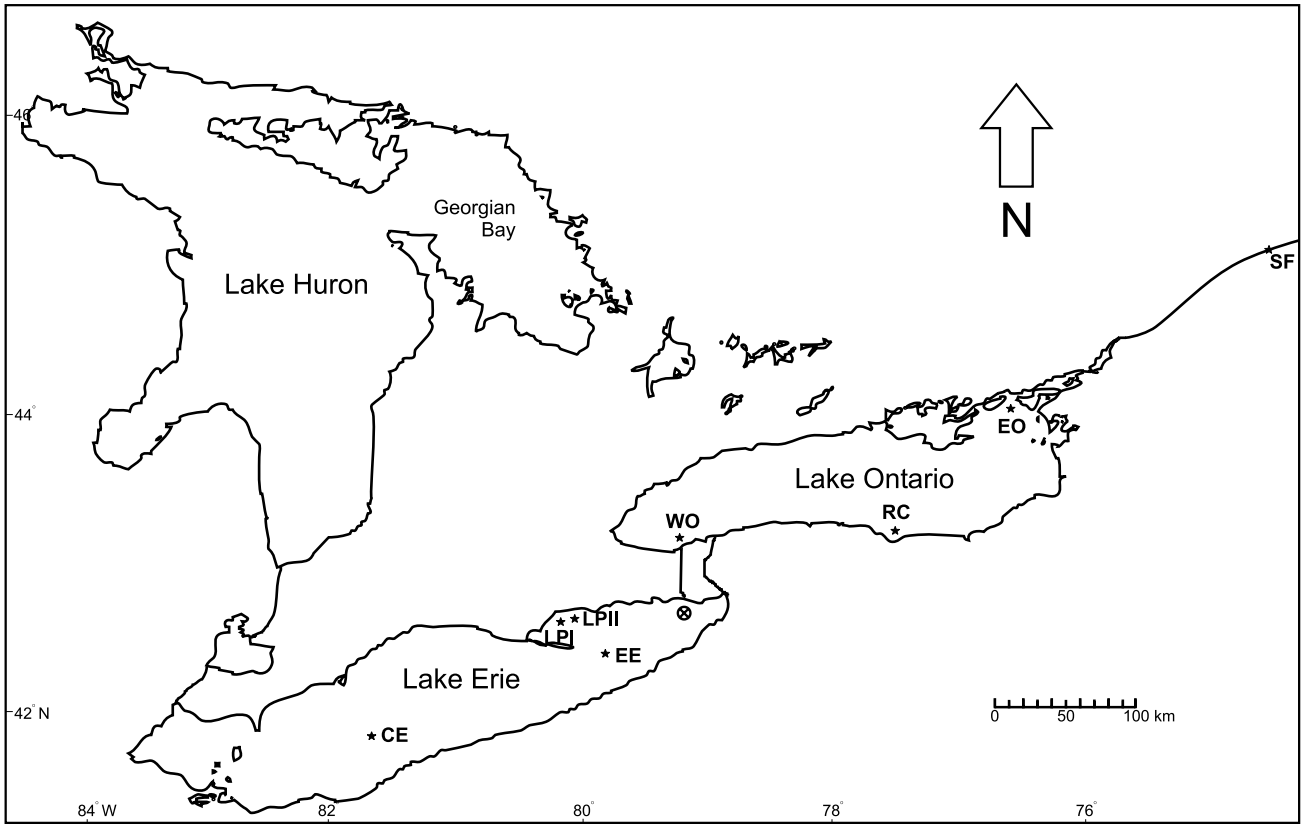
The quagga mussel (*Dreissena bugensis*) is native to the Dnieper drainage system in the Ukraine (Mills et al. 1996).

Received February 25, 1999. Accepted August 6, 1999. J15034

A.B. Wilson, K-A Naish, and E.G. Boulding. Department of Zoology, University of Guelph, Guelph, ON N1G 2W1, Canada.

[1]Author to whom all correspondence should be sent at the following address: Faculty of Biology, University of Konstanz, Konstanz, Germany, D-78457. e-mail: tony.wilson@uni-konstanz.de

Fig. 1. Map of *D. bugensis* sampling sites. See Table 1 for detailed information on sampling methods and locations. The encircled cross indicates the putative point of the 1989 introduction (Mills et al. 1993).



In 1989, *D. bugensis* individuals were identified in eastern Lake Erie, Canada (May and Marsden 1992; Mills et al. 1993; see Fig. 1). The quagga mussel spread rapidly following its initial colonization and by 1996 had spread through Lake Erie to Lake Ontario and the St. Lawrence River (Mills et al. 1996). Researchers have hypothesized that dreissenid mussels were introduced into the Laurentian Great Lakes following transoceanic transport of mussel larvae in the ballast water of ships (Hebert et al. 1989).

Studies on temporal variation in distribution of dreissenid mussels have benefited from an extensive survey program (U.S. Geological Survey 1997) that was established following the identification of the zebra mussel (*Dreissena polymorpha*) in Lake St. Clair, Ontario, in 1988 (Hebert et al. 1989). Census data suggest that the two dreissenid species may have colonized the Great Lakes by differing methods. While the spread of the zebra mussel has been characterized by several long-distance jump dispersal events within the Laurentian Great Lakes, the quagga mussel has exhibited a much more gradual downstream diffusion from its initial introduction in Lake Erie eastward into Lake Ontario and the St. Lawrence River (Mills et al. 1993).

Despite their apparent differential patterns of colonization, the radiation of both *D. bugensis* and *D. polymorpha* has been exceedingly rapid following their initial introduction. The quagga and zebra mussel share two notable life history characteristics that may have accelerated their colonization and spread through the Laurentian Great Lakes (Johnson and Carlton 1996). Firstly, both *D. bugensis* and *D. polymorpha* have a planktonic veliger stage, whereby free-living larvae can remain suspended in the water column for up to several weeks (Carlton 1993). During this time, larvae passively diffuse in lacustrine currents and, upon settlement, may found new populations.

Secondly, dreissenid mussels are byssate and can attach to hard substrate by producing byssal threads (Mills et al. 1996). *Dreissena* spp. have been found attached to the hulls of ships (Johnson and Carlton 1996). Movements of boats both within lakes (Johnson and Carlton 1996) and overland (Ricciardi et al. 1995) may result in the formation of new mussel populations in geographically disparate regions. Boat traffic and, to a lesser degree, birds and fish may have facilitated dispersal of the zebra mussel to upstream sites in Lake Superior, Lake Michigan, and Lake Huron from founding populations in Lake St. Clair (Carlton 1993).

While it is often difficult to directly track dispersal in aquatic environments, genetic techniques have often proven useful as indirect estimators of aquatic dispersal (Palumbi 1995; Parsons 1996). Classical population genetic models predict that geographically limited dispersal results in genetic isolation-by-distance even within large continuous populations (Wright 1946). Rousset (1997) further developed a model of isolation-by-distance using a regression approach to indirectly quantify demographic parameters such as population density and dispersal distance from genetic data.

Modelling studies by Ibrahim et al. (1996) have demonstrated that rare long-distance migration during range expansion can lead to the establishment of pocket populations in advance of the main invasion front, resulting in patterns of spatial clustering that can persist for hundreds of genera-

Table 1. *Dreissena bugensis* sampling site locations.

Sampling site	Coordinates	Depth (m)	Sampling technique	*N*	Date sampled, 1997
Central Erie (CE)	41°57.000′ N, 81°37.470′ W	10.0	Ponar grab (2)	54	October 7
Long Point Bay I (LPI)	42°42.960′ N, 80°15.084′ W	8.4	Ponar grab (3)	54	June 24
Long Point Bay II (LPII)	42°46.290′ N, 80°08.460′ W	8.4	Ponar grab (6)	54	June 22
Eastern Erie (EE)	42°30.394′ N, 79°53.798′ W	63.0	Ponar grab (2)	54	June 25
Western Ontario (WO)	43°13.471′ N, 79°16.356′ W	14.0	Ponar grab (3)	54	June 18
Rochester (RC)	43°24.500′ N, 77°53.000′ W	1.0	Shoreline	54	December 7
Eastern Ontario (EO)	44°09.020′ N, 76°38.000′ W	22.0	Ponar grab (3)	54	October 29
Lake St. Francis (SF)	45°07.140′ N, 74°21.010′ W	15.0	SCUBA	54	February 28

tions. Long-distance dispersal may have several important impacts on population genetic structure. Firstly, long-distance dispersal may lead to an upward bias in F_{ST} over that expected under normally distributed dispersal (Ibrahim et al. 1996). Secondly, a plot of isolation-by-distance regression in species that disperse over long distances should be skewed by lower differentiation at great distances (Rousset 1997). Finally, assuming that peripheral populations remain isolated in long-distance dispersers, higher inbreeding should be observed in these isolated populations (Ibrahim et al. 1996). Through an analysis of the deviation of genetic data from a model of isolation-by-distance, researchers can indirectly quantify the role of long-distance migration in range expansion of a species.

