Journal of Heredity 2010:101(1):97–106
doi:10.1093/jhered/esp078
Advance Access publication September 4, 2009

Temporal Variation in Population Genetic Structure of a Riverine African Cichlid Fish

Erika Crispo and Lauren J. Chapman

From the Department of Biology, McGill University, 1205 Avenue Dr Penfield, Montréal, QC, Canada H3A 1B1 (Crispo and Chapman).
Wildlife Conservation Society, 2300 Southern Boulevard, Bronx, NY (Chapman).

Address correspondence to Erika Crispo at the address above, or e-mail: erika.crispo@mail.mcgill.ca.

Population genetic structure in a riverine cichlid fish was recharacterized 2 years after patterns had been first described. We found that genetic structure changed, as evidenced by changes in F_{ST} between years among sites, significant F_{ST} between years "within" sites, and a significant proportion of the genetic variation partitioned between years. Most striking, signatures of isolation by distance were eradicated between years. Our study highlights that point-in-time estimates of population genetic structure might not be valid over longer time periods, particularly in systems exposed to strong seasonal or interannual variation in abiotic conditions.

Key words: *dispersal*, *isolation by distance*, *floods*, F_{ST}, *gene flow*, *genetic divergence*

Examining population structure and gene flow in nature is imperative for understanding a variety of phenomena, such as speciation (e.g., Bernatchez and Wilson 1998; Martin and McKay 2004; Barluenga et al. 2006) and biogeographical processes (e.g., Johnson and Taylor 2004; Fraser and Bernatchez 2005; Kotlik et al. 2008), and for establishing conservation units and priorities (DeSalle and Amato 2004; Pearse and Crandall 2004). Population structure is usually estimated using neutral genetic markers sampled at one point in time. When populations are sampled more than once, sampling is often performed over periods of time on the magnitude of 25–60 years (e.g., Meldgaard et al. 2003; Favé and Turgeon 2008; Sønstebø et al. 2008). Genetic structure can, however, fluctuate on time scales as short as 1–2 years (e.g., Lacson and Morizot 1991; Congdon 1995; González-Wangüemert et al. 2007; Beneteau et al. 2009), and thus studies are necessary for determining how often and under what conditions rapid changes in population genetic structure are expected to occur.

Events such as floods, droughts, and hurricanes could have profound and immediate effects on the population structure of aquatic organisms, either by physically moving individuals across a landscape or by changing the landscape so that dispersal corridors are altered, which might either prevent or enhance dispersal (e.g., Lacson and Morizot 1991; Huey et al. 2008; Jowers et al. 2008; Masci et al. 2008). Flooding in river drainages might influence variation in population structure in aquatic riverine organisms. For example, evidence for downstream-biased gene flow is common in rivers (e.g., Congdon 1995; Hernandez-Martich and Smith 1997; Barson et al. 2009), but it is unclear whether these patterns remain constant or vary temporally with changes in the environment.

The river systems of western Uganda are subject to biannual flooding that might influence the structure of fish populations (Figure 1). This region is characterized by a number of rivers, as well as connected swamps, inhabited by a variety of fish species including cichlids, catfishes, anabantoids, killifish, and barbs (Crispo E and Chapman LJ, personal observations). Some potential effects of flooding on riverine fishes might include increased gene flow, particularly in the downstream direction (e.g., Congdon 1995; Huey et al. 2008; Barson et al. 2009); declines in population sizes due to the washing out of fish or changes in abiotic or biotic conditions (e.g., Fausch et al. 2001; Grether et al. 2001); altered patterns of natural selection due to changes in abiotic or biotic conditions (e.g., Congdon 1995; Grether et al. 2001); or genetic drift (e.g., Congdon 1995; Barson et al. 2009). We thus used fish from western Uganda to test the prediction that population structure of riverine organisms can fluctuate on short time scales in areas afflicted by periodic flooding. We specifically examine genetic variability, inbreeding coefficients, estimated population sizes, genetic differentiation, the apportionment of genetic variation among sites and between years, and the proportion of shared alleles.

