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Genetic changes within freshwater bryozoan populations suggest temporal gene flow from statoblast banks

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Abstract

Temporal changes in the genetic structure of populations of the freshwater bryozoan *Cristatella mucedo* were investigated by genotyping colonies at five microsatellite loci. Colonies were collected from five different locations over time periods ranging from 1 month to 5 yr. Our findings identified substantial within-site genetic changes, both within and between years, that were not consistent with variation produced by sexual reproduction. There was a general trend toward a decrease in genetic diversity throughout the growing season, possibly due to clonal selection. In addition, 18 out of 21 within-site comparisons showed a significant difference in the allele frequencies at one or more loci. Two of the populations revealed identical clones in >1 yr. At one site, an apparent population crash occurred in 1993; nevertheless, numerous clones were present in 1994 and 1995, including two multilocus genotypes that were present at the same site in 1992. Since the populations from before and after the bottleneck remained genetically similar, the most plausible source of these clones is reintroduction from a statoblast bank rather than through introductions from other genetically distinct populations. These findings, in conjunction with earlier studies, indicate that propagule banks influence the population dynamics of a wide range of freshwater invertebrates in a number of ways, including the enhancement of within-population genetic diversity and a decreased likelihood of local extinctions.

Our understanding of a species' ecology and evolution can be greatly enhanced by studying patterns of population genetics. For example, data from a variety of nuclear and mitochondrial markers provide information on genetic variation within populations, and this in turn allows inferences about processes as diverse as evolutionary history, population viability, mating systems, and natural selection. Studies of population genetics also reveal levels of genetic differentiation and gene flow among populations. Dispersal and gene flow may be critical to the survival of individuals seeking to escape local threats such as pollution, predators, or parasites. Immigrants may also be important for introducing new genes into populations, thereby increasing levels of genetic variation (Freeland et al. 2000*a*) and possibly contributing to the long-term viability of local populations.

The increased accessibility of molecular markers over recent years has allowed population genetic studies on a wide variety of taxa including freshwater invertebrates. Much of the freshwater research has been conducted on zooplankton, including cladocerans (e.g., Hebert and Finston 1996), copepods (e.g., Boileau and Hebert 1991), and rotifers (e.g., Gómez et al. 1995). Studies of zooplankton have revealed varying levels of dispersal and gene flow across a variety of spatial scales, with some populations showing little genetic differentiation over wide geographic regions and others showing marked genetic discontinuity over small distances (e.g., Straughan and Leman 2000). In addition to dispersal in space, dispersal in time may occur when organisms produce dormant stages, resulting in temporal gene flow via egg banks (also termed propagule banks), which are analogous to the seed banks of plants (Leck et al. 1989). The importance of such long-term (>1 yr) temporal gene flow within freshwater zooplankton populations is highlighted by the fact that diapausing eggs of some cladocerans and copepods can remain viable in lake and pond sediments for up to several hundred years (Hairston et al. 1995; Caceres 1998). Temporal gene flow resulting from release of viable eggs from the sediments may provide the same benefits as spatial gene flow, including an escape from local threats, and a subsequent increase in within-population genetic variation. The genetic diversity harbored within a propagule bank can also influence the rate and direction of microevolution following directional and temporally fluctuating selection (Hairston et al. 1996).

Propagule banks may be particularly important to freshwater taxa that are incapable of actively dispersing among sites and therefore have only a limited potential for escape when local conditions within a site become unfavorable. However, temporal gene flow in freshwater invertebrates has to date been studied only in zooplankton (e.g., Caceres 1998; Gómez and Carvalho 2000). Further insight into the general significance of this process requires study of other freshwater organisms that are also incapable of active dispersal but that

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have contrasting life histories to those of the zooplankton taxa. One such organism is the bryozoan *Cristatella mucedo* (Phylum Bryozoa, Class Phylactolaemata). Here, we present evidence, based on investigations into the population genetics of *C. mucedo,* that temporal gene flow appears to be a widespread phenomenon among freshwater invertebrates and occurs in organisms with diverse life histories.

C. mucedo is a sessile, hermaphroditic, colonial species with predominantly asexual reproduction that includes colony growth through zooidal budding, colony fission, and, most commonly, the production of small (≈1 mm), buoyant, seedlike structures called statoblasts. Statoblasts remain dormant over winter and are resistant to desiccation and temperature extremes (Wood 1991; Okamura and Hatton-Ellis 1995). At least some can survive passage through duck, amphibian, and reptile digestive tracts (Brown 1933; Charambilidou pers. comm.). These propagules are the only means by which *C. mucedo* survives winter conditions and disperses to other sites. Hooks and spines on statoblast margins allow attachment to feathers and fur, thereby promoting dispersal of *C. mucedo* by animal vectors (Okamura and Hatton-Ellis 1995). Sexual reproduction occurs in early summer and results in the production of short-lived, mobile larvae that can achieve limited dispersal prior to settling on suitable substrata. Genetic characterization of maternal colonies and offspring has revealed the presence of extraparental genetic material (Jones et al. 1994), demonstrating that at least some larvae result from cross-fertilization, although self-fertilization cannot be discounted in all cases. The sexual phase is relatively brief and may be foregone in some years (Okamura 1997). The timing of sexual reproduction is unusual within facultatively sexual freshwater taxa. A more common reproductive pattern, as seen in many zooplankton groups, combines clonal reproduction throughout the growing season with sexual reproduction at the end of the summer, thereby generating genetically variable individuals for the reestablishment of populations in the following year (Lynch and Spitze 1994).