In this study, we use highly polymorphic microsatellite markers in an effort to clarify the present-day population genetic structure of the quagga mussel in the Laurentian Great Lakes. These data can serve as an indirect estimate of the dispersal of *D. bugensis* through North American waterways. As noted above, census data suggest a gradual diffusion of *D. bugensis* from its initial introduction into Lake Erie. Such a gradual diffusion should exhibit a strong fit to the isolation-by-distance model. Alternatively, departures from this model may indicate that additional dispersal mechanisms have also contributed to the range expansion of *D. bugensis*. Microsatellite markers will help to quantify this pattern of spread, clarifying the role that human-mediated dispersal vectors have played in the spread of the quagga mussel.

Materials and methods

Sample collection and microsatellite analysis

Adult *D. bugensis* were collected from a combination of nearshore and offshore habitats representing the extent of the species' range in the lower Laurentian Great Lakes (Fig. 1; Table 1). Upon collection, samples were extracted immediately or frozen at –90°C until further use. Population sample sizes were chosen following simulation study (Ruzzante 1998; Wilson 1998) in an effort to minimize statistical bias.

DNA extraction and amplification of the six microsatellite loci (*Dbug*1–6) was performed as outlined in Wilson et al. (1999). For each of the six loci, allelic ladders were constructed using a combination of polymerase chain reaction products from individual mussels. These allelic ladders were run every five lanes in each gel and helped to facilitate scoring of gels and to maintain consistency of scoring between different gel runs.

Statistical analyses

Linkage disequilibrium

Loci data were tested for genotypic disequilibrium using GENEPOP V3.1a (Raymond and Rousset 1995*a*). For the analysis, a 10 000-batch, 1000-iteration Markov chain analysis was preceded by a 10 000-step dememorization process.

Hardy–Weinberg equilibrium

Observed heterozygosities within populations were tested for departure from Hardy–Weinberg expectations using Weir and Cockerham's (1984) estimate as implemented by GENEPOP V3.1a (Raymond and Rousset 1995*a*). For loci with fewer than five alleles, an exact *P* value was calculated by a complete enumeration method, and for loci with a higher number of alleles, a 10 000-step, 1000-iteration Markov chain method (10 000 dememorization steps) was used to calculate an unbiased estimate of the *P* value.

Population differentiation

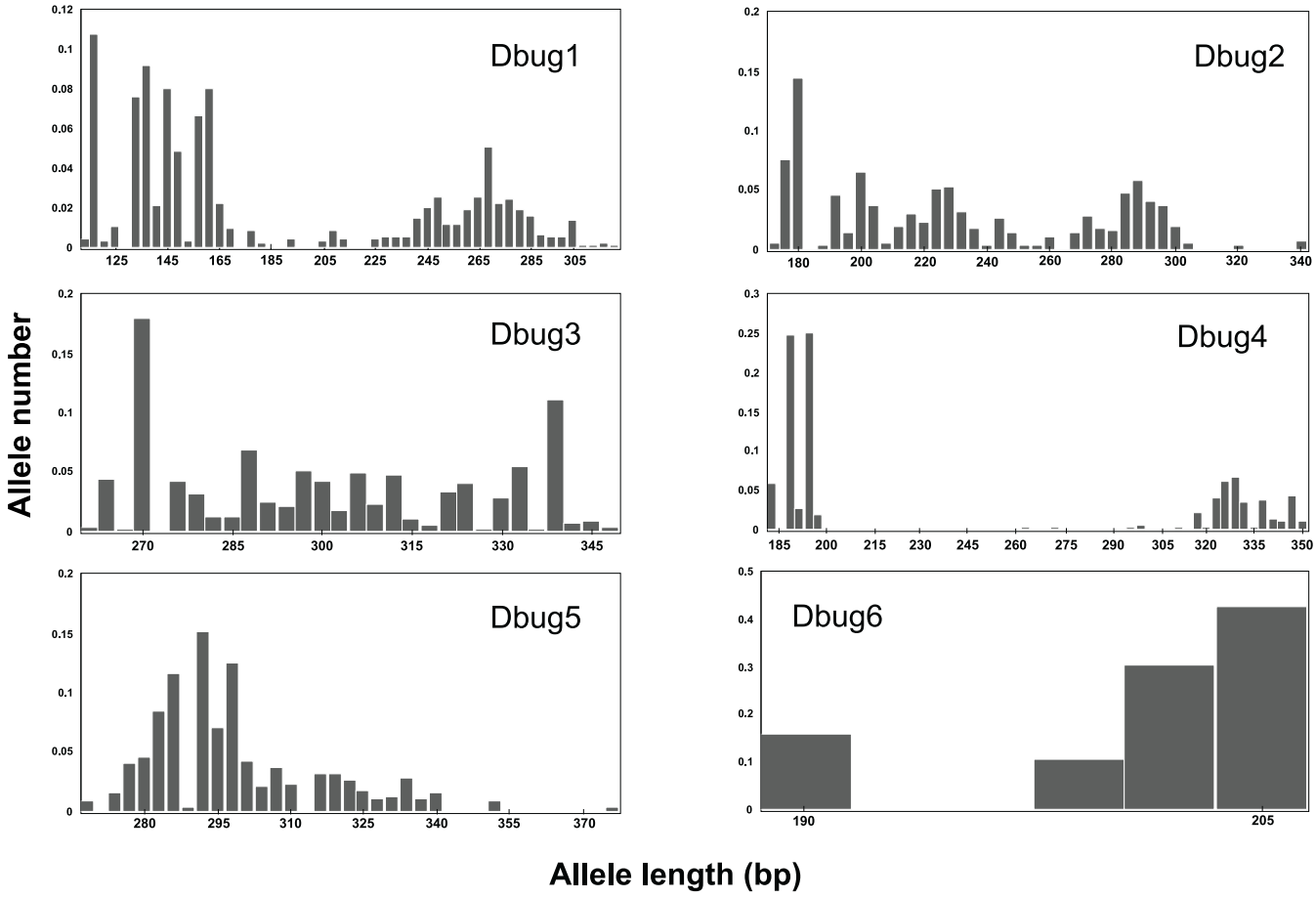
Tests for genotypic differentiation of populations are less powerful than those that assume random sampling of alleles (genic differentiation) but more appropriate when individuals exhibit nonrandom mating (Goudet et al. 1996). Both global and pairwise tests were performed, using GENEPOP V3.1a (Raymond and Rousset 1995*a*), in an effort to clarify the pattern of interpopulation differentiation. An unbiased estimate of the significance of the probability test was calculated through a 10 000-step, 1000-iteration Markov chain permutation (10 000 dememorization steps) of an R × C contingency table of allelic distribution for each population. The significance of the *P* values across the six loci was determined using Fisher's probability combination test (Raymond and Rousset 1995*b*).

Genetic distance between populations was measured by calculating F_{ST} and linearized with population divergence time via a transformation (linearized $F_{ST} = F_{ST}/(1 - F_{ST})$, Slatkin 1993) using Arlequin V1.1 (Schneider et al. 1997). The significance of these F_{ST} estimates was tested under the null hypothesis H_0 = "no difference between populations" by permuting genotypes between populations (10 000 iterations). The *P* value of the test is the proportion of permutations leading to an F_{ST} value larger or equal to that observed (Schneider et al. 1997).

Isolation-by-distance

Models of isolation-by-distance offer an empirical means to test patterns of dispersal from measures of population subdivision. Subdivided natural populations that fit a one-dimensional diffusive stepping-stone model should exhibit a strong fit to the isolation-by-distance model (Rousset 1997). Through simulation study, Rousset (1997) has found a linear relationship between $F_{ST}/(1 - F_{ST})$ and distance for one-dimensional habitats. Linearized pairwise F_{ST} estimates were plotted against the linear distance between populations. In addition to a computation of the linear regression between these two parameters, a Mantel permutation test (10 000 permutations)

Fig. 2. Allele frequency histograms for *D. bugensis* microsatellite loci.



was used to test the null hypothesis H_0 = "genetic distance and geographic distance are independent" using a rank correlation coefficient calculated from the pairwise F_{ST}–distance matrix. To aid in further interpretation of overall trends of population subdivision, pairwise linearized F_{ST} estimates were also plotted versus absolute distance separating populations from a sample representing the western extent of the species' range.