Materials and Methods

We reexamined population genetic structure of a widespread African cichlid fish (*Pseudocrenilabrus multicolor victoriae*) 2 years

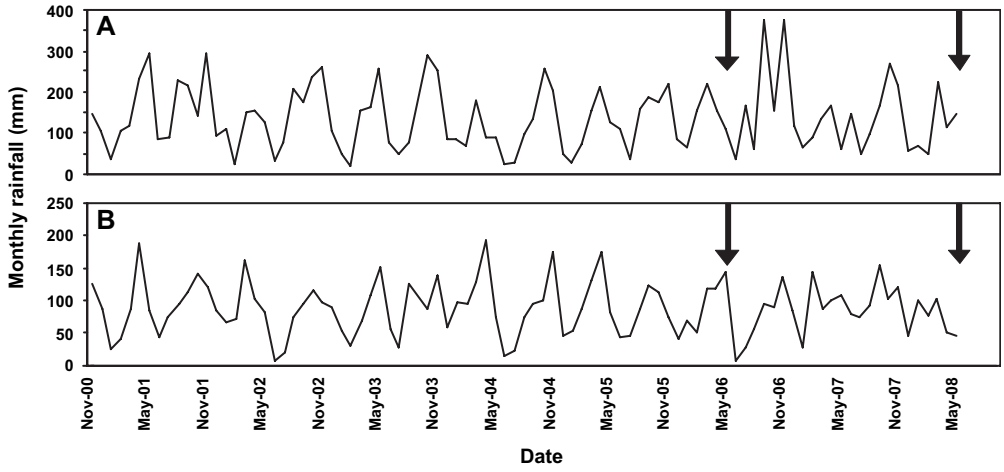

Figure 1. Total monthly rainfall at 2 locations, including one location upstream of the sampling sites (**A**) and one site centrally located within the study area (**B**). (**A**) Kibale National Park (N 00°56′52.29″, E 30°35′60.82″), located approximately 28 km northwest of Bunoga. (**B**) Kahunge (N 00°18′10.2″, E 30°31′57.5″). Black arrows indicate the periods of sampling. Data for Kibale were estimated using a rain gauge, and data for Kahunge were obtained from the Climate Prediction Center of the National Oceanic and Atmospheric Administration.

after it had been previously characterized (Crispo and Chapman 2008). This species can reproduce at an age of approximately 6 months in the laboratory and can survive for more than 2 years (i.e., individuals can survive longer than the period between sampling; Crispo E and Chapman LJ, personal observations). River and swamp populations of this species are subject to divergent selection related to dissolved oxygen, conductivity, and turbidity, and potentially prey availability, predation pressure, and competition with other species (Crispo E and Chapman LJ, personal observations). At least oxygen and conductivity are known to vary seasonally, and this variation might be attributable to rainfall (e.g., Chapman 1995; Chapman and Liem 1995; Chapman et al. 2002, 2004). Based on a previous study of population structure using neutral genetic markers, ecological barriers to gene flow were not uncovered in this species (i.e., no increased genetic divergence between river and swamp environments relative to within them), but physical barriers due to geographical separation and distance (i.e., isolation by distance) were present (Crispo and Chapman 2008).

In May–June 2008, we collected fin tissue from approximately 30 adult fish at each of 6 sites in the Mpanga watershed in western Uganda (Bunoga, Bwera, Rwebakwata, Kahunge, Kamwenge, and Kanyantale; Figure 2) using procedures described in Crispo and Chapman (2008). One swamp site was sampled in 2008 that had not previously been sampled (Kanyantale; Figure 2). Two sites that had previously been sampled in this watershed (Kiaragura and Kantembwe) were not sampled in 2008 due to logistic constraints. Contemporary dispersal among all sites should be possible at least during the wet season. Large portions of papyrus swamps in Uganda can become dry during the dry seasons, but these swamps can expand by nearly 300% during the wet seasons (Chapman 1995; Chapman and Liem 1995). These observations suggest that the Mpanga River and adjacent swamp might have increased connectivity during the wet seasons. Indeed, seasonal decreases in oxygen concentration at river sites downstream from the swamp in our study area suggest that swamp water, containing decomposing debris, flushes into the river during rainy periods (Chapman et al. 2008; Crispo and Chapman 2008). Sampling was performed throughout the range of *P. multicolor* within this one watershed. That is, *P. multicolor* could not be caught in abundance at sites located in the upstream areas of the drainage after extensive sampling (Aliganyira E, Omeja P, Twinomugisha D, personal communications; Chapman LJ, personal observations), and a large waterfall is located downstream, blocking upstream dispersal from other sites. No other watersheds lie close to the Mpanga in the area above the waterfall, making dispersal among watersheds unlikely, even during flooding.

We extracted DNA using DNeasy tissue kits (Qiagen) and genotyped 10 tetranucleotide microsatellite loci following procedures described in Crispo et al. (2007). To make between-year comparisons possible, we used loci and laboratory procedures identical to those used for the 2006 samples. That is, the same equipment, primers, and polymerase chain reaction conditions were used, and the same technician performed the laboratory work and identified the allele sizes (Crispo and Chapman 2008). Two loci that had previously been genotyped (Ppun2 and Ppun12) were not genotyped for this study because heterozygote deficits had previously been found, suggesting the possible presence of null alleles—these loci were not used in any analysis here or in the previous study (Crispo and Chapman 2008).