The unusual strategy of dispersing via asexually produced propagules undoubtedly contributes to the novel aspects of the population genetics of *C. mucedo.* Previous studies of *C. mucedo* populations have revealed negligible to low levels of spatial gene flow, low to high levels of within-population genetic diversity, and consistent deviations from Hardy–Weinberg equilibrium that were characterized by observed heterozygosity deficits (Freeland et al. 2000*a*,*b*). While some of the heterozygosity deficit may be explained by inbreeding among closely related genotypes, it could also at least partially result from the Wahlund effect following the mixing of individuals or genotypes from genetically dissimilar backgrounds (Freeland et al. 2000*a*). These earlier studies concluded that the high levels of genetic variation observed within many *C. mucedo* populations could not be explained entirely by sexual reproduction or spatial gene flow. However, a possible role of temporal gene flow that could allow for the reintroduction of diverse genotypes remained largely unexplored. Preliminary studies based on Random Amplified Polymorphic DNA (RAPD-PCR) suggested such a process by identifying changes in the genetic make-up of populations over time, although the mechanisms driving these changes remained unclear (Vernon et al. 1996; Hatton-Ellis 1997). The occurrence of complete *C. mucedo* statoblasts in sediment cores from various lakes (Bennike et al. 1998) supports the hypothesis of a statoblast bank, as does the hatching of a *C. mucedo* statoblast collected from shallow lake sediments (Freeland pers. comm.). In addition, statoblasts from several freshwater bryozoan genera are known to remain viable in sediment then germinate when conditions become favorable (Oda 1979; Wood and Marsh 1996), and statoblast viability for periods ≥2 yr has been demonstrated in the laboratory for multiple bryozoan species (Pennak 1989; Wood 1991).

The aim of this study was to use evidence from genetic data collected over varying time periods to establish whether *C. mucedo* genotypes are reintroduced into local populations from statoblast banks. In particular, we characterized temporal changes in allele frequencies, clonal genotypes, and levels of genetic diversity and inferred levels of gene flow within and among sites. Our results, based on analyses of microsatellite data from five different populations sampled over time scales ranging from 1 month to 5 yr, provide strong evidence for long-term temporal gene flow and suggest a role of statoblast banks in the maintenance of genetic diversity within populations.

Methods

Although bryozoan colonies are composed of multiple lophophores, they behave as cohesive units and may therefore be loosely defined as individuals. In this paper, the term ''colony'' can generally be interpreted as equivalent to the term ''individual.'' The term ''clone'' in this paper refers to the genotype of a colony and, because bryozoans can reproduce both sexually and asexually, distinct colonies may or may not equal distinct genotypes (*see below*).

Collection and genotyping—Colonies were collected from four populations in Great Britain and one in Scotland (Fig. 1), each of which was resampled on two to six separate occasions (Table 1). Beale Bird Park Lake was specifically targeted as a site to sample repeatedly due to the presence of myxozoan parasites in the population (Okamura 1996; Vernon et al. 1996). Standard collections of colonies (*see below*) from this site were made in 1991, 1994, and 1995 and three times in 1992. No samples were obtained in 1993 from this locality because only three colonies were located after prolonged searches during repeated visits to the site, and these exhibited extreme morbidity. Other sites were included because ongoing studies of the molecular ecology of *C. mucedo* resulted in the accumulation of collections from a subset of the many populations that have been the focus of genetic study over recent years. The particular set of populations included in this study reflects their proximity to various researchers involved in these genetic studies.

Approximately 30 colonies were collected from substrata around the edges of the ponds and lakes or from aquatic plants, branches, and other moveable objects that were retrieved with a grappling hook. Colonies were collected from haphazardly encountered areas within sites, collections being restricted by both the patchy distribution of colonies and the