Analysis of molecular variance (AMOVA)

In a further analysis of the correlation between genetic distance and topographic characteristics of the lower Great Lakes, an F_{ST}-based hierarchical AMOVA (Excoffier et al. 1992) was used to clarify the partitioning of molecular variance between three levels: variation among lakes, among populations within a lake, and among individuals within populations. Groupings were chosen in relation to putative point of introduction of *D. bugensis* (see Fig. 1).

Results

Descriptive statistics

A total of 167 alleles were observed for the six loci over eight populations (432 individuals), ranging from a low of four alleles at *Dbug*6 to a high of 44 at *Dbug*1 (Fig. 2). Allelic frequency data were plotted as a size-ranked histogram to clarify the sampling space of the allelic distribution (Fig. 2). The allelic distribution of the six microsatellites varied significantly across loci, with *Dbug*1 and *Dbug*4 exhibiting a bimodal distribution of alleles, *Dbug*5 displaying an approximately normal distribution, and *Dbug*2, *Dbug*3, and *Dbug*6 exhibiting a uniform distribution of alleles.

Linkage disequilibrium

Although exact tests for genotypic disequilibrium between microsatellite loci within populations gave five significant P values (χ^2 test, $P < 0.05$) out of 120 pairs of loci (4.17%), all values were rendered nonsignificant following sequential Bonferroni correction (Rice 1989). Global tests of linkage disequilibrium calculated from within-population data were not significant at the 5% level (χ^2 test), indicating that allelic variation at all six loci is in linkage equilibrium.

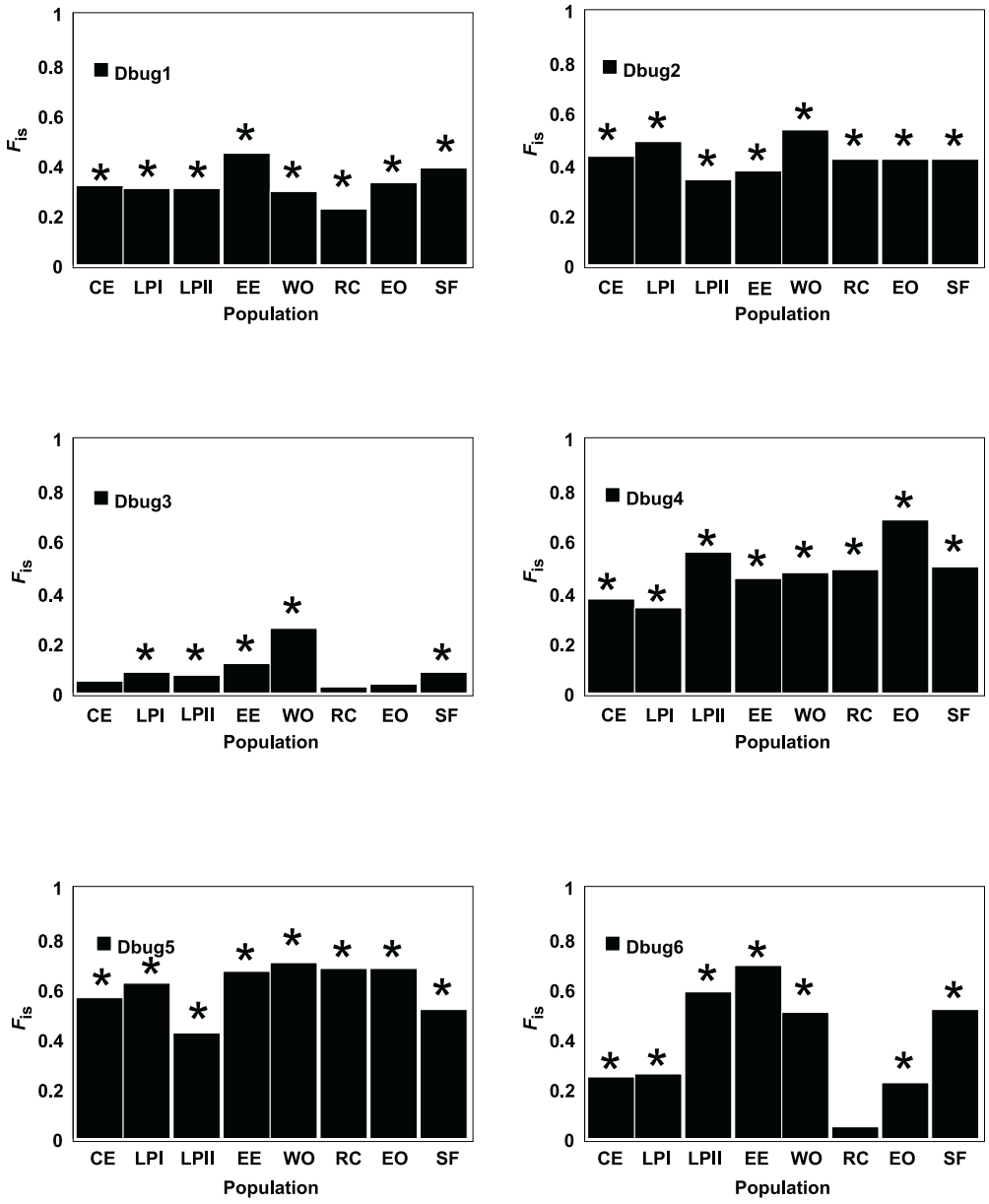
Hardy–Weinberg equilibrium

Departure from Hardy–Weinberg expectations was measured via a Markov chain permutation procedure for *Dbug*1–5 and a direct enumeration approach for *Dbug*6, which had fewer than five alleles. All six loci exhibit a significant heterozygote deficiency (χ^2 test, $P < 0.05$). The inbreeding coefficient (F_{IS}) was plotted by locus for each population (Fig. 3), graphically illustrating the departure from Hardy–Weinberg expectations.

Population differentiation

Global χ^2 tests of population differentiation indicated significant heterogeneity in gene frequencies among the eight populations ($P < 0.00001$). In an effort to further partition

Fig. 3. Hardy–Weinberg test for each locus in each *D. bugensis* population calculated using Weir and Cockerham's (1984) estimates as implemented by GENEPOP V3.1a (Raymond and Rousset 1995*a*). An asterisk indicates a significant heterozygote deficiency ($P < 0.05$). All loci exhibit a significant departure from Hardy–Weinberg equilibrium ($P < 0.00001$).



these data, pairwise estimates of genotypic differentiation were calculated (Table 2). Eighteen of 28 pairwise comparisons (75.0%) were significantly different at $P < 0.05$ (χ^2 test) following sequential Bonferroni correction (Rice 1989).

Pairwise F_{ST} estimates also showed high levels of genetic differentiation between populations (Table 3). Twenty-seven out of 28 population pairs (96.4%) were significantly differentiated on the basis of F_{ST}. While overall genetic differentiation was high, low levels of genetic differentiation were observed between several highly disjunct populations (e.g., central Erie – Lake St. Francis) under both exact tests for population differentiation and estimates based on F_{ST}.

Isolation-by-distance

While F_{ST} and exact tests of population differentiation both indicated significant differences between many populations, these differences are not correlated with the geographic distance between populations ($r^2 = 0.0344$; one-tailed Mantel test, $P = 0.32061$). The power of the significance of the regression slope (Zar 1984) was calculated to be 0.85. The high power of this test, coupled with the high P value observed, indicates that this result is not likely to be due to a type II error (i.e., falsely concluding that no significant difference occurs when, in fact, it does). These deviations from a pattern of isolation-by-distance suggest that diffusive dispersal of mussel larvae may not be the sole vector of gene flow between populations. The y-intercept of the regression line was positive and relatively large ($y = (8.818 \times 10^{-6}x) + 0.01395$), suggesting that gene flow maintained by long-distance dispersal has contributed to the poor overall fit to the isolation-by-distance model.

In an effort to clarify consistent trends contributing to the

Table 2. Fisher's exact test for genotypic differentiation: pairwise population comparisons.

