RICHARD J. NEVES

Biological Journal of the Linnean Society (1984), *22:* 157–185. With 5 figures

Molecular genetics of peripheral populations of Nova Scotian Unionidae (Mollusca: Bivalvia)

PIETER W. KAT*

Department of Earth and Planetary Sciences,
The Johns Hopkins University, Baltimore, Maryland 21218, U.S.A.

AND

GEORGE M. DAVIS

Department of Malacology, The Academy of Natural Sciences of Philadelphia,
19th and The Parkway, Philadelphia, Pennsylvania 19103, U.S.A.

Peripheral populations of eight species of freshwater bivalves (Unionidae) extending their geographic ranges into Nova Scotia, Canada, were examined electrophoretically to determine both the extent of genetic variability within such populations, and whether the hypothesized pathway of colonization across the Isthmus of Chignecto is reflected in patterns of genetic resemblance among these populations. The Nova Scotian species examined could be separated into two groups based on levels of observed heterozygosity and levels of variability in allele frequencies.

The first group is characterized by low levels of heterozygosity and polymorphism compared with north-eastern American populations, and in the case of one species, *Elliptio complanata*, considerable variability in allele frequencies among populations occurring in similar habitats in different drainages. Populations of *E. complanata* from Nova Scotia can be differentiated from conspecific populations on the southern Atlantic Slope by possession of fast alleles at two loci. Multivariate analyses define subgroups within populations of *E. complanata* consistent with hypothesis that the species invaded Nova Scotia by way of the Isthmus of Chignecto, and then split into two groups, one of which colonized Cape Breton to the north and the other of which colonized southern areas of the Province.

The second group of Nova Scotian species is characterized by little reduction in heterozygosity and polymorphism compared with values observed among north-eastern American conspecifics or congeners, little variability in allele frequencies from population to population, and little evidence to suggest that these species were dependent on the land bridge to invade the Province.

The type of dispersal is hypothesized to be responsible, in part, for these differences: larvae of species in the first group rely on a parasitic attachment to fish with territorial habits limited to fresh water, and are thus likely to invade new drainages separated by salt water by chance, in small numbers, and in stepping-stone fashion. Species in the second group parasitize anadromous or saltwater tolerant hosts, are likely to be introduced into new habitats in greater numbers and/or receive greater amounts of gene flow subsequent to colonization, and seem less dependent on land-bridges to colonize new habitats.

KEY WORDS:—Bivalva – genetics – peripheral populations – dispersal.

*Present address: Department of Malacology, National Museums of Kenya, P.O. Box 40658, Nairobi, Kenya.

0024–4066/84/060157+29 $03.00/0

CONTENTS

INTRODUCTION

Novel genetic combinations that arise as a result of gene-frequency changes in peripheral populations of a species remain central to theories on trans-specific change (Templeton, 1980; Futuyma & Mayer, 1980; Jones, 1981). Nevertheless, naturally-occurring peripheral populations (as opposed to insular populations) remain rather poorly studied despite their potential for information on changes in the frequences of alleles, levels of homozygosity, and degree of phenotypic variability (Kat, 1982).

Periperal populations are defined here as those at the border of a species' expanding geographic range. During range expansions, new populations can become established by a small number of founder individuals, with concomitant changes in the levels of genetic variability observed in central populations. This reduction can be pronounced during rapid range expansions, and depends on such parameters as the vagility, dispersability, and mode of reproduction of the species involved, as well as the size of the bottleneck and rate of population growth subsequent to the founder event. Computer simulations have predicted that the conditions under which a founder population can be expected to exhibit reductions in heterozygosity as well as a reduction of the number of alternative alleles per locus are rather stringent (Nei, Maruyama & Chakraborty, 1975). These stringent conditions, as well as other factors such as uncertainity as to when and how the populations were established, and uncertainity as to how and from where the populations were dispersed, perhaps account for the contradictory results from the few investigations of similar peripheral populations, which either document little or no reduction in variability (Babbel & Selander, 1974; Gottlieb, 1974; Lewontin, 1974; Levin, 1977; Ehrlich & White, 1980), or significant reductions (Avise & Selander, 1972; Schwaegerle & Schaal, 1979; Fuller & Lester, 1980; Schumaker & Babbel, 1980; Hoagland, in press). In all these cases, the possibility that changes in allele frequencies or levels of heterozygosity were established by selective forces in possibly marginal habitats, or resulted as a consequence of population size under the neutralist theory (Kimura & Crow, 1964; Kimura & Ohta, 1971) cannot be ignored.

Consequently, the system and the species examined have to be selected with care. Freshwater bivalves of the family Unionidae are well suited for a study of peripheral populations for a number of reasons. Unionids are sessile as adults,

and rely on a short-term parasitic attachment to fish hosts for dispersal. Distribution of a species by fish hosts might be unrestricted within a drainage system, but restriction of hosts by saline or brackish water will limit dispersal between drainages (Ortmann, 1913; Van der Schalie, 1945; Johnson, 1970). Successful introduction of unionids into new habitats probably involves few individual hosts. The rate and probability of successful colonization from a refugium is likely to decrease as distances between drainages increase. Dispersal probably involves a stepping stone model as discussed by Sepkoski & Rex (1974). Introduction would either involve the geomorphic process of stream capture or host migration from river to river through brackish or saline water.

The effects of Wisconsinan glacial ice, which covered all of Canada and large sections of the United States, still influence Holocene distributions of unionids. Native unionid populations in the glaciated areas were in all probability eliminated, although pockets of unglaciated land could have served as refugia for cold-adapted species (Baker, 1928; Clarke, 1973). Most northern Atlantic Slope species, however, probably had their ranges restricted to refuges in the temperate climates south of the maximal glacial advance. Post-glacial meltwaters and the resulting confluence of drainages provided rapid means of dispersal up the Mississippi–Missouri river systems, and there is evidence that species of *Anodonta* and *Lampsilis* were present in previously glaciated regions of Ontario more than 10 000 years B.P. (Miller, Karrow & Kalas, 1979). In contrast, recolonization of the essentially parallel and unconnected Atlantic Slope drainages has been relatively slow, considering that areas such as New England could have been deglaciated as long as 12 500 years ago (Borns, 1973). Sepkoski & Rex (1974) have demonstrated that species richness decreases geometrically with distance from source areas, while Athearn & Clarke (1961) have shown that recolonization of northern sections of the Atlantic Slope is still in progress.

We hypothesize that unionid species dispersed northward from refugia south of New England as glaciation subsided, and that colonization of Nova Scotia resulted from taxa dispersing through New Brunswick across a narrow land bridge (the Isthmus of Chignecto), assuming that freshwater fish hosts have limited capabilities of traversing saltwater barriers. This dispersal would have occurred within the past 4000 years, since the land-bridge only recently emerged as a result of isostatic rebound. The purpose of this study is to determine whether patterns of genetic variation among unionid species found on Nova Scotia substantiate this hypothesis when compared to north-eastern American populations, congeneric species, and among themselves. Do populations spreading out from the Isthmus of Chignecto demonstrate founder effects such as one might expect of expanding or recently expanded peripheral populations? Or do populations demonstrate a variety of alternative patterns (e.g. little reduction in levels of heterozygosity and polymorphism compared with north-eastern American populations) which suggest that colonization of the Province occurred via alternative pathways?

METHODS

Taxa studied

The species studied are listed in Appendix 1. Localities, ANSP (Academy of Natural Sciences of Philadelphia) catalogue numbers, and numbers of

individuals electrophoresed are given. The number of populations studied within a species as well as the number of individuals examined was determined by the frequency of occurrence of the species in Nova Scotia: *Elliptio complanata* is widespread and very abundant, and is therefore well represented in the samples, while species such as *Alasmidonta undulata*, *Lampsilis cariosa*, and *L. ochracea* are known from rather few localities, and have small populations. Southern populations of *Elliptio complanata* from various locations along the northern Atlantic Slope used for comparative purposes were analysed by Davis *et al.* (1981) and by Kat (1983a) and are also listed in Appendix 1. The location of the sample sites, exclusive of those in Davis *et al.* (1981), is illustrated in Fig. 1.

All specimens were collected in Nova Scotia during August 1980. Within a species, collection localities were chosen on the basis of accessibility of the site, as well as overall similarity of habitat. For example, all *Elliptio complanata* were collected from lakes with rather uniform bottom characteristics: mixtures of sand and small rocks. This reduces although does not rule out the possibility that the genetic differences observed between populations were habitat-induced rather than a reflection of genetic processes associated with colonization. Individuals of the common species were selected at random from a large area.

Electrophoresis

Electrophoretic methods are similar to those described by Davis *et al.* (1981). Horizontal starch gel electrophoresis was performed as described by Ayala *et al.* (1973). Individuals were brought back from Nova Scotia alive, and maintained in aquaria for at least three weeks before electrophoresis. Tissue samples were cut from the foot and viscera and homogenized by sonication. Small tabs of Whatman #3 filter paper were dipped in the homogenate, blotted, and applied to the gel. The formulae of the gel buffers and those used in the electrode trays are presented by Davis *et al.* (1981).

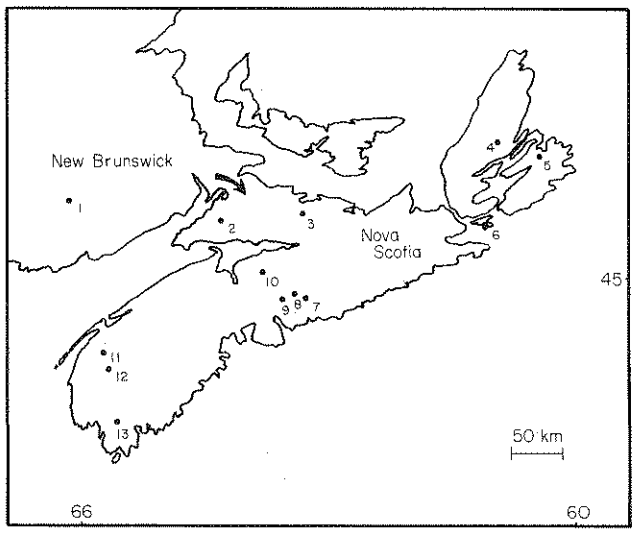

Figure 1. Location of the sample sites in Nova Scotia and New Brunswick. The arrow indicates a possible route of migration across the recently-formed land bridge at the Isthmus of Chignecto.