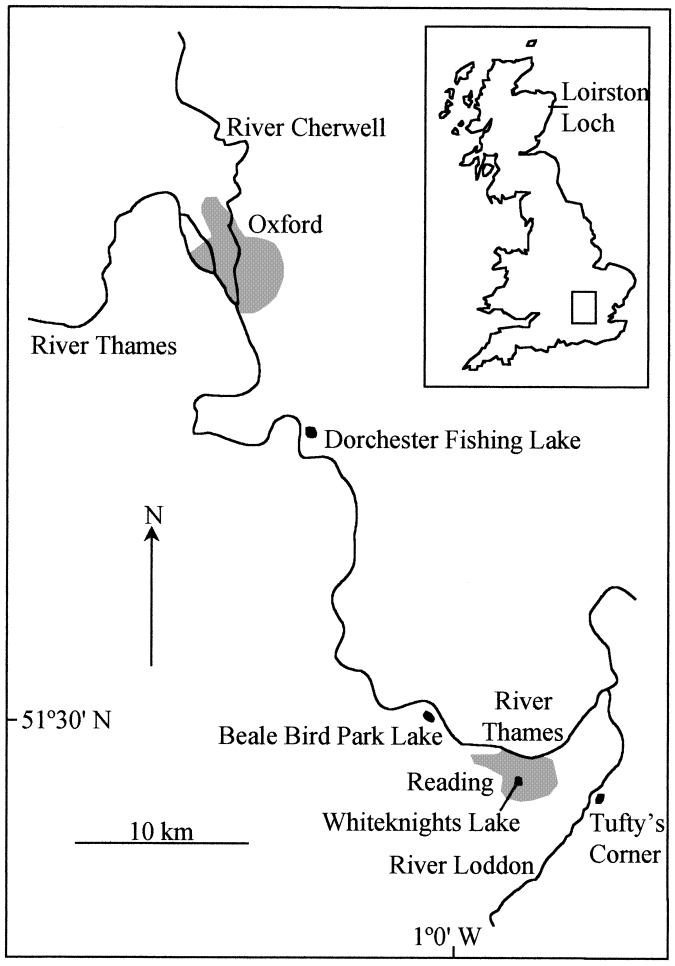

Fig. 1. Map showing the locations of sites.

difficulties associated with accessing some parts of the sites. The standard protocol was to spend up to 60 min searching for colonies in discrete habitat patches. In some cases, <30 colonies were encountered for collection during the standard 60-min period. To increase the likelihood that all of the colonies were derived from separate statoblasts or larvae, only one colony was taken from clumped distributions on a single substratum. However, due to the production of overwintering asexual statoblasts, colonies collected from separate substrata can be genetically identical. Collections were made from the same stretch of shoreline at each sampling date. The colonies were stored in 99% ethanol at 4°C.

DNA was extracted from ≈1 mm³ of tissue in 250 ml 5% Chelex (Bio-Rad) in ddH_2O, following the manufacturer's protocol. The colonies were genetically characterized on the basis of five polymorphic microsatellite loci (1.1, 2.2, 5.9, 6.7, and 9.4; Freeland et al. 1999). Amplification and visualization protocols are described in Freeland et al. (2000*a*).

Genetic characterization of populations—To characterize each population at each sampling date, we calculated the number of clones (identified as identical multilocus genotypes) and the levels of genetic diversity. There were three methods for describing genetic diversity within a population:

Table 1. Site location (national grid reference), date, relative date (number of days after 01 May), No. of colonies (N), No. of unique clones, No. of unique clones/number of colonies (D*), total No. of alleles (Na), expected heterozygosity (H_e), and observed heterozygosity (H_o).

Location and national grid reference	Date	Relative date	N	Clones	D*	Na	H_e (SD)	H_o (SD)
Beale Bird Park Lake, Great Britain (SU 618784)	09 Oct 91	162	30	18	0.60	12	0.35 (0.25)	0.17 (0.24)
Beale Bird Park Lake	09 Jul 92	70	30	8	0.27	10	0.27 (0.27)	0.03 (0.06)
Beale Bird Park Lake	19 Aug 92	111	29	2	0.07	6	0.01 (0.03)	0 (0)
Beale Bird Park Lake	03 Sep 92	126	28	5	0.18	8	0.01 (0.09)	0.05 (0.09)
Beale Bird Park Lake	04 Aug 94	96	20	8	0.40	11	0.37 (0.34)	0.12 (0.20)
Beale Bird Park Lake	25 Jul 95	68	31	28	0.90	22	0.49 (0.30)	0.10 (0.12)
Tufty's Corner, Great Britain (SU 774719)	19 Aug 97	111	30	13	0.43	16	0.46 (0.09)	0.10 (0.15)
Tufty's Corner, Great Britain	28 Jul 98	89	28	15	0.54	20	0.54 (0.19)	0.22 (0.36)
Tufty's Corner, Great Britain	19 Sep 98	142	30	21	0.70	22	0.56 (0.16)	0.09 (0.16)
Dorchester Fishing Lake, Great Britain (SU 580948)	11 Jun 92	41	21	7	0.33	15	0.47 (0.11)	0.20 (0.37)
Dorchester Fishing Lake	23 May 94	23	31	30	0.97	30	0.70 (0.14)	0.08 (0.14)
Whiteknights Lake, Great Britain (SU 737724)	10 Aug 93	102	22	2	0.09	10	0.35 (0.34)	0.60 (0.55)
Whiteknights Lake	06 Sep 93	129	30	9	0.30	12	0.35 (0.34)	0.27 (0.44)
Loirston Lake, Scotland (NJ 938009)	18 Aug 97	110	30	1	0.03	5	0 (0)	0 (0)
Loirston Lake, Scotland	06 Sep 98	129	30	1	0.03	5	0 (0)	0 (0)

expected heterozygosity (H_e), total number of alleles, and number of unique clones as a proportion of the total number of colonies collected (D*). Measures of linkage disequilibrium, Hardy–Weinberg equilibrium, and expected and observed heterozygosity values (H_o and H_e) were calculated in Popgene (Yeh et al. 1997). For each population and each sampling date, we calculated allele frequencies and assessed within-population changes in allele frequencies in Genepop using a *G*-based probability test, which applies a Markov chain method to obtain Fisher exact test statistics (Raymond and Rousset 1995). The interpretation of probability values was adjusted with the Bonferroni method.