Population–population	χ^2	*P* value
CE–LPI	18.951	0.0897
CE–LPII	49.326	0.0000*
CE–EE	15.617	0.2094
CE–WO	26.715	0.0085
CE–RC	69.710	0.0000*
CE–EO	43.590	0.0000*
CE–SF	16.912	0.1529
LPI–LPII	37.453	0.0002*
LPI–EE	27.296	0.0070
LPI–WO	31.517	0.0016*
LPI–RC	54.620	0.0000*
LPI–EO	41.404	0.0000*
LPI–SF	52.446	0.0000*
LPII–EE	46.171	0.0000*
LPII–WO	43.657	0.0000*
LPII–RC	29.308	0.0035*
LPII–WO	69.800	0.0000*
LPII–SF	62.704	0.0000*
EE–WO	22.172	0.0321
EE–RC	43.852	0.0000*
EE–EO	28.045	0.0054
EE–SF	19.155	0.0849
WO–RC	48.955	0.0000*
WO–EO	26.310	0.0097
WO–SF	22.523	0.0356
RC–EO	69.710	0.0000*
RC–SF	52.609	0.0000*
EO–SF	36.600	0.0003*

Note: Locus by locus pairwise *P* values were computed by GENEPOP V3.1a (Raymond and Rousset 1995*a*) and pooled using Fisher's χ^2 method for combining probabilities (Sokal and Rohlf 1981). Significance of pairwise comparisons was assessed following a sequential Bonferroni correction (Rice 1989). Global tests indicated that all loci were highly significantly differentiated among the eight populations ($P < 0.0001$). Asterisks indicate that allelic distributions are significantly different (χ^2 test, $P < 0.05$, df = 12).

deviation from isolation-by-distance, pairwise estimates of $F_{ST}/(1 - F_{ST})$ were plotted versus absolute distance (Fig. 4). Several key trends are observed over the range of the species. Firstly, and most significantly, even over short within-lake comparisons, there is no strong pattern of isolation-by-distance. Secondly, although some of the lowest levels of gene flow were detected between EO and the other sites, all populations exhibit a decrease in $F_{ST}/(1 - F_{ST})$ versus the Lake St. Francis population.

AMOVA

Populations were partitioned into two groups on the basis of their geographical relationship to the putative point of introduction of *D. bugensis* in eastern Lake Erie (see Fig. 1) in an effort to further clarify if the present-day genetic structure has been defined by historical colonization events. While significant ($P < 0.05$), less than 1% of the total genetic variation can be explained on the basis of these groupings (Table 4). These data are consistent with the isolation-by-distance test, indicating that while significant differences do exist, the current population genetic structure of *D. bugensis* in North America cannot be directly correlated with geographic distance between populations.

Discussion

The results of previous population census data (Mills et al. 1993) indicated that *D. bugensis* experienced a gradual diffusion from its initial point of introduction into Lake Erie. In contrast, the present population genetic study suggests that long-distance dispersal has also had a significant impact, maintaining gene flow between geographically disjunct populations. While significant differences were observed between many of the populations, tests of population subdivision and genotypic differentiation suggest that simple diffusion is not the sole factor underlying population structuring of the quagga mussel in the Laurentian Great Lakes. These empirical data support simulation study (Ibrahim et al. 1996), stressing the fact that the significance of long-distance dispersal must be taken into account in future theoretical estimates of species spread.

Descriptive statistics

The high number of alleles observed at five of six microsatellite loci (average allele number = 27.8 ± 7.5 alleles) allows the use of more powerful statistical tests for population structure than is possible using allozyme markers (Estoup et al. 1998). However, the overall low frequency of some of these alleles may confound analyses of population structure that place a higher significance on rare alleles (such as the private alleles method, see Slatkin 1985). While 14 putative private alleles were identified (Appendix), they are randomly distributed throughout the eight study populations and, given the low frequency at which they occur, may be present in one or more additional populations at a similar low frequency. In an effort to minimize bias associated with statistical estimators, Fisher's exact test for population differentiation and *F* statistics were chosen to estimate population structure (see Ruzzante 1998).

Distribution of alleles at each of the loci indicates that mutational modes at few, if any, of the loci fit strict stepwise mutation models. As the quagga mussel has only been present in the Laurentian Great Lakes for 8 years, the likelihood that de novo mutations have become established in North American populations appears unlikely. As the present-day allelic distribution in North American populations of *D. bugensis* may also be biased by historical founder effects, the use of F_{ST}, a more conservative estimator based on the infinite alleles model, is more appropriate than statistics that consider allelic size.

Deviation from Hardy–Weinberg equilibrium

All six loci exhibit significant heterozygote deficits, suggesting that nonrandom mating may be occurring in North American populations of *D. bugensis*. While outlying microsatellite loci may often exhibit significant heterozygote deficiencies due to differential amplification and (or) null alleles, the congruent pattern of heterozygote deficiency observed across all six loci demands a biological explanation. Three hypotheses relating to the life history of this species may help to explain this deviation. Firstly, *D.bugensis* is a

Table 3. Pairwise linearized F_{ST} estimates ($F_{ST}/(1 - F_{ST})$) (lower left) calculated by Arlequin V1.1 (Schneider et al. 1997) and distance between populations (km) (upper right) for eight study sites.

Population	CE	LPI	LPII	EE	WO	RC	EO	SF
CE	—	142	153	155	239	347	474	685
LPI	0.0059*	—	11	37	95	208	333	544
LPII	0.0089*	0.0116*	—	36	87	197	323	533
EE	0.0033	0.0150*	0.0146*	—	98	192	322	532
WO	0.0096*	0.0186*	0.0210*	0.0089*	—	115	236	446
RC	0.0097*	0.0134*	0.0117*	0.0127*	0.0189*	—	130	347
EO	0.0230*	0.0345*	0.0363*	0.0166*	0.0151*	0.0284*	—	211
SF	0.0113*	0.0305*	0.0230*	0.0058*	0.0105*	0.0182*	0.0172*	—

Note: Asterisks indicate that populations are significantly different (permutation test with sequential Bonferroni correction (Rice 1989), $P < 0.05$).

Fig. 4. Linearized F_{ST} ($F_{ST}/(1 - F_{ST})$) calculated by Arlequin V1.1 (Schneider et al. 1997) versus absolute linear distance from central Erie for each of six most westernly *D. bugensis* study populations.

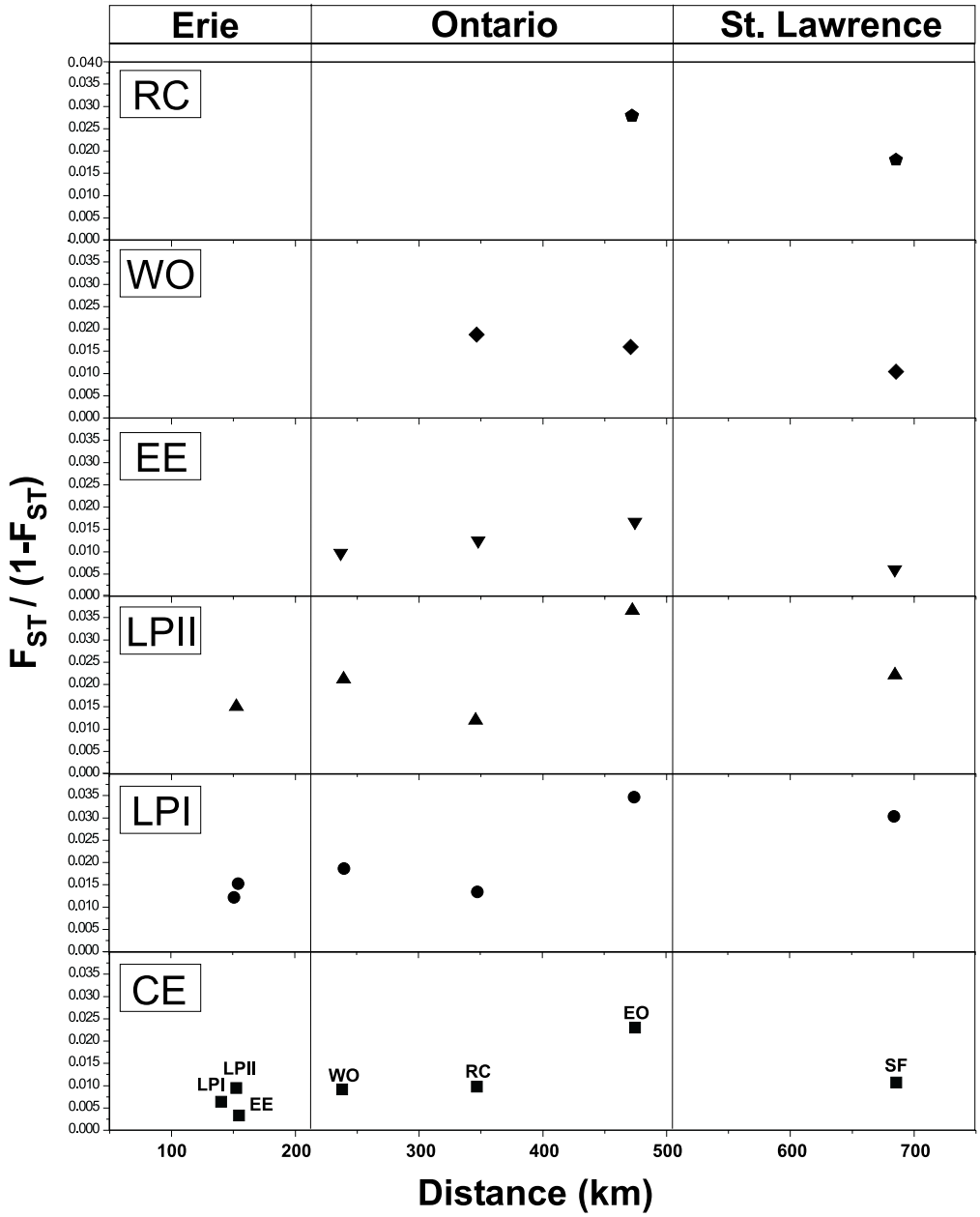


Table 4. AMOVA calculated by Arlequin V1.1 (Schneider et al. 1997).