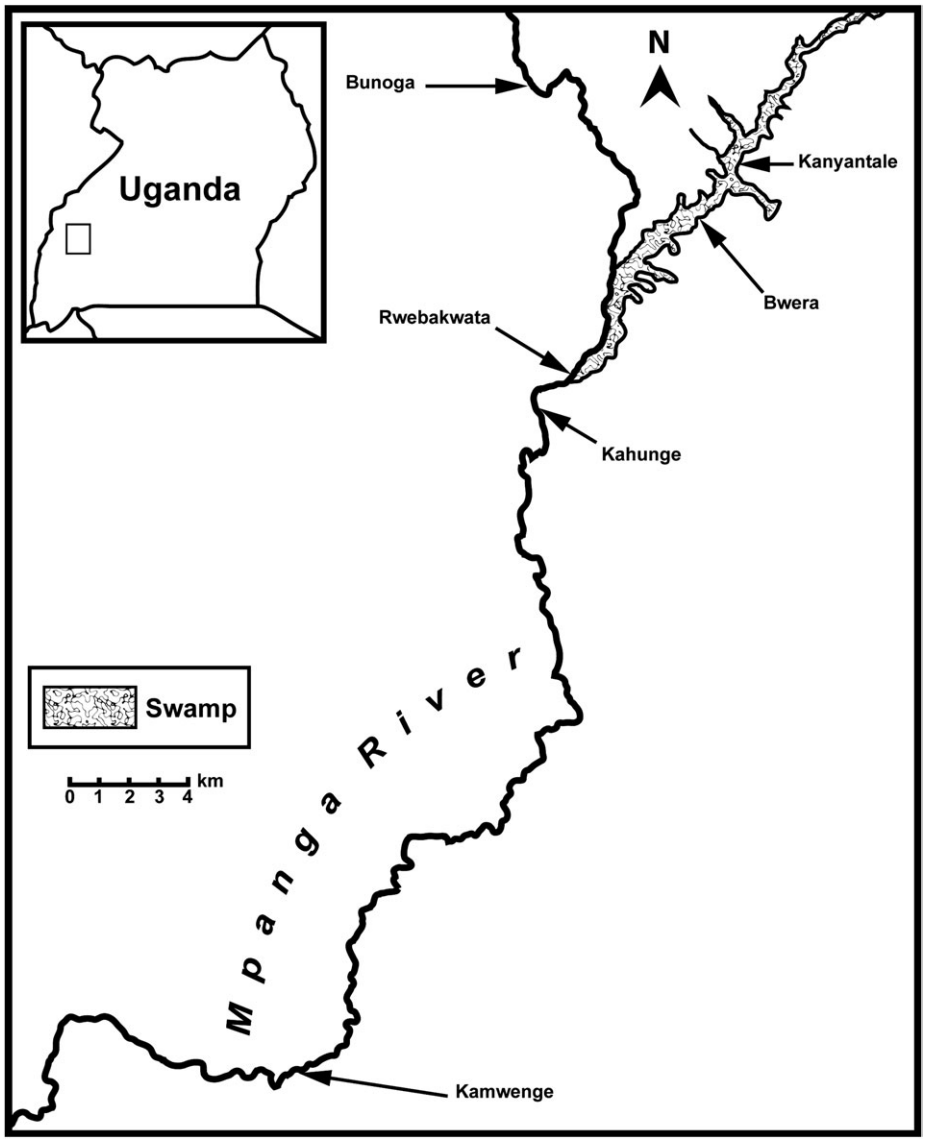

Figure 2. Sites sampled in summer 2008 within the Mpanga watershed. Inset shows the approximate area in Uganda depicted in the main figure. Modified from Figure 1 in Crispo and Chapman (2008).

To assess the suitability of the microsatellite markers, we tested for the possible presence of null alleles and the linkage of loci. To test for heterozygote deficits and linkage equilibrium (LE), we used Genepop on the Web (Raymond and Rousset 1995). Exact tests for Hardy–Weinberg equilibrium (HWE) (1-tailed tests for heterozygote deficits) were performed for each locus within each site and across all sites, and LE was tested for each pair of loci within each site and across all sites. We tested both HWE and LE using the Markov chain, with 1000 steps in the chain, 100 batches, and 1000 iterations per batch. We used the binomial likelihood approach for multiple tests (Chapman et al. 1999) to test for significant heterozygote deficits and departures from LE across all sites for each locus (heterozygotes) or pair of loci (linkage). This was done using the likelihood function in Chapman et al. (1999):

$$L = \sum_{i=r}^{n} C(1 - \alpha)^{n-r}(\alpha)^{r},$$

where n is the total number of tests, r is the number of significant tests, α is the significance level (0.05), and C is a factorial constant ($n!/r!(n - r)!$). Values are summed from r to n. If L is lower than α, we consider tests for departures from HWE and LE to be significant overall.