We assigned a number to each collection to represent the date on which it was collected. The start of the growing season varies, but it is unlikely to be earlier than 1 May, which we chose as day 1. All samples were given a date, termed the ''relative date,'' which was calculated as the number of days after day 1 on which each collection was made. We tested for relationships between the relative date and the levels of genetic diversity, measured as the total number of alleles, using a linear regression. For this comparison, the relative date was ln-transformed to normalize the residuals of the regression. In additional comparisons, we investigated possible relationships between observed heterozygosity (H_o) and both genetic diversity and relative date.

Investigation of gene flow—We inferred patterns of gene flow by examining the relationships among populations based on three independent methods: (1) reconstructing neighbor-joining trees based on genetic distances among sites and sampling dates; (2) indirectly estimating gene flow (N*m*) among populations based on an analog of F_{ST}; and (3) searching for instances of the same multilocus genotype occurring at more than one sampling date or location. A distance matrix of Nei's genetic distances (D: Nei 1978) among sites and sampling dates was imported into Phylip vers. 3.5 (Felsenstein 1995) to reconstruct neighbor-joining trees (Saitou and Nei 1987). This was conducted using both the full data set, which included data for all colonies sampled, and the data set from which genetically identical colonies (clonal replicates) were removed. To obtain estimates of N*m*, levels of population subdivision were calculated in RstCalc (Goodman 1997) as Rho, an unbiased measure of R_{ST} (an analog of F_{ST}) that corrects for potential biases that may result from unequal sample sizes and loci with unequal variances. N*m* values were calculated from Rho using the formula $Nm = ¼[(1/Rho) - 1]$, where N is the size of the local population, and *m* is the average rate of immigration per generation (Slatkin 1987). Although estimates of N*m* from F_{ST} (and analogs) have been criticized, as they make a number of assumptions that are often unrealistic (Whitlock and McCauley 1999), N*m* provides a useful measure here for comparing the relative rates of gene flow within and among sites. While there are a number of additional techniques for examining the distribution of genetic variation, we felt that these three methods for assessing gene flow were sufficient for our purposes because the results were consistent regarding the relative relationships of populations within and among sites (*see below*).

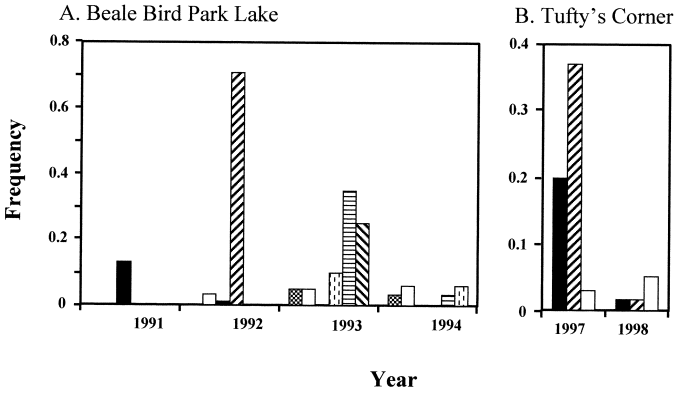

Fig. 2. Changes in the frequency of identical clones found in >1 yr at (A) Beale Bird Park Lake (six clones), and (B) Tufty's Corner (three clones).

Results

Genetic characterization of populations—There was considerable variation in the levels of genetic diversity within sites. Table 1 shows the number of clones, the number of alleles, D*, H_e, and H_o for each population. In four populations, a single locus revealed Hardy–Weinberg proportions. The remaining loci in all populations were in Hardy–Weinberg disequilibrium. The majority of H_o values were lower than H_e values (Table 1). Consistent heterozygosity deficits have been found in other *C. mucedo* populations, and proposed mechanisms underlying these patterns have been discussed elsewhere (Freeland et al. 2000*a*,*b*). When clonal replicates were removed from the data set, six pairs of alleles distributed among three populations showed linkage disequilibrium, a number that is unlikely to significantly affect our analyses.

Evidence for gene flow—No identical clones were found at more than one site, but there were several instances of the same clone occurring in the same site at different sampling times. The most striking example of this was seen in the Loirston Loch population, which was represented by a single clone in 1997 and 1998. In addition, there were six instances of the same clone occurring in >1 yr at Beale Bird Park Lake and three instances of the same clone occurring in >1 yr in Tufty's Corner (Fig. 2).

Values of Nei's D, Rho, and N*m* for within- and among-population comparisons are shown in Table 2. All within-population comparisons had smaller genetic distances than among-population comparisons, with the exception of Tufty's Corner, whose collection from August 1997 was more similar to Dorchester Fishing Lake in June 1992 than it was to Tufty's Corner in July 1998. As N*m* is derived from Rho, this naturally translates into all populations except Tufty's Corner having higher levels of within-population than among-population gene flow, a pattern that is corroborated by the finding of identical clones at different sampling dates but not at different locations. The low estimates of gene flow among sites are further supported by the finding of unique alleles at each location (3 at Beale Bird Park Lake, 13 at Tufty's Corner, 10 at Dorchester Fishing Lake, and 1

Table 2. Range, mean (±SE), and number of pairwise comparisons for within- and among-site measurements of Nei's D (genetic distances; Nei 1978), Rho (genetic differentiation; Goodman 1997), and N*m* (gene flow=¼[(1/Rho) − 1]).