Source of variation	df	Variance component	Percentage of variation
Among groups	1	0.00950 Va	0.39*
Among populations within groups	6	0.02526 Vb	1.03*
Among individuals within populations	424	0.86084 Vc	35.12*
Within individuals	432	1.55556 Vd	63.46*

Note: Populations were divided into two groups on the basis of location in relation to the putative point of introduction (Mills et al. 1993). Group 1: CE, LPBI, LPBII, EE; group 2: WO, RC, EO, SF. An asterisk indicates that the variance component is significant (permutation test, $P < 0.05$).

planktonic disperser. Thus, several cohorts of larvae that were spawned at different locations may settle at the same site. A genetic study of a single site, comprising several cohorts, could produce results that deviate from expected Hardy–Weinberg proportions, the homozygote excess known as the Wahlund effect (Hartl and Clark 1989).

A second explanation for the observed heterozygote deficiency is that clumps of adult mussels collected from the same site may often be half or full siblings produced by relatively few adults. Li and Hedgecock (1998) found differences in the genetic composition of oyster larvae within a single bay over an 11-day period that they attributed to temporal changes in the genetic composition of the adult population that produced the larvae. Their research highlights the fact that, as bivalves are capable of producing millions of larvae, only a few reproductive individuals may be needed to repopulate a large area.

Thirdly, heterozygote deficiencies among adult populations may arise due to selective pressures. Similarly high heterozygote deficiencies have been observed in adult populations of marine invertebrates with planktonic larvae (Singh and Green 1984; Beaumont 1991). Singh and Green (1984) hypothesized that heterozygous larvae may suffer greater mortality due to increased food requirements associated with higher growth rates, which could lead to an excess of homozygotes upon settlement. However, Haag and Garton (1995) found little support for this hypothesis in an examination of variation in allelic frequencies at a single allozyme locus during different life history stages of *D. polymorpha*. These data support the first hypothesis put forward here, that the high heterozygote deficiency that we observed at *D. bugensis* microsatellite loci may be more reasonably attributed to population-level effects than to differential mortality of larvae.

Because peripheral populations are often founded by rare, long-distance migrants, they may tend to show high levels of inbreeding within populations (Ibrahim et al. 1996). However, we did not observe the predicted pattern. No consistent population-level trends in inbreeding coefficient (F_{IS}) were observed across all six loci (Fig. 3). This may be due to the relatively continuous distribution of *D. bugensis* in the lower Great Lakes. While several peripheral populations may have originally been founded by low numbers of individuals, they may no longer be considered isolated given the present-day continuous distribution of the species in the lower Great Lakes.

High population differentiation

The F_{ST} values for *D. bugensis* ranged from 0.0033 to 0.0363 (mean = 0.016, SD = 0.0084) (Table 3). While population genetic data are limited for planktonic dispersers in freshwater systems, allozyme study has demonstrated comparable estimates of F_{ST} (in the range of 0.004–0.015) over a similar geographic range for marine species with extended planktotrophic development (Sarver and Foltz 1993; Holborn et al. 1994).

Both F_{ST} estimates and tests of population differentiation consistently demonstrate significant differences between 18 of 28 pairwise population comparisons. This high level of population differentiation does not correlate with any simple geographic relationship between populations, suggesting a complex pattern of interpopulation gene flow.

Deviations from isolation-by-distance and AMOVA

While the isolation-by-distance model is a useful tool for illuminating simple relationships between population structure and geography, it is limited in that it may be invalidated by as little as a single pairwise comparison that deviates from expectations (Rousset 1997). In this study, several geographically disjunct populations exhibit minimal population differentiation. While these major deviations from the isolation-by-distance model contribute to the poor overall fit of the genetic data to geography, a plot of pairwise F_{ST} versus absolute distance reveals a consistently higher level of gene flow between the Lake St. Francis site and all other populations than that that is expected under isolation-by-distance (Fig. 4). This congruent pattern adds additional strength to the hypothesis of long-distance dispersal of *D. bugensis*. The large positive *y*-intercept of the isolation-by-distance regression line also indicates a deviation from a normally distributed pattern of dispersal. We hypothesize that a pattern of leptokurtic dispersal (Ibrahim et al. 1996), involving a combination of rare long-distance dispersal and diffusive settlement of veliger larvae, has maintained high gene flow over long distances and contributed to a poor overall fit to the isolation-by-distance model.

The absence of any pattern of isolation-by-distance in *D. bugensis* over long distances in the present study may be due to the recent colonization of the Laurentian Great Lakes in addition to a deviation from a normally distributed pattern of dispersal. However, even when a group of populations are not at equilibrium, isolation-by-distance should remain detectable over short distances (Slatkin 1993). In the present study, no strong pattern of isolation-by-distance can be detected even over short within-lake comparisons (Fig. 4), providing additional evidence to suggest that long-distance dispersal has played a role in deviations from isolation-by-distance.

Although the AMOVA is more conservative than the isolation-by-distance test, it too indicates that the majority of the observed genetic variation cannot be explained on the basis of geography. The results of both of these tests indicate a more complex model of mussel dispersal than the diffusive radiation expected from survey data.

Significant local genetic subdivision

Studies of marine species with an extended larval stage have revealed genetic uniformity across wide distances but high levels of local genetic subdivision (Parsons 1996). Sim-

ilarly, while several disjunct populations have a similar allelic distribution and low F_{ST} in the present study, other more proximate sites, including two Long Point Bay populations (LPI and LPII, separated by only 11 km), are significantly different. These data suggest that while long-distance dissemination has had an impact on the population structure of the quagga mussel in North America, factors operating on a microgeographic level may contribute to significant genetic differences over short distances. Unfortunately, ecological study of dreissenid dispersal has been hampered by limitations in our ability to directly track larval dispersal. It is unclear how far mussel larvae may disperse in lacustrine environments and if dispersing larvae aggregate, as has been demonstrated in other bivalve species (Andrews 1979), factors that may also have a significant impact on population structuring. A recent study that investigated larval dispersal in a riverine metapopulation of *D. polymorpha* (Stoeckel et al. 1997) estimated that larvae may travel more than 300 km before settlement. While current flows vary significantly between riverine and lacustrine environments, these data may help to explain the differences observed between neighbouring populations in the present study, as larvae may travel large distances before settlement. A more detailed understanding of larval dynamics in lakes is essential to clarify the significance of random and nonrandom processes in the spread of dreissenid species.

Habitat shift: impact on future distributional modes

While the established range of the quagga mussel remains much more restricted than that of the zebra mussel, adult quagga mussels have recently been found in the Mississippi (Mills et al. 1996), Ohio (Brence and Miller 1994), and Rideau rivers (A. Martel, Canadian Museum of Nature, Ottawa, Ont., personal communication). The increased number of sightings of the quagga mussel outside the lower Great Lakes suggests an increasing dissemination of *D. bugensis*.

Dreissena bugensis was first identified in the offshore zone of Lake Erie (Mills et al. 1993) and became established quickly, presumably due to a lack of competition from the zebra mussel. While populations of *D. bugensis* grew to dominate the offshore zone (Mills et al. 1993), mussels from these areas were presumably much less likely to come into contact with boat traffic than individuals from zebra mussel populations in the nearshore zone. Boater-mediated transport of *D. polymorpha* may help to explain the more widespread distribution of the zebra mussel in North America.