Following electrophoresis, the gels were sliced and then stained for one of 12 enzymes which together encoded 14 loci- *Lap, Mdh* I and II, *Hex, Gpi, Pgm* I and II, *Mpi, 6Pgd, Oct, Aat, Sod, G3pdh,* and α-*Gpdh.* The compositions of the agar overlays and tray solutions can be found in Davis *et al.* (1981).

Data analysis

Allele frequencies were scored according to Ayala *et al.* (1973). At least four control specimens of *Elliptio complanata* from Swartswood Lake, New Jersey (ANSP 341949) were used on each gel to establish allele identity among populations.

Nei's (1972) genetic distances were computer generated, and this matrix of population relatedness was used in the multivariate analysis program NT–SYS (Rohlf, Kishpaugh & Kirk, 1972). Multidimensional scaling was performed to maximize goodness-of-fit to the regression of genetic distance and distance in three-dimensional space. Principal components analysis was then applied to the covariance matrix of the three-dimensional scaled distances followed by a computation of a minimum spanning tree (MST) for cluster analysis (Gower & Ross, 1969).

Allele frequences at six polymorphic loci of *Elliptio complanata* (*Lap, Pgm* I and II, *Gpi, Mpi,* and *Hex*) were compared using Fisher's exact test, a non-parametric statistical test to determine independence by pairwise comparisons.

RESULTS

Elliptio complanata

Allele frequencies of the eight polymorphic loci for the Canadian populations of *Elliptio complanata* are presented in Table 1. Table 2 lists values of Nei's (1972) genetic identity (*I*) and genetic distance (*D*) for all pairs of populations, including four populations examined by Davis *et al.* (1981). Their relationships are depicted graphically in an ordination diagram presented as Fig. 2. Stress in the multidimensional scaling (a statistic which measures goodness-of-fit of the distances in the configuration space to the monotone function of the original distances) after 32 iterations was 0.132. The cophenetic correlation (which measures the amount of distortion in phenetic relationships due to reduction from an *N*-dimensional space to a three-dimensional space) was 0.964. The first two axes depicted in Fig. 2 account for 47.9 and 43.4% of the variance, respectively. A minimum spanning tree (MST; Gower & Ross, 1969) is shown connecting all populations: it connects near neighbors in space and when superimposed on a principal components plot reveals the extent of local distortions. Hence populations C13 and C12, which seem to lie in close proximity, are not connected to each other by the MST but to C11, indicating the importance of the third principal component in describing the position of C11. Cluster analysis defines three major subdivisions within the Nova Scotian *Elliptio*: a southern, northern (Cape Breton), and a central group.

When the observed allele frequencies at several polymorphic loci for each population were compared by pairwise comparisons using Fisher's exact test to determine independence, the following patterns emerged (Table 3). *Lap* exhibits

Table 1. Allele frequencies of *Elliptio complanata*. Population site (e.g. C5, ect.) are depicted in Fig. 1; exact locality data are presented in Appendix 1

Enzyme	Allele	Populations														
		C5	C4	C11	C13	C12	C6	C9	C2	C3	C1	C8	ME*	NJ*	DEL*	MD*	SUS*
Lap	42						0.20		0.03								
	40			0.05	0.02				0.06		0.16						
	38	0.15	0.05		0.20	0.04		0.20	0.08	0.47	0.33	0.05					
	36			0.50	0.13	0.04	0.43	0.23	0.14	0.18	0.31	0.10			0.06	0.08	
	34	0.08	0.08	0.25	0.27	0.14		0.02	0.14	0.05	0.13	0.13				0.14	
	32	0.67	0.47	0.20	0.30	0.64	0.37	0.43	0.39	0.24	0.17	0.43	0.66	0.47	0.14	0.30	0.22
	30		0.26		0.07	0.14		0.10	0.08	0.05	0.02	0.02	0.14	0.28			0.16
	28	0.10	0.13					0.02	0.08			0.13	0.18	0.14	0.18	0.26	0.24
	26											0.10		0.11	0.52	0.06	0.30
	23											0.05			0.04	0.14	
Mdh I	11	0.06		0.16			0.05		0.05			0.20	0.12	0.16	0.26	0.10	
	8	0.94	1.00	0.84	1.00	1.00	0.95	1.00	0.95	1.00	1.00	0.80	0.88	0.81	0.72	0.90	1.00
Hex	37											0.20					
	34											0.65					
	31	0.32	0.25	0.10	0.22	0.26	0.15	0.22	0.36	0.40	0.25	0.15	0.12	0.58	0.60	0.26	0.22
	28	0.68	0.75	0.90	0.78	0.85	0.78	0.64	0.60	0.75		0.88	0.42	0.33	0.74	0.78	
Gpi	14	0.13	0.13	0.30	0.48	0.31	0.21	0.25	0.08	0.08				0.14	0.06	0.18	
	8	0.83	0.87	0.70	0.52	0.69	0.79	0.75	0.92	0.92	1.00	0.95	0.96	0.74	0.94	0.82	0.96
	2	0.05										0.05		0.06			
Pgm I	18		0.18				0.13	0.05	0.14	0.03	0.08	0.04		0.32			
	15	0.65	0.76	0.90	0.83	0.96	0.75	0.93	0.84	0.86	0.65	0.30	0.86	0.81	0.60	0.90	0.92
	12	0.35	0.06	0.10	0.17	0.04	0.13	0.02	0.16		0.32	0.50	0.10	0.16	0.04	0.10	0.04
	9											0.13					
Pgm II	34			0.03			0.25	0.05	0.10	0.05	0.08		0.04	0.08		0.08	
	32				0.13		0.05		0.13	0.05		0.05	0.04		0.04		0.18
	30	0.85	0.74	0.89	0.57	0.92	0.65	0.78	0.43	0.66	0.70	0.68	0.80	0.73	0.74	0.76	0.72
	28	0.15	0.26	0.08	0.30	0.08	0.05	0.05	0.20	0.08	0.03	0.10	0.10	0.08	0.14	0.04	0.10
	26							0.10	0.15	0.16	0.11	0.17		0.11	0.08	0.12	
Mpi	28		0.35	0.45	0.12	0.50		0.20	0.15	0.15	0.20	0.05					
	26	1.00	0.65	0.55	0.88	0.50	1.00	0.75	0.85	0.85	0.80	0.95	1.00	1.00	0.84	0.88	1.00
	24							0.05							0.16	0.12	
6*Pgd*	7																0.26
	5			0.10	0.05	0.15		0.10					0.24	0.28		0.04	0.34
	3	1.00	1.00	0.90	0.95	0.85	1.00	0.90	1.00	1.00	1.00	1.00	0.76	0.72	1.00	0.96	0.39

*Populations analysed by Davis *et al.* (1981).

Table 2. Nei's genetic identity (I; above the digonal) and distance (D; below the diagonal) for *Elliptio complanata*

Populations	SUS*	ME*	DEL*	MD*	C5	C4	C11	C13	C12	C6	C9	C2	C3	C1	C8
SUS	—	0.914	0.874	0.915	0.881	0.906	0.896	0.883	0.910	0.881	0.915	0.897	0.892	0.889	0.822
ME	0.090	—	0.934	0.982	0.983	0.976	0.949	0.958	0.965	0.972	0.981	0.974	0.962	0.956	0.913
DEL	0.135	0.068	—	0.958	0.948	0.946	0.915	0.928	0.916	0.939	0.943	0.954	0.956	0.944	0.933
MAR	0.089	0.018	0.043	—	0.981	0.978	0.968	0.976	0.967	0.981	0.988	0.985	0.978	0.973	0.924
C5	0.126	0.017	0.053	0.019	—	0.977	0.946	0.971	0.963	0.974	0.979	0.980	0.977	0.969	0.942
C4	0.098	0.024	0.055	0.022	0.023	—	0.967	0.973	0.984	0.968	0.983	0.983	0.977	0.971	0.916
C11	0.109	0.052	0.088	0.033	0.055	0.033	—	0.967	0.975	0.966	0.974	0.958	0.956	0.965	0.882
C13	0.124	0.043	0.075	0.024	0.029	0.027	0.033	—	0.966	0.974	0.982	0.978	0.971	0.966	0.902
C12	0.094	0.036	0.087	0.034	0.037	0.016	0.025	0.034	—	0.953	0.980	0.963	0.960	0.948	0.887
C6	0.127	0.028	0.063	0.019	0.026	0.032	0.034	0.026	0.048	—	0.985	0.980	0.972	0.974	0.914
C9	0.088	0.019	0.059	0.012	0.021	0.017	0.026	0.018	0.020	0.015	—	0.982	0.986	0.975	0.908
C2	0.109	0.026	0.047	0.015	0.020	0.017	0.042	0.022	0.037	0.020	0.018	—	0.986	0.982	0.931
C3	0.114	0.038	0.045	0.022	0.029	0.023	0.045	0.029	0.041	0.028	0.014	0.014	—	0.986	0.919
C1	0.117	0.045	0.057	0.027	0.031	0.029	0.035	0.034	0.053	0.026	0.025	0.018	0.014	—	0.927
C8	0.196	0.091	0.096	0.079	0.060	0.088	0.125	0.103	0.120	0.090	0.096	0.071	0.084	0.075	—

*Populations analysed by Davis *et al.*, (1981): SUS, Susquehanna River; ME, Maine; DEL, Delaware; MAR, Maryland.

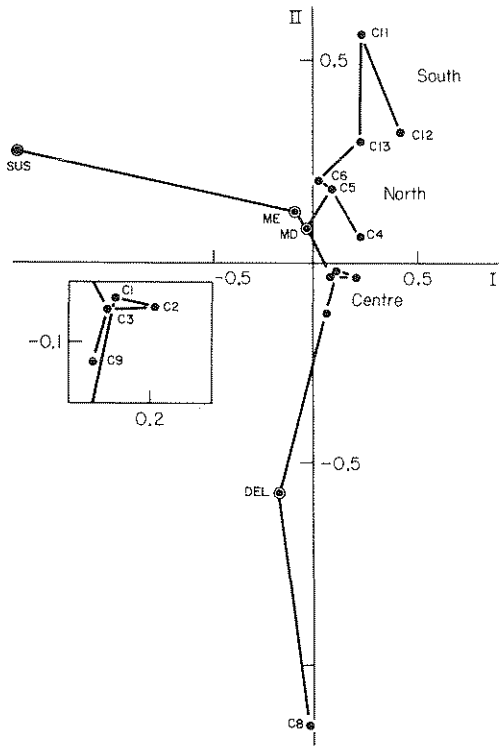

Figure 2. Multidimensional scaling of *Elliptio complanata* populations based on Nei's (1972) genetic distances. The magnified inset at left shows details of the central population group. All points are shown linked by a minimum spanning tree. Populations outside Canada are shown as concentric circles, and were analysed by Davis *et al.* (1981).

significant differences in distribution of alleles between Canadian and north-eastern American populations. In general, the northern populations possess a greater number of fast alleles when compared to southern populations. Within Nova Scotia, differences between populations seem almost randomly distributed; there is no clear pattern which indicates groupings on a geographic basis for this particular allele. A similar difference between Canadian and north-eastern American populations is noted for *Mpi*, which also differentiates between subgroups in Nova Scotia. *Hex* and *Pgm* I only show consistently significant differences between the CB population in Nova Scotia and all others compared, since CB exhibits unique alleles at these loci (Table 1).