	Within sites, among years			Among sites		
Location	Nei's D	Rho	N*m*	Nei's D	Rho	N*m*
Beale Bird	0.003–0.303	0.012–0.189	1.076–20.380	0.602–3.639	0.421–1.000	0.000–0.380
Park Lake	0.157±0.098	0.092±0.012	4.905±1.634	1.492±0.089	0.687±0.021	0.132±0.012
Tufty's	0.069–0.787	0.096–0.317	0.540–2.358	0.771–4.156	0.252–0.749	0.084–0.744
Corner	0.538±0.234	0.242±0.073	1.148±0.605	1.679±0.154	0.587±0.020	0.198±0.021
Dorchester				0.561–1.260	0.252–0.679	0.118–0.744
Fishing Lake	0.283	0.180	1.136	0.868±0.036	0.492±0.028	0.290±0.031
Whiteknights				0.478–23.90	0.259–0.796	0.047–0.716
Lake	0.129	0.256	0.726	1.327±0.115	0.613±0.031	0.193±0.030
Loirston Loch				0.478–4.156	0.422–1.000	0.000–0.343
	No within-population change			1.606±0.175	0.745±0.041	0.119±0.025

at each of Whiteknights Lake and Loirston Loch; *see* Web Appendix 1 at http://www.aslo.org/lo/pdf/vol_46/issue_5/1121a/.pdf).

Figure 3 shows the relationships among all sampling dates and locations, based on the calculations of Nei's D using all data. With the exception of Tufty's Corner, all populations clustered tightly according to location. The same relationships, although with slightly different distance measurements, are seen when clonal replicates are removed from the data set (tree not shown).

Allele frequencies are shown in Web Appendix 1. Figure 4 shows substantial changes in allele frequencies of the most variable loci within populations that were sampled during >1 yr (with the exception of Loirston Loch, which showed no change in genetic composition between years). For this comparison, data from the same year and the same site were added together to increase sample sizes. Of the 21 within-population pairwise comparisons, 18 exhibited significantly different allele frequencies in at least one locus. The three exceptions were comparisons between Beale Bird Park Lake in August 1992 and September 1992, Beale Bird Park Lake in August 1994 and July 1995, and Loirston Loch in August 1997 and September 1998.

Discussion

The data in this study illustrate that, with the exception of one site, the genetic composition and diversity of *C. mu-*

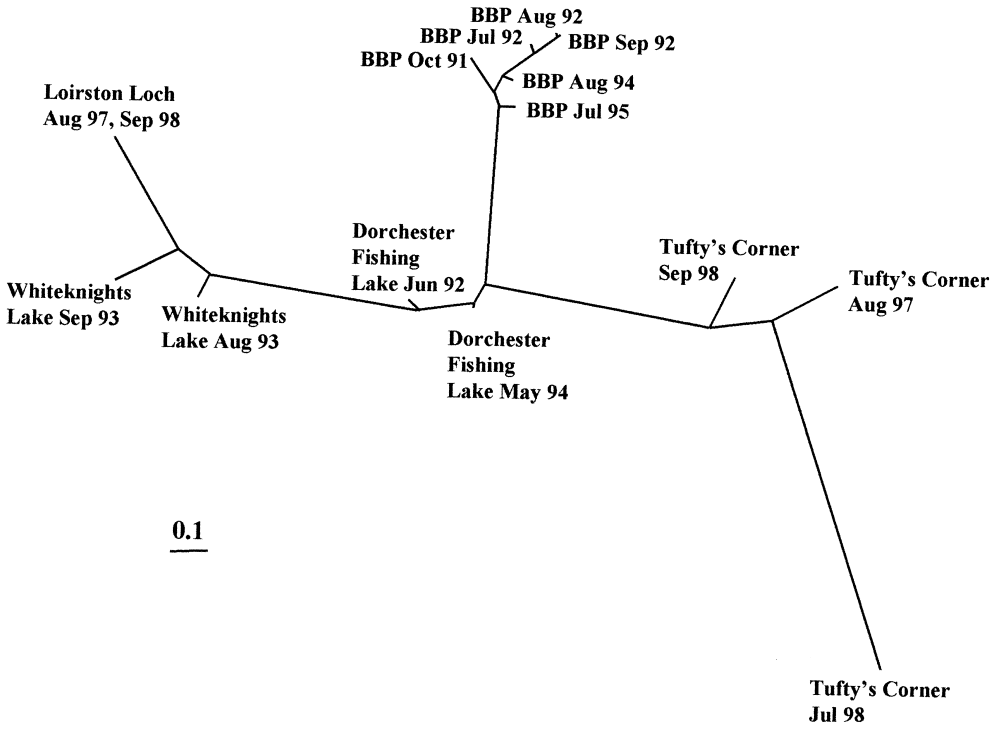

Fig. 3. Neighbor-joining tree of all sampling locations and dates based on Nei's genetic distance D. BBP, Beale Bird Park Lake. Branch lengths (scaled) are calculated following Saitou and Nei (1987).