Claxton (1998) and Mills et al. (1998) have suggested that *D. bugensis* may currently be displacing nearshore zebra mussels in eastern Lake Erie and on the south shore of Lake Ontario, perhaps due to a faster growth rate of the quagga mussel under resource-limited conditions (Mills et al. 1998). This pattern of competitive exclusion has also been described in eastern Europe, where *D. bugensis* has come to dominate populations of *Dreissena* at nearshore sites in several Ukrainian reservoirs (Mills et al. 1996). With the movement of quagga mussel populations into the nearshore zone, they are now much more likely to come in contact with local boat traffic. This hypothesis is consistent with recent mussel sightings (see above) and the results of the present study, which indicate high levels of gene flow between a number of disjunct populations. Although the quagga mussel may be limited by environmental factors or competitive exclusion by established zebra mussel populations, these results show that widespread dissemination of *D. bugensis* is not only possible but is presently occurring on a large scale.

Conclusions

The results of this study suggest that long-distance dispersal, possibly mediated by boater movement patterns, may be having a significant impact on quagga mussel dispersal patterns. The increased number of sightings of quagga mussels outside their established range, correlated with the recent movement of *D. bugensis* into the nearshore zone, may indicate an increased potential for the establishment of new quagga mussel populations. In contrast with these results, significant genetic differences were identified between populations separated by short distances, suggesting that microgeographic factors governing larval settlement and spread may play a role in limiting gene flow between populations.

Persistent founder effects often associated with range expansions may lead to a positive bias in genetic differentiation and a gradual decrease in within-population heterozygosity (Le Corre and Kremer 1998). These genetic consequences may be more pronounced under geographically limited dispersal than under an island model. Thus, while the present study offers an excellent snapshot view of quagga mussel population genetic structure, future work, including temporal as well as spatial variation, will help to reveal whether the present data form part of a persistent pattern of population subdivision or represent a transitory state as the system moves towards equilibrium.

As the zebra mussel became established in the Laurentian Great Lakes several years before the quagga mussel, concurrent molecular study on *D. polymorpha* (K.-A. Naish, unpublished data) may also provide an "eye to the future," illuminating what might be expected from the quagga mussel in the next 5–10 years. A comparison of quagga and zebra mussel data may help clarify large-scale patterns that have lead to the success of these molluscan invaders.

Acknowledgements

We are grateful to P.D.N. Hebert, G.L. Mackie, T.J. Crease, and three anonymous reviewers for their constructive comments on earlier drafts of this manuscript. Special thanks to W.G. Sprules, the captain and crew of the *Limnos*, and Y. Delafontaine and B. Cusson at Environment Canada in Montreal for assistance with collections. This research was funded in part by a Natural Sciences and Engineering Research Council of Canada collaborative research grant to E.G.B., P.D.N. Hebert, W.G. Sprules, and P. Yodzis and by a Natural Sciences and Engineering Research Council of Canada research grant to E.G.B. A.B.W. was partially supported by an Ontario graduate scholarship.

References

Andrews, J.D. 1979. Pelecypoda: Osteridae. *In* Reproduction of marine invertebrates. *Edited by* A.C. Giese and J.S. Pearce. Academic Press, Inc., New York.

Beaumont, A.R. 1991. Genetic studies of laboratory reared mussels, *Mytlius edulis*: heterozygote deficiencies, heterozygosity, and growth. Biol. J. Linn. Soc. **44**: 273–285.

Brence, A., and Miller, M. 1994. Quagga found in the Ohio River. *In* Zebra mussel update #21. *Edited by* C. Kraft. Gopher: //gopher.adp.wisc.edu:80/.browse/.METASGIZM/.data/.00000137.

Carlton, J.T. 1993. Dispersal mechanisms of the zebra mussel (Dreissena polymorpha). *In* Zebra mussels: biology, impacts, and control. Edited by T.F. Nalepa and D. Schloesser. Lewis Publishers, Boca Raton, Fla. pp. 677–697.

Claxton, W.T. 1998. Molecular systematics and ecology of deep and shallow water populations of *Dreissena polymorpha* and *Dreissena bugensis*. Ph.D. thesis, University of Guelph, Guelph, Ont.

Estoup, A., Rousset, F., Michalakis, Y., Cornuet, J.-M., Adriamanga, M., and Guyomard, R. 1998. Comparative analysis of microsatellite and allozyme markers: a case study investigating microgeographic differentation in brown trout (*Salmo trutta*). Mol. Ecol. **7**: 339–353.

Excoffier, L., Smouse, P.E., and Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics, **131**: 479–491.

Goudet, J., Raymond, M., de Meeus, T., and Rousset, F. 1996. Testing differentiation in diploid populations. Genetics, **144**: 1933–1940.

Haag, W.R., and Garton, D.W. 1995. Variation in genotype frequencies during the life cycle of the bivalve, *Dreissena polymorpha*. Evolution, **49**: 1284–1288.

Hartl, D.L., and Clark, A.G. 1989. Principles of population genetics. Sinauer Associates, Inc., Sunderland, Mass.

Hastings, A. 1996. Models of spatial spread: is the theory complete? Ecology, **77**: 1675–1679.

Hebert, P.D.N., Muncaster, B.W., and Mackie, G.L. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusc in the Great Lakes region. Can. J. Fish. Aquat. Sci. **46**: 1587–1591.

Holborn, K., Johnson, M.S., and Black, R. 1994. Population genetics of the corallivorous gastropod *Drupella cornus* at Ningaloo Reef, Western Australia. Coral Reefs, **13**: 33–39.

Ibrahim, K., Nichols, R.A., and Hewitt, G.M. 1996. Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. Heredity, **77**: 282–291.

Johnson, L.E., and Carlton, J.T. 1996. Post establishment spread in large-scale invasions: dispersal mechanisms of the zebra mussel *Dreissena polymorpha*. Ecology, **77**: 1686–1690.

Le Corre, V., and Kremer, A. 1998. Cumulative effects of founding events during colonisation on genetic diversity and differentiation in an island and stepping-stone model. J. Evol. Biol. **11**: 495–512.

Li, G., and Hedgecock, D. 1998. Genetic heterogeneity, detected by PCR-SSCP, among samples of larval Pacific oysters (*Crassostrea gigas*) supports the hypothesis of large variance in reproductive success. Can. J. Fish. Aquat. Sci. **55**: 1025–1033.

May, B., and Marsden, J.E. 1992. Genetic identification and implications of another invasive species of dreissenid mussel in the Great Lakes. Can. J. Fish. Aquat. Sci. **49**: 1501–1506.

Mills, E.L., Dermott, R.M., Roseman, E.F., Dustin, D., Mellina, E., Conn, D.B., and Spidle, A.P. 1993. Colonization, ecology, and population structure of the "quagga" mussel (Bivalvia: Dreissenidae) in the lower Great Lakes. Can. J. Fish. Aquat. Sci. **50**: 2305–2314.

Mills, E.L., Rosenberg, G., Spidle, A.P., Ludyanskiy, M., Pligin, Y., and May, M. 1996. A review of the biology and ecology of the quagga mussel (*Dreissena bugensis*), a second species of freshwater dreissenid introduced to North America. Am. Zool. **36**: 271–286.

Mills, E.L., Chrisman, J.R., Baldwin, B.S., Owens, R.W., O'Gorman, R., Howell, T., Roseman, E.F., and Raths, M.K. 1998. Recent changes in the dreissenid community and a shift toward the quagga mussel in Lake Ontario. 41st Conference of the International Assocation for Great Lakes Research, Hamilton, Ont., May 1998. International Assocation for Great Lakes Research, Ann Arbor, Mich. p. 118. (Abstr.)

Palumbi, S.R. 1995. Using genetics as an indirect estimator of larval dispersal. *In* Ecology of marine invertebrate larvae. *Edited by* L.R. McEdward. CRC Press, Inc., Boca Raton, Fla. pp. 369–387.

Parsons, K.E. 1996. The genetic effects of larval dispersal depend on spatial scale and habitat characteristics. Mar. Biol. **126**: 403–414.

Raymond, M., and Rousset, F. 1995*a*. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. **83**: 239.

Raymond, M., and Rousset, F. 1995*b*. An exact test for population differentiation. Evolution, **49**: 1280–1283.

Ricciardi, A., Serrouya, R., and Whoriskey, F.G. 1995. Aerial exposure tolerance of zebra and quagga mussels (Bivalvia: Dreissenidae): implications for overland dispersal. Can. J. Fish. Aquat. Sci. **52**: 470–477.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution, **43**: 223–225.

Rousset, F. 1997. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. Genetics, **145**: 1219–1228.

Ruzzante, D.E. 1998. A comparison of several measures of genetic distance and population structure with microsatellite data: bias and sampling variance. Can. J. Fish. Aquat. Sci. **55**: 1–14.

Sarver, S.K., and Foltz, D.W. 1993. Genetic population structure of a species' complex of blue mussels (*Mytilus* spp.). Mar. Biol. **117**: 105–112.