The summed number of alleles present in each *Elliptio complanata* population at seven polymorphic loci (*Lap*, *Pgm* I and II, *Mpi*, *Gpi*, *Hex*, and *Mdh* I) is presented in Fig. 3. Two enigmatic populations deserve mention; those of ME and C8. The ME population contains two monomorphic loci among the seven mentioned above, and consequently has a very low number of alleles for its position within the geographic range. The C8 population, on the other hand, has no monomorphic loci among the above seven, and the number of alleles at these loci is among the highest observed for any population of *E. complanata*. This was the only population examined which was sampled from a river instead of a

Table 3. Fisher's exact test to determine significant differences of allele frequencies between pairs of populations of *Elliptio complanata* at two loci: *Lap* (above the diagonal) and *Mpi* (below the diagonal). Populations other than those from Nova Scotia and New Brunswick analysed by Davis *et al.*, (1981). *, Indicates a probability of 0.05 or less

Population	C1	C2	C3	C4	C5	C6	C8	C9	C11	C12	C13	ME	NJ	DE	MD1	MD2	SUS
C1	—	*			*	*	*	*			*	*	*	*	*	*	*
C2		—										*	*	*		*	*
C3			—		*	*	*				*	*	*	*	*	*	*
C4				—		*			*	*		*	*	*		*	*
C5	*			*	—	*			*	*		*	*	*		*	*
C6				*		—				*	*	*	*	*	*	*	*
C8				*		*	—		*	*		*	*	*		*	*
C9					*			—			*	*	*	*		*	*
C11					*	*			—		*	*	*	*	*	*	*
C12					*	*				—		*	*	*		*	*
C13		*	*		*	*	*			*	—	*	*	*		*	*
ME		*	*	*		*		*	*	*	*	—			*		
NJ	*	*	*	*	*		*	*	*	*	*		—		*		
DE	*	*	*	*	*	*	*	*	*	*	*			—			
MD1	*	*	*	*	*	*	*	*	*		*				—		
MD2	*	*	*	*	*	*	*	*	*		*					—	
SUS																	—

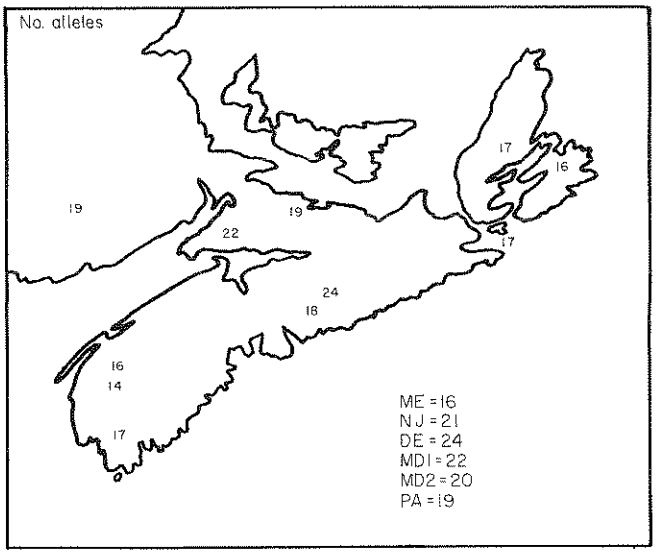

Figure 3. Number of alleles present at six polymorphic loci (*Lap*, *Pgm* I and II, *Mpi*, *Hex*, and *Gpi*) of *Elliptio complanata* from Nova Scotia and more southern sample sites. Position of the number of alleles on the map corresponds to the sample sites.

lake, and it is possible that this habitat difference contributed to the observed divergence from the other Nova Scotian populations.

Levels of heterozygosity and polymorphism for the various populations are presented in Table 4, and levels of heterozygosity are depicted in Fig. 4. Heterozygosity, and to a much lesser extent polymorphism, are below the levels of more southern populations: Nova Scotian populations exhibit an average observed heterozygosity of 0.060 ± 0.011 and an average polymorphism of 0.425 ± 0.051, compared to an average observed heterozygosity of 0.145 ± 0.019 and a polymorphism of 0.482 ± 0.106 for the more southern populations (Davis *et al.*, 1981). Heterozygosity decreases further within Nova Scotia: central populations exhibit an average observed heterozygosity of 0.069 ± 0.009, while populations at the southern end of the Province exhibit an average observed heterozygosity of 0.048 ± 0.006.

Lampsilis radiata

Allele frequencies of the seven polymorphic loci of the Canadian populations of *Lampsilis radiata* are presented in Table 5. Table 6 lists values of Nei's genetic identity I and distance D for all pairs of populations: their relationships are depicted graphically in Fig. 5. Stress in multidimensional scaling after 45 iterations was 0.056 and the cophenetic correlation was 0.99. The first two axes in Fig. 5 account for 93.7 and 4.9% of the variance, respectively; thus there is little distortion evident in the MST, which defines a cluster formed by the central Nova Scotian populations (C2, C9, and C7); those from New Brunswick (C1) and the northern part of the Isthmus of Chignecto (C3) are rather widely separated.

Levels of heterozygosity and polymorphism for the populations examined are

Table 4. Values of observed heterozygosity (H) and polymorphism (P) for the species and populations studied

					Elliptio complanata					
	C1	C2	C3	C4	C5	C6	C8	C9	C11	C12	C13
H	0.071	0.062	0.065	0.058	0.053	0.055	0.084	0.063	0.041	0.051	0.054
P	0.428	0.500	0.428	0.428	0.357	0.357	0.500	0.500	0.428	0.428	0.428

				Lampsilis radiata	
	C1	C2	C3	C7	C9
H	0.041	0.021	0.037	0.004	0.006
P	0.357	0.143	0.357	0.071	0.214

			Lampsilis ochracea
	C5	C12	
H	0.045	0.052	
P	0.286	0.286	

		Lampsilis cariosa
	C5	
H	0.070	
P	0.430	

			Anodonta cataracta fragilis
	C2	C4	C6
H	0.078	0.085	0.082
P	0.214	0.214	0.214

			Anodonta implicata
	C1	C5	C9
H	0.056	0.057	0.069
P	0.357	0.357	0.357

		Alasmidonta undulata
	C8	C10
H	0.011	0.015
P	0.071	0.071

listed in Table 4. Heterozygosity and polymorphism both decrease with distance from the Isthmus of Chignecto, but the pattern is different from that observed in *Elliptio complanata*.

Relationships within other taxa

The other taxa examined in this study, *Anodonta implicata, A. cataracta fragilis, Alasmidonta undulata, Lampsilis ochracea,* and *L. cariosa* are represented by three, three, two, two, and one population, respectively, and were thus not subjected to multidimensional scaling. Levels of heterozygosity and polymorphism are presented in Table 4. To determine relationships of these peripheral populations to more southern populations or congeneric species, *A.c. fragilis* was compared with *A.c. cataracta* populations from New Jersey; Nova Scotian *L. ochracea* with a population from Lake Waccamaw, North Carolina; and Nova Scotian *L. radiata* and *A. implicata* with populations from eastern Maryland as well as several congeneric species.

Anodonta cataracta fragilis was named by Clarke & Rick (1963) on the basis of intermediacy of beak sculpture and shell shape between *A.c. cataracta* from the northern Atlantic Slope and *A. fragilis* from Newfoundland. Electrophoretic analyses reveal that *A.c. cataracta* from New Jersey only resembles *A.c. fragilis* at

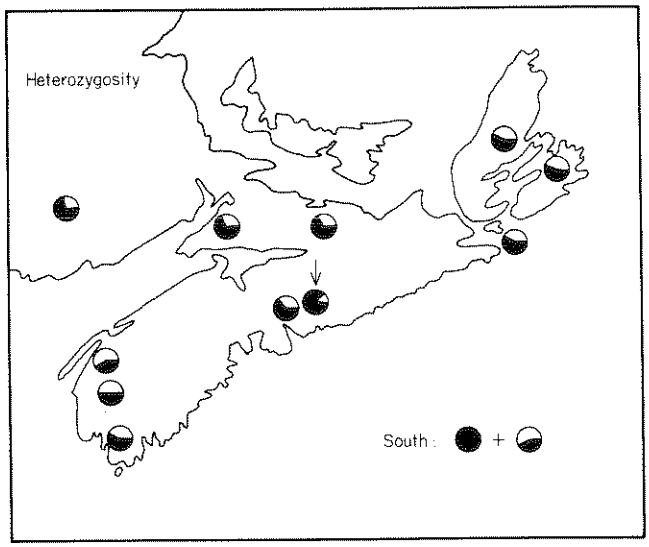

Figure 4. Levels of heterozygosity among Canadian and more southern populations of *Elliptio complanata*. The amount of the circle filled represents the level of heterozygosity multiplied by 100; e.g. for $H = 0.075$, the circle is 75% filled. For actual levels of observed heterozygosity, see Table 4. By this method, more southern populations have an average heterozygosity of 145% (see text), depicted here as a full circle (100%) and one filled to 45%. The arrow indicates the river population of *E. complanata* (CB).

a level of 0.608 ± 0.020, and examination of stomach structure (Kat, 1983b) indicates that *A.c. fragilis* has a distinct affinity with the Eurasian *A. cygnea*. *Anodonta cataracta fragilis* could therefore represent a taxon with European affinities which survived glaciation in a local refuge, and is presently colonizing