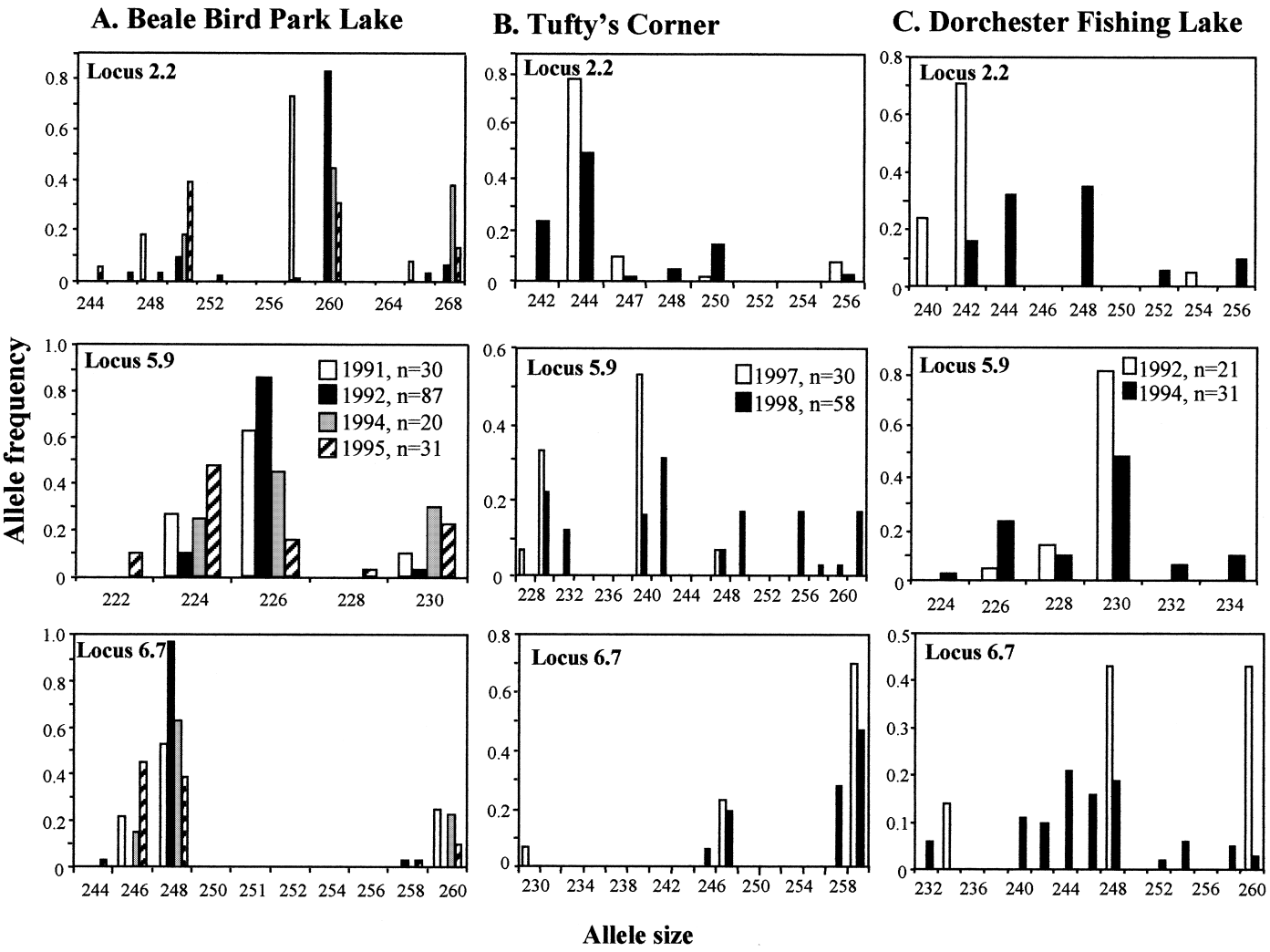

Fig. 4. Changes in allele frequencies of the three most polymorphic loci (2.2, 5.9, and 6.7) shown for the three variable populations that were sampled in >1 yr. Within-year samples were added together, and single frequency values were calculated for each year. (A) Beale Bird Park Lake. (B) Tufty's Corner. (C) Dorchester Fishing Lake.

cedo populations change substantially both within growing seasons and among years. In addition, as found in earlier studies (Hatton-Ellis et al. 1998; Freeland et al. 2000*a*,*b*), populations at different sites were genetically distinct and had variable, and often high, levels of genetic diversity. An exceptional population was found in Loirston Loch, which showed no evidence of temporal genetic change and lacked genetic variation. This population is likely to be near the northern limit of the range of *C. mucedo* in Great Britain and was apparently founded by a single clone that has subsequently proliferated asexually via colony fission and statoblast production. Since this population is not typical of the populations investigated in this or in other studies (Hatton-Ellis et al. 1998; Freeland et al. 2000*a*,*b*), it is largely excluded from subsequent discussion.