Schneider, S., Kueffer, J.-M., Roessli, D., and Excoffier, L. 1997. Arlequin V1.1: a software for population genetic analysis. Genetics and Biometry Laboratory, University of Geneva, Geneva, Switzerland.

Singh, S.M., and Green, R.H. 1984. Excess of allozyme homozygosity in marine molluscs and its possible biological significance. Malacologia, **25**: 569–581.

Slatkin, M. 1985. Rare alleles as indicators of gene flow. Evolution, **39**: 53–65.

Slatkin, M. 1993. Isolation by distance in equilibrium and nonequilibrium populations. Evolution, **47**: 264–279.

Sokal, R.R., and Rohlf, F.J. 1981. Biometry. 2nd ed. W.H. Freeman & Co., San Francisco, Calif.

Stoeckel, J.A., Schneider, D.W., Soeken, L.A., Blodgett, K.D., and Sparks, R.E. 1997. Larval dynamics of a riverine metapopulation: implications for zebra mussel recruitment, dispersal, and control in a large-river system. J. North Am. Benthol. Soc. **16**: 586–601.

U.S. Geological Survey. 1997. National zebra mussel and aquatic nuisance species clearinghouse: United States distribution maps. http://www.nfrcg.gov/zebra.mussel.

Weir, B.S., and Cockerham, C.C. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution, **38**: 1358–1370.

Wilson, A.B. 1998. Dispersal patterns of *Dreissena bugensis* in the Laurentian Great Lakes as inferred from highly polymorphic microsatellite markers. M.Sc. thesis, University of Guelph, Guelph, Ont.

Wilson, A.B., Boulding, E.G., and Naish, K.-A. 1999. Characterization of tri- and tetra-nucleotide microsatellite loci in the invasive mollusc, *Dreissena bugensis*. Mol. Ecol. **8**: 692–693.

Wright, S. 1946. Isolation by distance under diverse systems of mating. Genetics, **31**: 39–59.

Zar, J.H. 1984. Biostatistical analysis. 2nd ed. Prentice-Hall, Inc., Englewood Cliffs, N.J.

Appendix. Allele frequency data for *D. bugensis* microsatellite data.

Population	Allele											
Locus: *Dbug*1	1	2	3	4	5	6	7	8	9	10	11	12
CE-54	0.000	0.139	0.000	0.009	0.019	0.176	0.000	0.074	0.028	0.000	0.111	0.102
EE-54	0.000	0.102	0.000	0.000	0.083	0.093	0.019	0.074	0.028	0.019	0.167	0.056
LPI-54	0.000	0.140	0.010	0.000	0.030	0.100	0.030	0.100	0.020	0.000	0.060	0.080
LPII-54	0.009	0.104	0.000	0.028	0.179	0.113	0.057	0.038	0.085	0.000	0.000	0.019
WO-54	0.000	0.127	0.000	0.000	0.029	0.069	0.010	0.098	0.029	0.000	0.049	0.196
RC-54	0.000	0.132	0.000	0.047	0.132	0.047	0.057	0.085	0.123	0.009	0.019	0.009
EO-54	0.028	0.075	0.019	0.000	0.019	0.094	0.000	0.113	0.019	0.000	0.057	0.066
SF-54	0.000	0.125	0.000	0.000	0.115	0.038	0.000	0.067	0.058	0.000	0.067	0.125
	23	24	25	26	27	28	29	30	31	32	33	34
CE-54	0.000	0.000	0.037	0.019	0.028	0.000	0.000	0.009	0.037	0.028	0.028	0.009
EE-54	0.000	0.000	0.009	0.028	0.037	0.019	0.037	0.000	0.000	0.046	0.019	0.009
LPI-54	0.000	0.020	0.010	0.030	0.010	0.020	0.040	0.010	0.010	0.010	0.050	0.030
LPII-54	0.019	0.028	0.000	0.000	0.000	0.028	0.000	0.066	0.028	0.057	0.000	0.000
WO-54	0.010	0.000	0.000	0.029	0.039	0.010	0.000	0.029	0.059	0.059	0.020	0.039
RC-54	0.019	0.000	0.009	0.009	0.000	0.009	0.000	0.028	0.028	0.094	0.000	0.038
EO-54	0.000	0.000	0.038	0.019	0.009	0.009	0.019	0.000	0.028	0.085	0.009	0.047
SF-54	0.000	0.000	0.019	0.029	0.087	0.000	0.000	0.010	0.019	0.029	0.058	0.029
Locus: *Dbug*2	1	2	3	4	5	6	7	8	9	10	11	12
CE-54	0.028	0.083	0.176	0.000	0.046	0.009	0.046	0.046	0.000	0.000	0.019	0.037
EE-54	0.019	0.047	0.132	0.000	0.057	0.009	0.066	0.057	0.000	0.019	0.019	0.009
LPI-54	0.000	0.077	0.183	0.010	0.058	0.058	0.029	0.010	0.010	0.010	0.000	0.010
LPII-54	0.000	0.074	0.130	0.009	0.019	0.000	0.065	0.037	0.028	0.028	0.019	0.028
WO-54	0.000	0.077	0.096	0.000	0.077	0.010	0.058	0.077	0.010	0.019	0.029	0.029
RC-54	0.000	0.070	0.190	0.000	0.040	0.020	0.070	0.000	0.000	0.050	0.080	0.000
EO-54	0.000	0.080	0.200	0.000	0.010	0.000	0.090	0.050	0.000	0.010	0.060	0.050
SF-54	0.000	0.108	0.049	0.010	0.059	0.010	0.098	0.020	0.000	0.020	0.029	0.029
	23	24	25	26	27	28	29	30	31	32	33	34
CE-54	0.000	0.009	0.009	0.000	0.046	0.046	0.046	0.028	0.028	0.037	0.000	0.000
EE-54	0.000	0.028	0.047	0.019	0.038	0.038	0.057	0.000	0.047	0.019	0.028	0.000
LPI-54	0.000	0.010	0.010	0.019	0.000	0.077	0.087	0.077	0.038	0.029	0.000	0.010
LPII-54	0.009	0.000	0.019	0.019	0.009	0.046	0.083	0.028	0.046	0.009	0.000	0.000
WO-54	0.000	0.000	0.038	0.058	0.010	0.029	0.010	0.115	0.038	0.010	0.010	0.010
RC-54	0.000	0.010	0.020	0.000	0.010	0.040	0.070	0.050	0.030	0.010	0.000	0.010
EO-54	0.000	0.040	0.020	0.030	0.000	0.050	0.050	0.010	0.060	0.040	0.000	0.000
SF-54	0.000	0.020	0.069	0.000	0.020	0.059	0.069	0.029	0.010	0.010	0.010	0.000
Locus: *Dbug*3	1	2	3	4	5	6	7	8	9	10	11	12
CE-54	0.000	0.037	0.000	0.139	0.000	0.019	0.046	0.009	0.037	0.074	0.019	0.019
EE-54	0.000	0.009	0.000	0.113	0.000	0.047	0.028	0.000	0.028	0.113	0.009	0.038
LPI-54	0.000	0.028	0.000	0.226	0.000	0.009	0.009	0.038	0.009	0.085	0.057	0.019
LPII-54	0.010	0.087	0.010	0.077	0.010	0.048	0.019	0.019	0.019	0.038	0.019	0.029
WO-54	0.019	0.056	0.000	0.278	0.000	0.056	0.065	0.028	0.009	0.093	0.000	0.019
RC-54	0.000	0.046	0.000	0.222	0.000	0.093	0.028	0.000	0.000	0.037	0.019	0.009
EO-54	0.000	0.019	0.000	0.208	0.000	0.019	0.047	0.009	0.000	0.047	0.057	0.000
SF-54	0.000	0.074	0.009	0.167	0.000	0.056	0.019	0.009	0.000	0.065	0.028	0.037
	23	24	25	26	27	28	29	30	Genes			
CE-54	0.000	0.028	0.074	0.000	0.083	0.019	0.000	0.009	108			
EE-54	0.000	0.038	0.085	0.000	0.057	0.000	0.000	0.019	106			
LPI-54	0.000	0.000	0.057	0.000	0.123	0.000	0.028	0.000	106			
LPII-54	0.010	0.038	0.038	0.000	0.144	0.010	0.029	0.000	104			
WO-54	0.009	0.000	0.046	0.000	0.074	0.019	0.009	0.000	108			
RC-54	0.009	0.093	0.019	0.009	0.093	0.000	0.009	0.009	108			
EO-54	0.000	0.028	0.094	0.000	0.189	0.009	0.000	0.000	106			
SF-54	0.000	0.009	0.028	0.009	0.130	0.009	0.000	0.000	108			

Appendix (*continued*).