Table 5. Allele frequencies for *Lampsilis radiata*

Enzyme	Allele	Populations				
		C9	C2	C7	C1	C3
Lap	34		0.47		0.21	0.03
	32	0.92	0.53	1.00	0.75	0.67
	30	0.08			0.04	0.30
Mdh I	19				0.13	
	15	1.00	1.00	1.00	0.87	1.00
Hex	35				0.06	
	32	1.00	1.00	1.00	0.94	1.00
Pgm I	18			0.05	0.04	0.32
	15	1.00	1.00	0.95	0.87	0.63
	13				0.09	0.05
Pgm II	28	1.00	1.00	1.00	0.94	0.95
	26				0.06	0.05
6*Pgd*	4	0.96	1.00	1.00	1.00	0.90
	2	0.04				0.10
G3*pdh*	11				0.13	0.05
	9	0.05			0.19	0.35
	7	0.95	1.00	1.00	0.68	0.60

Table 6. Nei's genetic identity (I; above the diagonal) and distance (D; below the diagonal) for *Lampsilis radiata*

Populations	C9	C2	C7	C1	C3
C9	—	0.986	0.999	0.991	0.979
C2	0.014	—	0.984	0.987	0.967
C7	0.001	0.016	—	0.989	0.975
C1	0.009	0.013	0.033	—	0.986
C3	0.021	0.033	0.025	0.014	—

Nova Scotia from the north. It is possibly hybridizing with *A.c. cataracta* in the Province, although this remains to be determined: unlike other authors (e.g. Athearn & Clarke, 1961; Wiles, 1975) we found no individuals with an unequivocal *A.c. cataracta* phenotype on Nova Scotia. Levels of heterozygosity and polymorphism ($H = 0.081 \pm 0.004$; $P = 0.237 \pm 0.041$) for the populations of *A.c. fragilis* examined are within the range characteristic of other *Anodonta* (Kat, 1983b), and higher than those which characterize northeastern American *A.c. cataracta* ($H = 0.028 \pm 0.006$; $P = 0.113 \pm 0.039$) (Davis, unpublished data). Allele frequencies among Nova Scotian *A.c. fragilis* populations differ only to a small extent. A similar pattern of overall homogeneity in allele frequencies and levels of heterozygosity and polymorphism also occurs in *A. implicata*, and this species in fact exhibits the highest level of polymorphism among *Anodonta* thus far examined (Kat, 1983b).

Lampsilis ochracea and *L. cariosa* are only known from a few localities on Nova Scotia (Athearn & Clarke, 1961; Clarke & Rick, 1963), and are thus only represented by a small number of populations in this study. However, the populations of *L. ochracea* exhibit similar allele frequencies and no reduction in heterozygosity or polymorphism even when compared to populations at the other extreme of the geographic range in North Carolina, and levels of heterozygosity and polymorphism are well within the range typical of other *Lampsilis* (Kat, 1983c). In fact, *L. cariosa* exhibits the highest level of heterozygosity and the second highest level of polymorphism thus far encountered among five species of *Lampsilis* (Kat, 1983). Thus, while

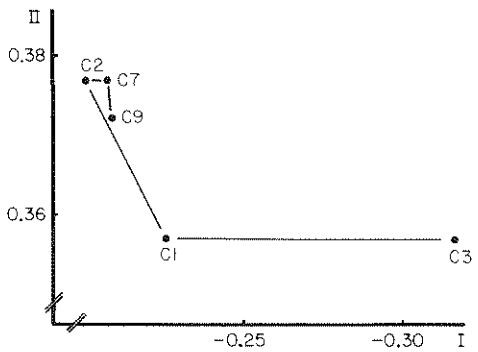

Figure 5. Multidimensional scaling of *Lampsilis radiata* based on Nei's (1972) genetic distances. All points are shown linked by a minimum spanning tree.

these species of *Lampsilis* and *Anodonta* are represented by small sample sizes, the patterns of resemblance among populations as well as levels of heterozygosity and polymorphism exhibit distinct differences when compared to those characteristic of *L. radiata* and *Elliptio complanata* from the Province.

Alasmidonta undulata has colonized the central region of Nova Scotia, but population sizes remain rather small. This species has the lowest heterozygosity observed among the species examined ($H = 0.013$) and only exhibits polymorphism at a single locus (*Gpi*). Interestingly, this species exhibits two types of simultaneous hermaphroditism in Nova Scotia: scattered spermatogenic regions within female follicles as well as well-defined oogenic and spermatogenic gonadal regions. The former type is encountered among very low-density lake populations, while the later type is observed among pure males and females in higher-density river populations. In source areas such as Maryland and Virginia, all populations thus far examined reproduce dioeciously (Kat, unpubl. data). Van der Schalie (1970) also found no evidence of hermaphroditism in this species (although his sample size was extremely small), but reported a similar occurrence of scattered spermatogenic regions within female follicles for the closely-related Interior Basin species *A. marginata*. Comparative electrophoretic data from other *Alasmidonta* or southern populations of *A. undulata* are not currently available.

DISCUSSION

Limitations of the method

The use of sequential electrophoretic analyses, varied gel pore sizes and concentrations, heat denaturation, and isoelectric focusing has recently demonstrated that the standard electrophoretic methods used here will underestimate variability, and may thereby underestimate levels of heterozygosity and polymorphism (Coyne & Felton, 1977; Johnson, 1977; Ramshaw, Coyne & Lewontin, 1979). However, as Davis *et al.* (1981) point out, data from standard methods are still of considerable value, mainly because of the availability of comparative data critical to this study. The data published here should thus be considered indicative of trends within these peripheral populations rather than as indicative of fine-scale processes.

In addition, it is widely recognized that electrophoretic data can be indicative of phenotypic rather than genotypic characters. For instance, Oxford (1975) has shown that esterases in *Cepaea nemoralis* may be food-induced, and Dillon & Davis (1980) note that synthesis of *Lap* might be under environmental control in *Goniobasis proxima*. It could therefore be argued that differences in these Nova Scotian populations could be environmentally induced and hence the entire pattern of geographic relationships spurious, depending only on similarity of proximal habitats. Ideally, transplants of juveniles should be performed to test these hypotheses, but trends in the data and precautions taken before electrophoresis consitute strong circumstantial evidence that environmentally induced differences play a minor role. First, geographic proximity has little to do with electrophoretic similarity: while geographic subgroups are defined for *Elliptio complanata*, relationships within these subgroups are contrary to

predictions based on geographic proximity. Second, all populations were maintained under identical conditions for at least three weeks before electrophoresis, reducing the possibility of food and other environmentally induced differences. Habitats sampled were as similar as possible.

Patterns observed among peripheral populations

The eletrophoretic data (Tables 1, 4, and 5) suggest the existence of two groups among the peripheral populations of eight Nova Scotian unionid species. The first group contains species which exhibit considerable reduction in heterozygosity and/or the existence of fluctuations in allele frequencies and novel allelic combinations and consists of *Alasmidonta undulata, Lampsilis radiata,* and *Elliptio complanata*. The second group either shows no such trends, or shows no evidence of such trends when data from congeneric species are compared. This group consists of *Anodonta implicata, A. cataracta fragilis, Lampsilis ochracea,* and *L. cariosa*.

Alasmidonta undulata falls within the first group. This species has the lowest heterozygosity observed among the species examined ($H = 0.013$) and only exhibits polymorphism at a single locus (*Gpi*). The observation of spermatogenic regions within female follicles as well as separated male and female gonadal regions within a single individual, and the completely dioecious central-range populations indicate that members of this genus possess a wide variety of reproductive modes, perhaps including self-fertilization. A similar reduction in heterozygosity and polymorphism has been observed in other potentially self-fertilizing molluscs and plants (Selander & Hudson, 1976; Hornbach *et al.,* 1980; McLeod & Sailstad, 1980; Shumaker & Babbel, 1980). This potential for self-fertilization could have been instrumental in estalishment of this species' wide geographic range (North Carolina to Nova Scotia), although the existence of wide-ranging dioecious species indicate that the method of reproduction is only one of several variables important in range expansions.

Lampsilis radiata has attained a Nova Scotian distribution similar to that of *Alasmidonta undulata*, yet the pattern of electrophoretic similarities among populations of this species suggests that it could have been one of the first to colonize the Province (see below). Heterozygosity and polymorphism progressively decrease with distance from the Isthmus of Chignecto, and one population, C7, is almost completely homozygous (Table 3). Low heterozygosity in *L. radiata* is almost entirely due to loss of rare alleles, and only populations C1 and C3 exhibit moderate frequencies of these alternative alleles (Table 5). Levels of heterozygosity for populations of *L. radiata* from Nova Scotia ($H = 0.022 \pm 0.017$) are considerably below those of northeastern American populations ($H = 0.059 \pm 0.007$) (Kat, unpubl. data), congeners such as *L. ochracea* ($H = 0.048 \pm 0.005$) and *L. cariosa* ($H = 0.070$) in the Province, *L. teres* ($H = 0.056$) from Florida (Davis *et al.,* 1981), *L. splendida* ($H = 0.059$) from Georgia and the closely-related *Lampsilis* sp. from Lake Waccamaw ($H = 0.039$) (Kat, 1983c).

Within the first group, the clearest pattern emerges with an examination of *Elliptio complanata*. Davis *et al.,* (1981) conducted a survey of species of *Elliptio* occurring from New Jersey to Florida, and noted that the *E. complanata* species 'group' is apparently of recent origin, appears to be actively radiating today,

and is characterized by considerable heterozygosity and polymorphism ($H = 0.145 \pm 0.019$ and $P = 0.482 \pm 0.106$ for *E. complanata*). Nova Scotian populations, on the other hand, especially those at the northern and southern peripheries of the Province, are characterized by low heterozygosity and some reduction in polymorphism resulting from loss or reduction in frequency of alternative alleles (Tables 1 and 4; $H = 0.060 \pm 0.011$ and $P = 0.435 \pm 0.051$). Peripheral populations, however, are far from consistent in expression of these trends. Some populations exhibit increased frequencies of otherwise rare alleles (e.g. *Lap* 42 and *Pgm* II 34 in C6) while other lose them (e.g. *Pgm* II 34 in C5 and C12), and some populations show high levels of heterozygosity at a single locus when compared to other populations (e.g. *Lap* and *Pgm* I in C8).