Causes of temporal genetic change—The four generally cited explanations for temporal variation in allele frequencies are sampling drift, genetic drift, selection, and migration. We cannot statistically rule out the effects of sampling drift and genetic drift because the assayed loci were too polymorphic to be analyzed with a program that is specifically designed to test for these processes (Temptest; Waples 1989). In addition, it is extremely difficult to predict genetic drift (or lack thereof) from population size because the large number and patchy distribution of colonies at some sites, combined with restricted access to lakes, means that counting colonies is unrealistic. Furthermore, even if a thorough census were obtained, it would not provide an estimate of the effective population size because an unknown proportion of these would be clonal replicates. However, while we cannot empirically estimate the influence of drift on our results, we suggest that there are several reasons for the unlikelihood of accounting for all of the changes that we observed. First, the fluctuation in clonal frequency was often pronounced. For example, one clone in Beale Bird Park Lake went from a frequency of 0% in October 1991 to 71% in August 1992, 10% in August 1994, and then back to 0% in July 1995 (Fig. 2). This range in variation is unlikely to have resulted from genetic drift. Similarly marked fluctuations in allele frequen-

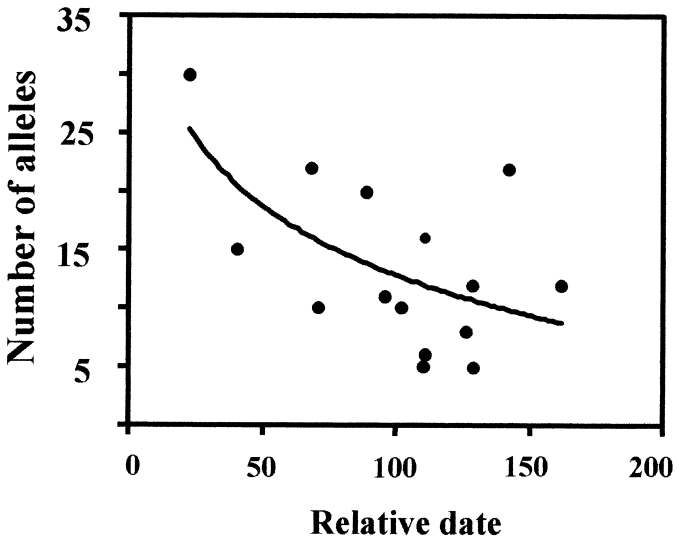

Fig. 5. Linear regression of ln-relative data versus number of alleles. $P = 0.019$, $r = -0.60$, $n = 15$. The untransformed values are plotted, displaying the curved line from the resulting exponential relationship.

cies were also seen (*see Results and Web Appendix 1*). Second, overall genetic diversity within a site was inversely correlated with the relative date (Fig. 5), and this reflects a nonrandom influence on the temporal changes in genotypes. Third, there was no correlation between number of colonies sampled and number of alleles recovered ($P = 0.49$, $r = 0.19$, $n = 15$). This shows that the number of genotypes identified was not simply a function of the number of colonies that were collected and therefore counters the argument that sampling drift compromises the interpretation of our results.

Selection is a likely explanation for some of the temporal changes in *C. mucedo* as, unlike genetic drift, it can affect each locus differently (Lewontin and Krakauer 1973), a pattern that was seen in our data. Decreasing genetic diversity throughout the growing season in cladoceran populations has been interpreted as clonal selection (Lynch and Spitze 1994), and seasonal changes in clonal frequencies within freshwater zooplankton populations have been linked to differences in, for example, temperature (Rossi and Menozzi 1990) and oxygen concentration (Weider and Lampert 1985). While there are few known examples of selection acting directly on microsatellites (Charlesworth et al. 1994), the loci that we targeted may be linked to genes that are subject to selection. The decrease in genetic diversity that occurs throughout the growing season in *C. mucedo* populations may result from clonal selection.

A potential explanation for the temporal patterns of genetic changes in *C. mucedo* populations thus entails differential selection throughout the growing season and short-term dormancy ($\leq$1 yr) that allows for the reintroduction of genotypes in the following year. However, as it is highly unlikely that every genotype will successfully reproduce each year, a population relying on this annual renewal of diversity should be prone to a gradual decay in genetic variation. Novel genotypes may appear within a population following somatic mutations or sexual reproduction, although we can exclude the former, as somatic mutations were unlikely to have accumulated over the time scales that we investigated. Sexual reproduction is also unlikely to have played a major role in the creation of novel genotypes because there was no correlation between observed heterozygosity and genetic diversity, a pattern that would be expected if novel genotypes were a result of sex. Furthermore, populations of facultatively sexual organisms whose genetic diversity results primarily from sex are generally in Hardy–Weinberg equilibrium (Lynch and Spitze 1994; Grebelnyi 1996), and none of the *C. mucedo* populations were in Hardy–Weinberg proportions. This lack of conformation to Hardy–Weinberg equilibrium is unlikely to result from a chance founder effect, as it is a pattern consistent in numerous populations throughout Europe (Freeland et al. 2000*a*), North America (Freeland et al. 2000*b*), and Great Britain (this study). Therefore, alternate processes influencing the genetic profiles of populations must be explored.