Allele										
13	14	15	16	17	18	19	20	21	22	
0.009	0.000	0.009	0.009	0.009	0.000	0.000	0.009	0.000	0.000	
0.019	0.019	0.000	0.000	0.000	0.009	0.019	0.009	0.000	0.009	
0.040	0.000	0.010	0.000	0.010	0.000	0.000	0.020	0.000	0.020	
0.009	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.009	
0.029	0.010	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.010	
0.009	0.000	0.028	0.009	0.000	0.019	0.000	0.000	0.009	0.000	
0.057	0.028	0.028	0.000	0.000	0.000	0.000	0.000	0.028	0.000	
0.010	0.019	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000	
35	36	37	38	39	40	41	42	43	44	Genes
0.019	0.019	0.000	0.000	0.009	0.037	0.009	0.009	0.000	0.000	108
0.028	0.009	0.000	0.000	0.000	0.028	0.000	0.000	0.009	0.000	108
0.040	0.030	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	100
0.019	0.038	0.000	0.019	0.009	0.000	0.000	0.000	0.000	0.000	106
0.010	0.010	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	102
0.000	0.019	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	106
0.019	0.000	0.038	0.009	0.009	0.028	0.000	0.000	0.000	0.000	106
0.019	0.010	0.000	0.010	0.019	0.000	0.000	0.000	0.010	0.010	104
13	14	15	16	17	18	19	20	21	22	
0.093	0.037	0.019	0.009	0.000	0.037	0.037	0.000	0.000	0.028	
0.085	0.057	0.028	0.019	0.009	0.019	0.019	0.009	0.000	0.000	
0.019	0.048	0.010	0.019	0.019	0.048	0.000	0.000	0.000	0.029	
0.046	0.111	0.037	0.009	0.000	0.037	0.009	0.028	0.009	0.009	
0.019	0.000	0.048	0.029	0.000	0.019	0.038	0.000	0.000	0.000	
0.070	0.020	0.020	0.030	0.010	0.030	0.020	0.000	0.020	0.000	
0.030	0.060	0.040	0.000	0.000	0.010	0.000	0.000	0.000	0.000	
0.049	0.088	0.059	0.029	0.000	0.020	0.000	0.000	0.000	0.020	
35	36	Genes								
0.000	0.000	108								
0.000	0.000	106								
0.000	0.000	104								
0.000	0.000	108								
0.000	0.038	104								
0.010	0.000	100								
0.000	0.010	100								
0.000	0.010	102								
13	14	15	16	17	18	19	20	21	22	
0.093	0.074	0.019	0.056	0.037	0.056	0.000	0.009	0.028	0.019	
0.038	0.057	0.038	0.047	0.009	0.075	0.009	0.009	0.057	0.075	
0.047	0.066	0.019	0.047	0.000	0.047	0.000	0.000	0.038	0.047	
0.048	0.019	0.048	0.029	0.029	0.048	0.029	0.010	0.029	0.058	
0.009	0.028	0.009	0.028	0.037	0.056	0.009	0.000	0.028	0.019	
0.065	0.037	0.009	0.037	0.028	0.028	0.028	0.019	0.037	0.019	
0.047	0.009	0.009	0.047	0.019	0.038	0.009	0.000	0.038	0.057	
0.065	0.056	0.000	0.102	0.028	0.037	0.009	0.000	0.019	0.037	

Appendix (*concluded*).

Population	Allele											
Locus: *Dbug*4	1	2	3	4	5	6	7	8	9	10	11	12
CE-54	0.046	0.222	0.093	0.194	0.028	0.000	0.009	0.009	0.000	0.000	0.000	0.000
EE-54	0.019	0.264	0.000	0.245	0.009	0.019	0.000	0.000	0.009	0.000	0.000	0.000
LPI-54	0.019	0.415	0.009	0.217	0.028	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LPII-54	0.073	0.208	0.031	0.208	0.052	0.000	0.010	0.000	0.000	0.000	0.000	0.000
WO-54	0.148	0.194	0.000	0.296	0.009	0.000	0.000	0.000	0.000	0.000	0.028	0.000
RC-54	0.058	0.298	0.000	0.154	0.000	0.000	0.000	0.029	0.000	0.029	0.000	0.010
EO-54	0.085	0.198	0.000	0.481	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000
SF-54	0.037	0.194	0.093	0.213	0.037	0.000	0.009	0.000	0.000	0.000	0.000	0.000
	23	24	25	26	Genes							
CE-54	0.000	0.019	0.056	0.037	108							
EE-54	0.038	0.038	0.085	0.009	106							
LPI-54	0.028	0.000	0.009	0.028	106							
LPII-54	0.021	0.010	0.031	0.000	96							
WO-54	0.019	0.000	0.037	0.000	108							
RC-54	0.000	0.019	0.048	0.000	104							
EO-54	0.019	0.009	0.009	0.000	106							
SF-54	0.000	0.000	0.083	0.019	108							
Locus: *Dbug*5	1	2	3	4	5	6	7	8	9	10	11	12
CE-54	0.000	0.041	0.020	0.000	0.051	0.153	0.010	0.143	0.071	0.143	0.051	0.010
EE-54	0.010	0.000	0.010	0.030	0.080	0.130	0.000	0.230	0.100	0.070	0.020	0.020
LPI-54	0.021	0.000	0.074	0.011	0.064	0.160	0.000	0.138	0.011	0.213	0.000	0.021
LPII-54	0.034	0.034	0.102	0.045	0.114	0.068	0.000	0.182	0.000	0.125	0.091	0.000
WO-54	0.011	0.000	0.033	0.011	0.087	0.098	0.000	0.217	0.033	0.196	0.065	0.022
RC-54	0.010	0.000	0.070	0.020	0.190	0.080	0.010	0.080	0.030	0.080	0.020	0.020
SF-54	0.000	0.010	0.051	0.031	0.020	0.092	0.010	0.143	0.112	0.061	0.112	0.010
	23	24	25	26	27	Genes						
CE-54	0.020	0.051	0.020	0.010	0.000	98						
EE-54	0.010	0.020	0.000	0.000	0.000	100						
LPI-54	0.011	0.021	0.000	0.000	0.032	94						
LPII-54	0.000	0.000	0.011	0.000	0.000	88						
WO-54	0.000	0.022	0.000	0.000	0.000	92						
RC-54	0.023	0.000	0.011	0.000	0.000	88						
EO-54	0.000	0.030	0.000	0.000	0.000	100						
SF-54	0.010	0.000	0.020	0.000	0.000	98						
Locus: *Dbug*6	1	2	3	4	Genes							
CO-54	0.148	0.148	0.343	0.361	108							
EE-54	0.194	0.102	0.435	0.269	108							
LPI-54	0.167	0.093	0.472	0.269	108							
LPII-54	0.093	0.130	0.287	0.491	108							
WO-54	0.102	0.083	0.315	0.500	108							
RC-54	0.296	0.056	0.278	0.370	108							
EO-54	0.194	0.083	0.139	0.583	108							
SF-54	0.102	0.157	0.167	0.574	108							

Appendix (*concluded*).

Allele									
13	14	15	16	17	18	19	20	21	22
0.000	0.000	0.009	0.037	0.028	0.046	0.130	0.028	0.009	0.000
0.000	0.000	0.019	0.000	0.000	0.085	0.047	0.047	0.009	0.057
0.000	0.000	0.038	0.000	0.038	0.019	0.075	0.028	0.000	0.047
0.000	0.000	0.042	0.000	0.083	0.104	0.031	0.042	0.000	0.052
0.009	0.000	0.019	0.000	0.037	0.037	0.056	0.074	0.000	0.037
0.000	0.019	0.019	0.000	0.058	0.144	0.096	0.010	0.000	0.010
0.000	0.000	0.019	0.000	0.038	0.009	0.038	0.019	0.000	0.057
0.009	0.019	0.009	0.000	0.056	0.065	0.056	0.037	0.009	0.056

13	14	15	16	17	18	19	20	21	22
0.020	0.010	0.010	0.020	0.041	0.041	0.010	0.000	0.031	0.020
0.060	0.030	0.000	0.060	0.040	0.020	0.000	0.020	0.000	0.040
0.096	0.000	0.000	0.000	0.032	0.021	0.021	0.000	0.021	0.032
0.011	0.045	0.000	0.000	0.057	0.000	0.045	0.000	0.011	0.023
0.011	0.098	0.000	0.033	0.000	0.011	0.033	0.000	0.022	0.000
0.050	0.040	0.000	0.080	0.080	0.040	0.020	0.000	0.000	0.050
0.010	0.041	0.000	0.092	0.010	0.051	0.051	0.041	0.000	0.020