This pattern of variability in allele frequencies at various loci among peripheral populations contrast with the results of Highton & Webster (1976) for salamander populations occurring in previously glaciated areas. In fact, these salamanders exhibited the reverse relationship between variability of central and peripheral populations noted here: populations that occur in previously glaciated regions were found to be very uniform with respect to allele frequences, while those that occur in unglaciated regions exhibited considerable divergence. Highton & Webster (1976) also did not find the type of divergence among peripheral salamander populations which involves establishment of unique alleles and/or allele frequencies as is the case among some *E. complanata* peripheral populations (see below). Why these differences occur is not entirely clear, but can be explained to some extent by uncertainty as to pathways of dispersion, and the existence of cryptic species and well-differentiated races among salamanders from unglaciated regions (e.g. Highton, 1980). In contrast, Pierce & Mitton (1980) found evidence of similar genetic heterogeneity to that noted for peripheral *Elliptio complanata* among recently-established tiger salamander populations, and postulated a combination of genetic drift and founder effects to be responsible for such differentiation. Heterogeneity among recently established populations was also noted by Brown & Marshall (1981) and Harding & Barnes (1977) for some colonizing plant species, and could thus be indicative of a common phenomenon among populations of polymorphic species recently founded by few individuals.

Despite the fluctuation in allele frequencies among peripheral populations of *Elliptio complanata*, Fig. 2 reveals the existence of distinct trends in overall relatedness among populations of the species. The MST defines northern, central, and southern subgroups, and reveals that within a subgroup, genetic distances between populations in peripheral subgroups are larger than those in the more cohesive central group. Relationships between individual genetic identity values (Table 2) indicate that *E. complanata* most likely colonized Nova Scotia across the Isthmus of Chignecto and then split into two groups, one colonizing Cape Breton to the north, and the other colonizing southern regions of the Province. Average genetic identity values between Nova Scotian populations ($I = 0.966$) are well within those suggested by Avise (1976) for levels of difference between conspecific populations, but are rather low compared to levels of similarity observed by Davis *et al.* (1981) for southern populations ($I = 0.98$ to 0.99).

One population of *Elliptio complanata*, that from C8, is quite different from other Nova Scotian conspecifics in both levels of heterozygosity and allele

frequencies. In fact, the C8 population exhibits novel alleles for *Lap*, *Hex*, and *Pgm* I (Table 1). This population differs from all others in that it was collected in a river instead of a lake, but whether this difference is due to fundamental differences between river and lake environments remains to be determined. On the other hand, this population only resembles other Nova Scotian populations at a level of 0.912, which is well within the range indicated by Davis *et al.* (1981) as characteristic of interspecific differences within the radiating genus *Elliptio*, and could indicate an early stage of divergence of this population from others in Nova Scotia.

Patterns of resemblance between populations of *Elliptio complanata* and those of *Lampsilis radiata* differ somewhat, indicating that these species could have colonized Nova Scotia *via* different pathways. Alternatively, these species may have been introduced into the new habitats under different conditions of initial colonizer population numbers, or different patterns of drainage connections. The host fish of *E. complanata* is most frequently cited as *Perca flavescens*, the yellow perch. Livingstone (1951) has shown that while this species occurs on the Nova Scotian peninsula, it is not known from Cape Breton Island to the north. Recently, Wiles (1975) demonstrated that *E. complanata* also parasitizes the banded killifish, *Fundulus diaphanus*, a species which not only occurs on Cape Breton Island, but has a wide distribution on the rest of Nova Scotia. Both fish, however, are restricted to fresh water (Fowler, 1906; Mansueti, 1960; Hynes, 1970), and Livingstone (1951) surmises that colonization of Nova Scotia occurred through the Isthmus of Chignecto. Patterns of resemblance among populations of *E. complanata* substantiate this hypothesis. However, patterns of resemblance among populations of *L. radiata* as defined by the MST indicate that population C3 is distinct in this species, and is linked to population C1 in New Brunswick (Fig. 5). Since the outflow of C3 drains into the Northumberland Strait, a relationship to C1 (connected to the Bay of Fundy) could only come about if these populations were established before the Isthmus of Chignecto formed; *L. radiata* parasitizes a large number of fish species (Fuller, 1974), some of which are tolerant of saline conditions (Hynes, 1970). Evidence for rapid range expansions by this species is provided by occurrence of subfossil specimens from the Transitional (10 000–6000 B.P.) and Nipissing (6000–4000 B.P.) stages in Ontario (Miller *et al.*, 1979). It is difficult, however, to reconcile a long-standing presence of *L. radiata* in the Province with its limited degree of colonization; further studies of *L. radiata* populations are necessary to elucidate these relationships.

The remaining four species examined (*Anodonta implicata*, *A. cataracta fragilis*, *Lampsilis cariosa*, and *L. ochracea*) show little variability in levels of heterozygosity and polymorphism, exhibit little variability in allele frequencies among widely-separated populations in eastern Canada, and in the cases of *L. ochracea* and *A. implicata*, populations from opposite range extremes. The type of dispersal of these species also differs from that characteristic of the first group, and it is hypothesized that this type of dispersal could affect both the size of the initial founder population and subsequent rates of genetic exchange among demes.

Glochidia of *Anodonta implicata* are parasitic on the alewife, *Alosa pseudoharengus*, an anadromous fish (Johnson, 1946). Consequently, this unionid occurs only in habitats with a direct link to the ocean (Johnson, 1946), and the tolerance of its host to saline conditions probably facilitates inter-drainage dispersal.

Colonization of new habitats by this species is presumably accomplished by a relatively large number of individuals because of schooling behavior of the host. Although their host fish are as yet unknown, a similar hypothesis can be constructed for *Lampsilis ochracea* and *L. cariosa*, since they are similarly restricted to habitats close to the ocean (Johnson, 1970). These species are additionally characterized by a highly sporadic occurrence of populations in drainages within their overall geographic ranges. For example, *L. cariosa* apparently occurs at only one site in eastern Canada, the Sydney River drainage; *L. ochracea* is only known from three widely separated sites in Nova Scotia, and *A. implicata* occurs sporadically in the Province (Athearn & Clarke, 1961; Clarke & Rick, 1963; D. Davis, Nova Scotia National Museum, pers. comm.). Without erecting a highly complex hypothesis involving elimination of all or most intervening populations, this pattern is highly suggestive of introduction by a saltwater-tolerant host which can bypass drainages.

Examples of the high molecular genetic cohesion among populations of species dispersed by such saltwater tolerant hosts are provided by the levels of genetic similarity observed between populations of *Lampsilis ochracea* and *Anodonta implicata* sampled from the northern and southern extremes of their geographic ranges. A population of *L. ochracea* from Lake Waccamaw, North Carolina, resembles Nova Scotian populations at a level of 0.954 ± 0.009 (Kat, 1983), and Nova Scotian *A. implicata* resemble those from eastern Maryland at a level of 0.968 ± 0.014 (Kat, unpubl. data). This level of divergence is commonly observed among populations of *Elliptio complanata* sampled from neighboring drainages (see Table 2).

Founder events and effects

The probability that a fish host will introduce unionids into new habitats depends on several factors.

Since production of glochidia is seasonal, and glochidia are only carried for a period of a few weeks to a month (Lefevre & Curtis, 1910), introduction by fish hosts can only occur during a restricted period of time. Chance events which lead to introduction of fish hosts, if they do not occur during the limited time during which they are infected with unionid glochidia, will not result in introduction of unionids. It is therefore not uncommon to encounter fish hosts with larger geographic ranges than their unionid parasites (e.g. Clarke, 1973).

Frequency of infection by glochidia is poorly understood, but available studies indicate that it is very low among natural fish populations (see summary by Coker, 1921). Consequently, the probability that an infected fish host will be among those introduced into a new habitat, if such a chance introduction were to take place, is equally low.

Under laboratory conditions, fish hosts tolerate infection by about 2500 hooked glochidia (e.g. those of *Anodonta*) which attach mainly to fins, and about 500 unhooked glochidia (e.g. those of *Elliptio*) which attach mainly to gills (Lefevre & Curtis, 1910). Natural infections occur at a much lower frequency (average of about 125 per host; see Coker *et al.*, 1921). Mortality among both pre-metamorphosed glochidia and juveniles is very high (Coker *et al.*, 1921).

Propagation of the unionid population depends on survival of the host in the new habitat, and successful re-infection of these hosts.

Considering these factors, we provide a model for unionid population growth following an initial introduction into a new habitat (Appendix 2). In this model we compare unionids to a hypothetical population of *Drosophila,* which is regularly used in models of colonizing species. The model indicates that the rate of unionid population growth can be extremely slow, and that even when juveniles are introduced into virgin habitats in large numbers, none might survive to reproductive age.

Given the probability that unionids might be introduced in small numbers, and that population growth can be slow, Nei *et al.* (1975) discussed two effects of founder events important to this study. First, a reduction in heterozygosity associated with establishment of a population by a small number of founders is often substantial, and continues to decrease during initial generations with loss of rare alleles due to genetic drift. Once reduced to a low level, an increase in heterozygosity to original levels by mutation occurs at a rate roughly equal to the reciprocal of the mutation rate per generation. For Nova Scotian unionids with at most one generation per year, this rate could be extremely slow (on the order of 10^5 years for a mutation rate of 10^{-5} per locus per generation). The amount of reduction in heterozygosity depends primarily on the number of founding individuals, but as Nei *et al.* (1975) point out, with a slow rate of population growth, the amount of reduction in heterozygosity can be substantial even with a moderate number of founders. This decrease is observed among the Nova Scotian populations of *Elliptio complanata,* for example. Second, founders can only introduce a small amount of the total genetic variability, so that the loss of alleles in the first generation can be drastic if the number of founders is small. Nei *et al.* (1975) mention, however, that the average number of alleles per locus increases faster than the level of heterozygosity once population size is restored. Since population sizes of *E. complanata* in Nova Scotia do not differ substantially from other populations sampled from central geographic range locations, much difference between the number of alleles present in these Nova Scotian populations would not be expected. In fact, Nova Scotian *E. complanata* average 18.0 ± 2.3 alleles for seven polymorphic loci while southern populations average 20.3 ± 2.7 alleles; the difference only becomes statistically significant ($F_{1,\ 13} = 11.65$, $P < 0.01$) when only those populations at the northern and southern extremes of the Province (which hypothetically represent the youngest populations and thus possess fewer alleles; see Fig. 3) are compared with north-eastern American populations.