The fourth factor that may contribute to changes in allele frequencies over time is spatial gene flow following the transport of statoblasts on vectors such as waterfowl, mammals, and boats, which may introduce novel genotypes into populations. While gene flow based on N*m* values among sites was very low in this study, these values must be interpreted with caution, as N*m* estimates may be unreliable because of unrealistic assumptions about the stability of populations (Whitlock and McCauley 1999). In addition, we sampled only a small proportion of potential sites, and it is possible that measures of gene flow would be higher if we had sampled from more locations. Nevertheless, the fact that no identical clones were found in more than one site, combined with the presence of up to 13 unique alleles within sites (Web Appendix 1), provides evidence for the occurrence of relatively low levels of spatial gene flow among sites (*see also* Hatton-Ellis et al. 1998; Freeland et al. 2000*a–c*). Since there is little evidence for spatial gene flow, the additional factor that may explain at least some of the genetic changes in *C. mucedo* populations is long-term ($>$1 yr) temporal gene flow via statoblast banks. Delayed hatching of statoblasts within sites could contribute to the observed changes in population genetic profiles, such as the introduction of novel genotypes both within and among growing seasons.

Effects of temporal versus spatial gene flow on genetic changes through time—Our data imply that temporal gene flow exerts a stronger influence than spatial gene flow in shaping *C. mucedo* populations. Since populations of *C. mucedo* at different sites are genetically distinct (this study; Hatton-Ellis et al. 1998; Freeland et al. 2000*a*,*b*), the pattern of genetic similarity maintained within a site over time would not be expected if the genetic make-up of a population were largely a result of immigration. This premise is exemplified by Beale Bird Park Lake, a site that appeared to experience a tremendous population crash in 1993 following high levels of parasitism of the *C. mucedo* population by a myxozoan parasite (Vernon et al. 1996). The site was subsequently repopulated by numerous clones in 1994. If these clones had originated elsewhere, then the 1991, 1992,

and 1994 populations should show higher levels of genetic differentiation than those reflected in Fig. 3. The most parsimonious explanation for the similarities before and after the population crash is that the site was repopulated from a statoblast bank containing clones that are genetically similar to, and in some cases indistinguishable from, those that populated Beale Bird Park Lake in previous years. Under this scenario, the population crash refers only to adult colonies in that particular year and does not take into account dormant, viable statoblasts. The hypothesis of a statoblast bank means that apparently small or extinct *C. mucedo* populations may actually harbor a relatively large effective population size.

The hypothesis of long-term temporal gene flow is reinforced by the finding of two identical clones before and after the apparent bottleneck at Beale Bird Park Lake. Surveys of a total of 10 North American, 12 continental European, and 11 Great Britain locations in this and other studies using microsatellite (Freeland et al. 2000*a,b*) and RAPD-PCR data (Hatton-Ellis et al. 1998) have revealed only one case of identical clones occurring in more than one unconnected site. It should be noted that Beale Bird Park Lake is located <20 m from the River Thames, which flooded in January 1994. Although *C. mucedo* rarely occurs in rivers (Okamura 1997), statoblasts could conceivably have been transported by the river into Beale Bird Park Lake from other sites. However, arguments against appreciable levels of spatial gene flow remain relevant whether the proposed vector is waterfowl or a flooding river.

The importance of temporal gene flow to freshwater invertebrates—The results of this study significantly extend our understanding of the general importance of propagule banks by providing evidence that freshwater invertebrates with varying life histories incorporate the added dimension of temporal dispersal. The production of asexual dormant propagules by the benthic *C. mucedo* contrasts with the production of sexual dormant propagules by the mobile zooplankton taxa. It is notable that the production of asexual dormant propagules is common to a number of other sessile and highly clonal freshwater taxa, including sponges, the hydrozoan *Cordylophora*, ctenostome bryozoans, and other phylactolaemate bryozoans. Whether propagule banks are important in the population dynamics of these other taxa is unknown, in part because very little is known about any aspect of the population genetics of these organisms. However, prolonged (>1 yr) statoblast viability occurs in a number of other phylactolaemate bryozoans (Oda 1979; Pennak 1989; Wood 1991), and in one species, statoblasts appear to be regularly released from at least several centimeters below the sediment surface (Wood and Marsh 1996). Prolonged dormancy followed by temporal gene flow in these taxa may allow clones to achieve escape through time from biological enemies and harsh but fluctuating environmental conditions.

This study and earlier studies on zooplankton taxa provide evidence that the interplay between gene flow in time and space both within and among populations may contribute to a complex metapopulation structure for many freshwater invertebrates. Notably, many freshwater taxa inhabiting lakes and ponds, including bryozoans and zooplankton, rely on passive dispersal to achieve colonization of new sites. Such passive dispersal, which contributes to metapopulation dynamics, is likely to be a relatively rare event, resulting in the low levels of gene flow that are typically observed (Freeland et al. 2000*a–c*). Periodic reintroduction to the natal site following long-term residency in sediment may be a much more reliable means of achieving dispersal. Temporal gene flow may therefore play a more significant role than spatial gene flow in the maintenance of genetic diversity and the avoidance of extinction in local populations. In addition, in those taxa such as *C. mucedo* that produce asexual dormant propagules, temporal gene flow may promote the long-term survival of clones. Investigation of the incidence of propagule banks in a diversity of taxa would greatly contribute to our understanding of the conditions associated with the evolution of prolonged diapause and its consequences for ecology and population genetics. Finally, determining the relative importance of spatial versus temporal gene flow to levels of genetic variation and long-term population persistence will be of great importance if we are to successfully conserve biodiversity and ecosystem function in freshwater habitats.

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