While there seems to be strong evidence of the importance of founder effects on levels of heterozygosity in peripheral populations of *Elliptio complanata*, it is uncertain to what extent allele frequencies are affected by founder events since the relative contribution of selective factors and chance factors to such allele frequencies cannot be determined. Using a large-scale comparison, it seems that fast alleles at loci which code for variable-substrate enzymes (Sarich, 1977) such as *Lap* and *Gpi* are more common in Nova Scotia than among north-eastern American populations of *E. complanata*, which could indicate differences in selective regimes. However, a recent study of *E. complanata* from Delaware and Maryland indicates that populations from north-eastern Maryland possess close affinities to those from Nova Scotia in electrophoretic as well as morphological characters, while those from south-eastern Maryland and Delaware are highly similar to populations from Virginia and North Carolina; thus these differences

in alleles could well indicate racial rather than selective differences: there is no *a priori* reason to postulate that selective regimes between Nova Scotia and north-eastern Maryland (separated by more than 1000 km) should be more similar than selective regimes between north-eastern and south-eastern Maryland (separated by less than 75 km) (Kat, 1983a). Similarly, there is no evidence of variability of thermally variable enzymes such as *α-Gpdh* (Johnson, 1976) when north-eastern American and Canadian populations are compared. Comparison of individual *E. complanata* populations of Nova Scotia, however, reveals considerable heterogeneities in allele frequencies at several loci. If these differences were the result of differences in selective regime experienced by these populations, it would require erection of a highly complex patchwork of differentially operating selective forces, which given the overall similarity and geographic proximity of habitats, seems inapplicable. Interestingly, a similar pattern of fluctuation in allele frequencies has been noted in isolated populations of wingless water striders (Zera, 1981), cave fish (Avise & Selander, 1972), and cave beetles (Laing, Cormandy & Peck, 1976), in which limited dispersal capabilities and small founder populations were thought to contribute to the observed patterns. In contrast, Zera (1981) found no such fluctuation in allele frequencies among winged water striders occuring in the same habitats as the wingless variety; these winged species were hypothesized to disperse more easily and colonize in larger numbers than their wingless congeners.

Variability in allele frequences among populations was not consistently observed for all unionid species in Nova Scotia; *Lampsilis ochracea, Anodonta implicata,* and *A. cataracta fragilis* are not characterized by such variability, and *L. cariosa* does not appear to exhibit substantial loss of heterozygosity. Several non-inclusive hypotheses can account for this difference.

These species do not perceive differences in their environment in the same fashion as *Elliptio complanata,* and thus do not respond to the same selective factors which might have caused variability among populations of *E. complanata.* These species would thus possess a "general purpose genotype" (Selander & Hudson, 1976) and perceive their environment in a "fine grained" fashion (Valentine, 1976), resulting in a rather small number of adaptive alleles. The data, however, do not support with this particular hypothesis. For example, *L. cariosa* is one of the most heterozygous species among *Lampsilis* thus far examined (Kat, 1983c), *A. implicata* exhibits the highest level of polymorphism among *Anodonta* thus far examined (Kat, 1983b), and all three species exhibit higher levels of heterozygosity and polymorphism than do species which possess the "general purpose genotype" (e.g. Selander & Kaufman, 1973; Selander & Hudson, 1976; Smith *et al.,* 1979; Halliday, 1981). Nevertheless, the possibility remains that *E. complanata* perceives its environment as very 'coarse grained'.

Lampsilis ochracea, L. cariosa, A. implicata, and *A.c. fragilis* do not exhibit the same degree of polymorphism at the loci surveyed that *E. complanata* does, but variability could be equally great if more loci were surveyed, or if more sensitive techniques were used. For example, the apparently monomorphic locus *Sod* exhibits considerable polymorphism for *A. implicata* when run at pH 8 instead of pH 9.

Since at least *L. ochracea, A. implicata,* and *L. cariosa* parasitize anadromous or salt-tolerant hosts, they are more likely to colonize new habitats in greater numbers and/or receive greater amounts of subsequent gene flow than unionids like *E. complanata* which parasitize territorial and freshwater hosts. In the

absence of strong divergent selection, unionids with saltwater tolerant hosts will therefore show higher levels of resemblance among widely separated populations than will the more isolated unionids that parasitize hosts limited to fresh water.

We suspect that all three hypotheses contribute to the observed pattern, but stress the importance of the last.

The hypothesis that host dispersal capability affects levels of genetic resemblance among populations of a species along a geographic range is testable. Most Atlantic Slope unionids appear to be expanding their geographic ranges in response to post-Wisconsinan climatic amelioration, and while there exists widespread ignorance as to the identity of potential fish hosts for most unionids, such hosts have been recognized for a number of common species with relatively large geographic ranges (e.g. Fuller, 1974). Widely-separated populations of such species could be examined electrophoretically to determine if the proposed hypothesis is sound, or if other hypotheses apply.

SUMMARY

Our data indicate that dispersing unionid species colonized Nova Scotia via a more complex series of pathways than the initially hypothesized simple invasion across a land-bridge. We recognize four distinct categories of species or species groups characterized by differences in hypothesized avenues of colonization:

(a) *Alasmidonta undulata* and *Elliptio complanata*, for which patterns of resemblance among populations indicate colonization by way of the Isthmus of Chignecto;

(b) *Anodonta implicata*, *Lampsilis ochracea*, and *L. cariosa* which either possess or have been implicated to possess andromous or saltwater-tolerant hosts and which could have colonized the Province via oceanic routes possibly before the land-bridge was formed;

(c) *Lampsilis radiata*, which shows evidence of colonization of Nova Scotia before the Isthmus of Chignecto was formed and subsequent recolonization across this land bridge;

(d) *Anodonta cataracta fragilis* (and presumably *Margaritifera margaritifera* of the family Margaritiferidae) which might have survived glaciation in a local refuge, and which are now expanding their ranges southward into Nova Scotia.

Accordingly, founder effects such as reductions in heterozygosity and variability in allele frequencies from population to population in similar habitats are not observed to the same extent for all species examined. While differences in perception of selective regime among species occurring in these habitats cannot be ruled out, the means of colonization could be primarily responsible for the presence of such variability: habitats which are isolated for species which parasitize strictly freshwater hosts are hypothesized to be much less so for species which parasitize saltwater-tolerant or anadromous hosts. Species in the latter category could consequently colonize such habitats in greater initial numbers and/or receive greater amounts of subsequent gene flow.

ACKNOWLEDGEMENTS

Discussions with Arthur Clarke led to the initial design of this study. Derek Davis kindly identified some newly-discovered populations of less-common

species on Nova Scotia, and the help of Lucy Look was invaluable in the field. Caryl Hesterman and Robert Dillon gave freely of their time and expertise during electrophoresis. Arthur Cain, Elaine Hoagland, Douglas Futuyma, and Bryan Clarke commented on various drafts of the manuscript. Funds were provided, in part, by grants from the National Science Foundation (DEB 78-01550 to GMD), the National Institutes of Health (BRSG 5-S07-RR07041-14 to PWK), the Consolidated Gifts Fund of the Department of Earth and Planetary Sciences (to PWK) and the Jessup Fund of the Academy of Natural Sciences of Philadelphia (to PWK). We are grateful to all.

REFERENCES

ATHEARN, H. D. & CLARKE, A. H., 1961. The freshwater mussels of Nova Scotia. *National Museum of Canada Bulletin, 183:* 11–41.

AVISE, J. C., 1976. Genetic differentiation during the speciation process. In F. J. Ayala, (Ed.), *Molecular Evolution:* 106–122. Massachusetts: Sinauer Press.

AVISE, J. C. & SELANDER, R. K., 1972. Evolutionary genetics of cave dwelling fishes of the genus *Astyanax*. *Evolution, 26:* 1–19.

AYALA, F. J., HEDGECOCK, D., ZUMWAULT, G. S. & VALENTINE, J. W., 1973. Genetic variation in *Tridacna maxima*, an ecological analog of some unsuccessful evolutionary lineages. *Evolution, 27:* 177–191.

BABBEL, G. R. & SELANDER, R. K., 1974. Genetic variability in edaphically restricted and widespread plant species. *Evolution, 30:* 213–233.

BAKER, F. C., 1928. The freshwater Mollusca of Wisconsin. Part II. Pelecypods. *Wisconsin Academy of Sciences, Arts, and Letters*, Madison, Wisconsin.

BORNS, H. W. Jr., 1973. Late Wisconsin fluctuation of the Laurentide ice sheet in southern and eastern New England. In R. F. Black, R. I. Goldthwait & H. B. Willman (Eds), *The Wisconsinan Stage*. Geological Society of America Memoir No. 136.

BROWN, A. H. D. & MARSHALL, D. R., 1981. Evolutionary changes accompanying colonization in plants. In G. G. E. Scudder & J. L. Reveal (Eds.), *Evolution Today*. Proceedings of the Second International Congress of Systematic and Evolutionary Biology: 351–363.

CLARKE, A. H., 1973. The freshwater molluscs of the Canadian Interior Basin. *Malacologia, 13:* 1–509.

CLARKE, A. H. & RICK, A. M., 1963. Supplementary records of Unionacea from Nova Scotia with a discussion of the identity of *Anodonta fragilis* Lamarck. *National Museum of Canada Bulletin, 199:* 2–14.

COKER, R. E., 1921. *Fresh water mussels and mussel industries of the United States*. Report of the Division of Scientific Inquiry for Fiscal Year 1921, Appendix VIII of the Report to the U.S. Commissioner of Fisheries. Bureau of Fisheries Document No. 911.

COKER, R. E., SHIRA, A. F., CLARK, H. W. & HOWARD, A. D., 1921. Natural history and propagation of fresh-water mussels. *Bulletin of the United States Bureau of Fisheries, 34:* 383–405.

COYNE, J. A. & FELTON, A. A., 1977. Genetic heterogeneity at two alcohol dehydrogenase loci in *Drosophila pseudoobscura* and *D. persimilis*. *Genetics, 87:* 285–304.

DAVIS, G. M., HEARD, W. H., FULLER, S. L. H. & HESTERMAN, C., 1981. Molecular genetics and speciation in *Elliptio* and its relationships to other taxa of North American Unionidae (Bivalvia). *Biological Journal of the Linnean Society, 15:* 131–150.

DILLON, R. T. & DAVIS, G. M., 1980. The *Goniobasis* of southern Virginia and northwestern North Carolina: Genetic and shell morphometric relationships. *Malacologia, 20:* 83–98.

EHRLICH, P. R. & WHITE, R. R., 1980. Colorado checkerspot butterflies: Isolation, neutrality, and the biospecies. *American Naturalist, 115:* 328–341.

FOWLER, H. W., 1906. *Fresh and salt water fish found in the waters of New Jersey*. Annual Report of the New Jersey State Museum for 1905, Part II.

FROST, S. W., 1959. *Insect Life and Insect Natural History* (2nd revised edition). New York: Dover Publications.

FULLER, B. & LESTER, L. J., 1980. Correlations of allozymic variation with habitat parameters using the grass shrimp, *Paleomonetes pugio*. *Evolution, 34:* 1099–1104.

FULLER, S. L. H., 1974. Clams and mussels (Mollusca: Bivalvia) In C. W. Hart & S. L. H. Fuller (Eds), *Pollution Ecology of Freshwater Invertebrates*. New York: Academic Press.

FUTUYMA, D. J. & MAYER, G. C., 1980. Non-allopatric speciation in animals. *Systematic Zoology, 29:* 254–271.

GOTTLIEB, L. D., 1974. Genetic stability in a peripheral isolate of *Stephanomeria exigua* ssp. *coronaria* that fluctuates in population size. *Genetics, 76:* 551–556.

GOWER, J. C. & ROSS, G. J. S., 1969. Minimum spanning trees and single linkage cluster analysis. *Applied Statistics, 18:* 54–64.

HALLIDAY, R. B., 1981. Heterozygosity and genetic distance between sibling species of meat ants (*Iridomyrmex purpureus* Group). *Evolution, 35:* 234–242.
HARDING, J. & BARNES, K., 1977. Genetics of *Lupinus*. X. Genetic variability, heterozygosity, and outcrossing in colonial populations of *Lupinus succulentus*. *Evolution, 31:* 247–255.
HIGHTON, R., 1980. Comparison of microgeographic variation in morphological and electrophoretic traits. *Evolutionary Biology, 10:* 397–436.
HIGHTON, R. & WEBSTER, T. P., 1976. Geographic protein variation and divergence in populations of the salamander *Plethodon cinereus*. *Evolution, 30:* 33–45.
HORNBACH, D. J., MCLEOD, M. J., GUTTMAN, S. I. & SEILKOP, S. K., 1980. Genetic and morphological variation in the freshwater clam *Sphaerium* (Bivalvia: Sphaeriidae). *Journal of Molluscan Studies, 46:* 158–170.
HYNES, H. B. N., 1970. *The Ecology of Running Waters*. Toronto: University of Toronto Press.
JOHNSON, G. B., 1976. Genetic polymorphism and enzyme function. In F. J. Ayala (Ed.), *Molecular Evolution:* 46–59. Massachusets: Sinauer Press.
JOHNSON, G. B., 1977. Assessing electrophoretic similarity. *Annual Review of Ecology and Systematics, 8:* 309–328.
JOHNSON, R. I., 1946. *Anodonta implicata* Say. *Occasional Papers on Mollusks, 1:* 109–116.
JOHNSON, R. I., 1970. The systematics and zoogeography of the Unionidae (Mollusca: Bivalvia) of the southern Atlantic Slope region. *Bulletin of the Museum of Comparative Zoology, 140:* 263–450.
JONES, J. S., 1981. Models of speciation—the evidence from *Drosophila*. *Nature, 289:* 773–774.
KAT, P. W., 1982. The relationship between heterozygosity for enzyme loci and development homeostasis in peripheral populations of freshwater bivalves (Unionidae). *American Naturalist, 119:* 96–103.
KAT, P. W., 1983a. Patterns of electrophoretic and morphologic variability in a widely distributed unionid: an initial survey. *Netherlands Journal of Zoology, 33:* 21–40.
KAT, P. W., 1983b. Genetic and morphological divergence among nominal species of North American *Anodonta*. *Malacologia, 23:* 361–374.
KAT, P. W., 1983c. Morphologic divergence, genetics and speciation among *Lampsilis* (Bivalvia: Unionidae). *Journal of Molluscan Studies, 49:* 133–145.
KIMURA, M. & CROW, J. F., 1964. The number of alleles that can be maintained in a finite populations. *Genetics, 49:* 725–738.
KIMURA, M. & OHTA, T., 1971. *Theoretical Aspects of Population Genetics*. Princeton: Princeton University Press.
LAING, C., CORMANDY, G. R. & PECK, S. B., 1976. Population genetics and evolutionary biology of the cave beetle *Ptomophagus hirtus*. *Evolution, 30:* 484–498.
LEFEVRE, G. & CURTIS, C. C., 1910. Studies on the reproduction and artificial propagation of freshwater mussels. *Bulletin of the United States Bureau of Fisheries, 30:* 105–201.
LEVIN, D. A., 1977. The organization of genetic variability in *Phlox drummondi*. *Evolution, 31:* 477–494.
LEWONTIN, R. C., 1974. *The Genetic basis of Evolutionary Change*. New York: Columbia University Press.
LIVINGSTON, D. A., 1951. The freshwater fishes of Nova Scotia. *Bulletin of the Nova Scotia Institute of Science, 23:* 1–90.
MANSUETI, R., 1960. Comparison of the movements of stocked and resident yellow perch, *Perca flavescens*, in tributaries of the Chesapeake Bay, Maryland. *Chesapeake Science, 1:* 21–35.
MCLEOD, M. J. & SAILSTAD, D. M., 1980. An electrophoretic study of *Corbicula fluminea* (Bivalvia: Corbiculidae) in the Catawba River. *Bulletin of the American Malacological Union, Inc. 1980:* 17–19.
MILLER, B. B., KARROW, P. F. & KALAS, L. L., 1979. Late Quaternary mollusks from glacial Lake Algonquin, Nipissing, and Transitional sediments from southwestern Ontario, Canada. *Quaternary Research, 11:* 93–112.
NEI, M., 1972. Genetic distance between populations. *American Naturalist, 106:* 282–292.
NEI, M., MARUYAMA, T. & CHAKRABORTY, R., 1975. The bottleneck effect and genetic variability in populations. *Evolution, 29:* 1–10.
ORTMANN, A. E., 1913. The Alleghenian divide and its influence upon the freshwater fauna. *Proceedings of the American Philosophical Society, 52:* 287–390.
OXFORD, G. S., 1975. The nature and distribution of food-induced esterases in helicid snails. *Malacologia, 17:* 331–339.
PIERCE, B. J. & MITTON, J. B., 1980. Patterns of allozyme variation in *Ambystoma tigrinum mavortium* and *A. t. nebulosum*. *Copeia, 1980:* 594–605.
RAMSHAW, J. A. M., COYNE, J. A. & LEWONTIN, R. C., 1974. The sensitivity of gel electrophoresis as a detector of genetic variation. *Genetics, 93:* 1019–1037.
ROHLF, E. J., KISHPAUGH, J. & KIRK, D., 1972. *NT-SYS: Numerical Taxonomy System of Multivariate Statistical Programs*. Stony Brook: State University of New York.
SARICH, V. M., 1977. Rates, sample sizes, and the neutrality hypothesis for electrophoresis in evolutionary studies. *Nature, 265:* 24–28.
SCHWAEGERLE, K. E. & SCHAAL, B. A., 1979. Genetic variability and founder effect in the pitcher plant *Sarracenia purpurea* L. *Evolution, 33:* 1210–1218.
SELANDER, R. K. & HUDSON, R. O., 1976. Animal population structure under close inbreeding: The land snail *Rumina* in southern France. *American Naturalist, 110:* 695–718.

SELANDER, R. K. & KAUFMAN, D. W., 1973. Genetic variability and strategies of adaptation in animals. *Proceedings of the National Academy of Sciences of the United States of America, 70:* 1875–1877.
SEPKOSKI, J. J. Jr. & REX, M. A., 1974. Distribution of freshwater mussels: Coastal rivers as biogeographic islands. *Systematic Zoology, 23:* 165–188.
SHUMAKER, K. M. & BABBEL, G. R., 1980. Patterns of allozymic similarity in ecologically central and marginal populations of *Hordeum jubatum* in Utah. *Evolution, 34:* 110–116.
SMITH, M. H., BRITTON, J., BURKE, P., CHEUER, R. K., SMITH, M. W. & HAGEN, J., 1979. Genetic variability in *Corbicula*, an invading species. In J. Britton (Ed.), *Proceedings, First International Corbicula symposium:* 243–248. Texas: Texas Christian University.
TEMPLETON, A. R., 1980a. The theory of speciation via the founder principle. *Genetics, 94:* 1011–1021.
TEMPLETON, A. R., 1980b. Modes of speciation and inferences based on genetic distances. *Evolution, 34:* 719–729.
VALENTINE, J. W., 1976. Genetic strategies of adaptation. In F. J. Ayala, (Ed.) *Molecular Evolution:* 78–94. Sunderland, Massachusetts: Sinauer Press.
VAN DER SCHALIE, H., 1945. The value of mussel distribution in tracing stream confluence. *Papers of the Michigan Academy of Arts and Letters, 20:* 355–373.
VAN DER SCHALIE, H., 1970. Hermaphroditism among North American freshwater mussels. *Malacologia, 10:* 93–112.
WILES, M., 1975. The glochidia of certain Unionidae (Mollusca) in Nova Scotia and their fish hosts. *Canadian Journal of Zoology, 53:* 33–41.
ZERA, A., 1981. Genetic structure of two species of water striders (Gerridae: Hemiptera) with differing degrees of winglessness. *Evolution, 35:* 218–225.

APPENDIX 1: TAXA STUDIED

Classification, ANSP catalog numbers, and collection localities of populations studied. N = number of individuals electrophoresed. Unless otherwise noted, all localities from Nova Scotia, Canada; refer to Figure 1 for location of stations (C1 = Canadian Station 1, etc.).

Unionidae
Anodontinae

Anodonta cataracta fragilis

1. ANSP 354362; $N = 20$. First Lake O' Law (C4), *c.* 25 km NW Baddeck, Victoria Co.
2. ANSP 354564; $N = 20$. Shaw Lake (C6), *c.* 5 km NNE Arichat, Isle Madame, Richmond Co.
3. ANSP 254566; $N = 20$. Newville Lake (C2), Halfway River East, Cumberland Co.

Anodonta implicata

1. ANSP 354582; $N = 20$. French Lake (C1) at Sunbury–Oromocto State Park, *c.* 10 km S Oromocto, Sunbury Co., New Brunswick.
2. ANSP 356702; $N = 20$. Sydney River (C5), Blachette Lake, *c.* 6 km SW Sydney, Cape Breton Co.
3. ANSP 354574; $N = 20$. Shubenacadie Grand Lake (C9), Grand Lake, Halifax Co.

Alasmidonta undulata

1. ANSP 354559; $N = 15$. Kennetcook River (C10), Riverside Corner, Hants Co.

2. ANSP 354575; $N = 20$. Nine Mile River (CB), Elmsdale, Hants Co.

Ambleminae
Lampsilini

Lampsilis radiata

1. ANSP 354583; $N = 20$. French Lake (C1) at Sunbury–Oromocto State Park, *c.* 10 km S Oromocto, Sunbury Co., New Brunswick.
2. ANSP 354572; $N = 20$. Shubenacadie Grand Lake (C9), Grand Lake, Halifax Co.
3. ANSP 354560; $N = 20$. Lake Egmont (C7), *c.* 3 km S Cooks Brook, Halifax Co.
4. ANSP 354577; $N = 20$. Mattatall Lake (C3), *c.* 6 km E Wentworth Centre, Cumberland Co.
5. ANSP 354567; $N = 20$. Newville Lake (C2), Halfway River East, Cumberland Co.

Lampsilis ochracea

1. ANSP 354579; $N = 10$. Placide Lake (C12), *c.* 1 km SW Havelock, Digby Co.
2. ANSP 354570: $N = 15$. Sydney River (C5), Blachette Lake, *c.* 6 km SW Sydney, Cape Breton Co.

Lampsilis cariosa

1. ANSP 354571; $N = 10$. Sydney River (C5), Blachette Lake, *c.* 6 km SW Sydney, Cape Breton Co.

Pleurobemini

Elliptio complanata

1. ANSP 354581; $N = 20$. French Lake (C1) at Sunbury Oromocto State Park, *c.* 10 km S Oromocto, Sunbury Co., New Brunswick.
2. ANSP 354578; $N = 20$. Mattatall Lake (C3), *c.* 6 km E Wentworth Centre, Cumberland Co.
3. ANSP 354573; $N = 20$. Shubenacadie Grand Lake (C9), Grand Lake, Halifax Co.
4. ANSP 354568; $N = 20$. Newville Lake (C2), Halfway River East, Cumberland Co.
5. ANSP 354580; $N = 20$. Placide Lake (C12), *c.* 1 km SW Havelock, Digby Co.
6. ANSP 354565; $N = 20$. Shaw Lake (C6), *c.* 5 km NNE Arichat, Isle Madame, Richmond Co.
7. ANSP 354576; $N = 20$. Nine Mile River (C8), Elmsdale, Hants Co.
8. ANSP 354569; $N = 20$. Sydney River (C5), Blachette Lake, *c.* 6 km SW Sydney, Cape Breton Co.

9. ANSP 354563; $N = 20$. First Lake O' Law (C4), *c.* 25 km NW Baddeck, Victoria Co.
10. ANSP 354561; $N = 20$. Nowlans Lake (C11), Havelock, Digby Co.
11. ANSP 354558; $N = 20$. Lac A Pic (C13), *c.* 1 km E Springhaven, Yarmouth Co.
12. ANSP 350069; $N = 25$. Susquehanna River, Cumberland Co. Pennsylvania, U.S.A.
13. ANSP 349150; $N = 25$. Kennebec River, Somerset Co. Maine.
14. ANSP 349333; $N = 25$. Nanticoke River, Sussex Co. Delaware.
15. ANSP 345056; $N = 25$. Sassafras River, Kent Co., Maryland.

APPENDIX 2. POPULATION GROWTH RATE AMONG INTRODUCED UNIONIDS

According to Nei *et al.* (1975), the rate of population growth following a bottleneck is a critical determinant of how much heterozygosity will be lost in a newly-established population initiated by a few founder individuals. In order to determine rate of population growth among unionids, a hypothetical model of such growth was constructed which incorporates actual life-history data gathered by Lefevre and Curtis (1910), Coker (1921), and Coker *et al.* (1921). In this model, unionids are compared with a hypothetical insect species with five generations per year, such as is encountered among some *Drosophila*, an insect frequently studied in colonization experiments. The model was constructed with the following assumptions:

(1) Fish hosts carry about 500 glochidia parasitic on gills per individual. A higher level of infection can induce mortality among the fish, and this level of infection was considered maximal by Lefevre & Curtis (1910) for natural populations. Actual levels of infection calculated from 3671 fish of 46 species averaged roughly 125 glochidia per individual fish (range—1–416) (Coker *et al.*, 1921). The higher number is used in the model.

(2) Coker *et al.* (1921) stress that infection of the fish host by glochidia is a matter of chance. Glochidia will die if they do not attach to the fish host soon after release from the maternal individual, and will not complete metamorphosis unless they attach to one of a very limited number of suitable hosts. These rather stringent conditions seem to contrast with a very low level of selectivity among the glochidia: both Coker (1921) and personal observations indicate that glochidia will attach to almost any substratum, including slivers of wood. Coker *et al.* (1921) mention that less than 3% of the 3671 fish examined were infected with glochidia. In the model an artificially high number of fish (20) was chosen to carry glochidia into the new habitat, which, according to the frequencies observed by Coker *et al.* (1921), would represent a total introduction of roughly 700 fish into the new habitat. An identical number of insects form their founder population.

(3) High mortality is associated with the first year class in the model, after which mortality decreases slowly. When juveniles complete metamorphosis (after a period of 1–3 weeks according to Coker *et al.*, 1921) and drop off the fish, they are no more than 2–3 mm in length, and are susceptible to predation by a diverse group of animals (e.g. turbellarians, chaetopods, crayfish, insects, fish, turtles, birds, etc.) as well as other types of mortality (Coker *et al.*, 1921). In

carefully controlled culture conditions, Coker (1921) and Coker *et al.* (1921) were able to achieve about 10% survival of the first year class. This level of survival is incorporated in the model, and increases by 10% for each successive year class. Identical rates of mortality were imposed on the insect for comparative purposes.

(4) The numbers of glochidia produced by females in natural populations vary considerably with size of the individuals and from species of species. Coker *et al.* (1921) observed a range of 75 000 large glochidia to 3 000 000 small glochidia for females of different species. In the model, it is assumed that 4000 glochidia per female will survive to the initial juvenile stage. Again, this number is probably highly exaggerated; frequency of attachment of glochidia per female to the proper fish host is extremely low (Coker, 1921; Coker *et al.*, 1921), and only 25% of those which attach survive to metamorphosis under carefully controlled laboratory conditions (Coker, 1921). The hypothetical insect was arbitrarily assigned 200 offspring per reproductive event, an average number according to Frost (1959).

(5) The hypothetical unionid does not begin reproducing until its fifth year (range among unionids: 2–8 years according to Coker *et al.*, 1921), reproduces once per year, and reproduces six times before dying. Average lifespan for unionids is not known, and probably varies tremendously from habitat to habitat. To counter the effects of a possibly shorter-than-average lifespan in the model, females were assigned an extremely high reproductive rate of 9000 females/female. The hypothetical insect reproduces after two months, and lives one year.

(6) An unlimited number of fish hosts is present subsequent to colonization; females do not have to compete for hosts. Actual data (Coker *et al.*, 1921) indicate that fish hosts acquire immunity to glochidial infection, and this could be an important limiting factor for colonizing unionids, which conceivably have to rely on the limited number of concurrently introduced hosts several times in order to reproduce successfully. This limitation is ignored in the model.

When these assumptions, a life-history table was constructed for the unionid (Table A1) and the insect (Table A2), and growth of the unionid population is followed for 20 years. It becomes apparent that unionid populations grow very slowly: after 20 years with a net reproductive rate (R_0) of 9000 females/female, 92 adult females are present and 186 279 juvenile females, 90% of which are in the highest-mortality age class. The rapidly reproducing insect population can form a population of comparable size in less than two years. Since mortality rates among juvenile unionids are probably much higher in nature than here assumed (Fuller, 1974), it becomes apparent that even when juveniles are introduced into a habitat in large numbers, none might survive to maturity. Also once a large number of adults become established in a population, it is conceivable that the number of non-immune fish hosts could become limiting to further population growth. In the model, 920 fish carrying 500 glochidia each are required to serve as hosts for the 92 adult females present in the population in year 20 of population growth; such host densities might not be encountered in the area of introduction during the initial reproductive seasons of the unionids.

Table A1. Life-history table for a hypothetical unionid population

	Age:	1	2	3	4	5	6	7	8	9	10	11
	l_x*	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	0
	m_x**	0	0	0	0	2000	2000	2000	2000	2000	2000	0
Age†		0	1	2	3	4	5	6	7	8	9	10
Year‡												
0		5000										
1			500									
2				100								
3					30							
4						12						
5		12000					6					
6		8000	1200					4				
7		6000	800	240					3			
8		4000	600	160	72					2		
9		4000	400	120	48	28					2	
10		4000	400	80	36	19	14					2
11		28 000	400	80	24	14	9		8			
12		34 000	2800	80	24	9	7		5	5		
13		34 000	3400	560	24	9	4		4	3	3	
14		28 000	3400	680	168	9	4		2	3	2	3
15		28 000	2800	680	204	67	4		2	1	2	2
16		28 000	2800	560	204	81	33		2	1	1	2
17		82 000	2800	560	168	81	33		20	1	1	1
18		116 000	8200	560	168	67	40		20	14	1	1
19		154 000	11 600	1640	168	67	33		24	14	11	1
20		168 000	15 400	2320	492	67	33		20	17	11	10

*l_x, Fraction of females surviving to each age from previous cohorts.
**m_x, Average number of female offspring produced per female at each age.
†Age, age of the individuals in each cohort.
‡Year, number of years the population has been established.
The vertical line indicates the division between juveniles to the left and reproducing females to the right.

Table A2. Life-history table for a hypothetical insect population

Age (in fifths)		1	2	3	4	5	6
l_x*		0.1	0.2	0.3	0.4	0.5	0
m_x**		0	100	100	100	100	0
Age† (in fifths)	0	1	2	3	4	5	6
Year (in fifths)‡							
0	5000						
1		500					
2	10 000		100				
3	3 000	1000		30			
4	21 200	300	200		12		
5	12 600	2120	60	60		6	
6	44 600	1260	424	18	24		0
7	39 600	4460	252	127	7	10	
8	106 100	3960	932	75	50	4	0
9	112 700	10 610	792	280	30	25	0
10	248 700	11 270	2122	238	112	15	0

*l_x, Fraction of females surviving to each age from previous cohorts.
**m_x, Average number of female offspring produced per female at each age.
†Age, age of the individuals in each cohort.
‡Year, number of years the population has been established. Since the lifespan of each insect was limited to one year, the years are here broken up into fifths.
The vertical line indicates the division between juveniles to the left and adult females to the right.