To detect changes in genetic variability, we examined allelic richness and heterozygosity. To estimate allelic richness (i.e., the number of alleles standardized to a common sample size), we used HP-Rare version 8-16-2004 (Kalinowski 2005), assuming 30 genes per site (as in Crispo and Chapman 2008). We estimated within-site observed (direct count) and expected (unbiased)

heterozygosities across all loci using Tools for Population Genetic Analysis version 1.3 (Miller 1997). Inbreeding coefficients (F_{IS}) were calculated at each site using the equation $F_{IS} = 1 - (H_O/H_E)$, where H_O and H_E are the observed and expected heterozygosities, respectively.

To examine whether effective population sizes (N_e) changed between years, we used the program LDNE (Waples and Do 2008). This program estimates N_e using information on linkage disequilibrium (LD), based on point-in-time samples, and corrects for biases resulting from the presence of rare alleles. Because alleles with very low frequencies can bias results (Waples 2006), we performed analyses after removing alleles with frequencies (P_{crit}) lower than 0.05, 0.02, and 0.01 (i.e., 3 separate analyses). We used a model of random mating and used both the parametric and jackknife procedures to construct 95% confidence intervals. Similarly, we estimated the effective number of breeding individuals (N_b) using the program Nb_HetEx (Zhdanova and Pudovkin 2008). This analysis is based on the assumption that the excess of heterozygotes increases with a decreasing number of breeders. We used 1000 iterations for the bootstrap analysis. All of these analyses were performed on both the 2006 and 2008 data. Note, however, that these 2 methods assume that populations are closed (i.e., that no gene flow occurs among populations), which is probably not the case in the present system (see below). Therefore, the results from these analyses should be interpreted with caution.

To detect changes in genetic structure, we estimated F_{ST} values for all site pairs within a year, and between years within each site, based on distance matrices using Arlequin version 3.01 (Excoffier et al. 2005). Significance levels of F_{ST} values were based on 1000 permutations. Next, we performed 2 Mantel tests using Fstat version 2.9.3.2 (Goudet 2001). The first Mantel test compared matrices of within-year F_{ST} values, considering only sites that were common between years (2008 values used as the dependent variable, 2006 values used as the explanatory variable). The second Mantel test tested for isolation by distance using the 2008 data set, comparing matrices of F_{ST} values and geographical distance (results from the 2006 data set are presented in Crispo and Chapman 2008). Geographical distance separating sites was measured as the physical distance along the waterway. Significance levels for the Mantel tests were based on 20,000 randomizations.

To examine the relative effects of spatial versus temporal variation, we performed an analysis of molecular variance (AMOVA; locus-by-locus and over all loci) using Arlequin version 3.01, considering only the 5 sites that were common between years. Genetic variation was partitioned 3 ways: among sites across years (i.e., years nested within sites), among years within sites, and within sites within years. Significance levels for the AMOVA groupings were based on 1000 permutations. This analysis will inform whether structuring is significant among sites irrespective of time (i.e., spatial structure) versus among years within sites (i.e., temporal structure).

Genetic structure was too low to obtain consistent and meaningful results using the programs GENECLASS or MIGRATE—the former would have detected first-generation dispersers and the latter would have estimated directional gene flow between pairs of sites and between years within sites. We attempted to use both of these programs, but GENECLASS indicated that over 80% of sampled individuals were first-generation dispersers (accurate detection should be low when F_{ST} is low; Manel et al. 2005), and MIGRATE provided inconsistent results even after very long runs (results not shown). In addition, MIGRATE assumes that population dynamics are at a stable equilibrium, which is not the case in the present system, making its use inappropriate for the current data set (see Kuhner 2009). Instead, we estimated the proportion of shared alleles (D_{ps}; Bowcock et al. 1994), which provides some information on population admixture (assuming that identical alleles are a result of common ancestry and are not due to homoplasy). We performed this analysis using Microsatellite Analyzer version 4.05 (Dieringer and Schlötterer 2003), where D_{ps} was calculated using 1 − (similarity factor).

Results

We detected significant heterozygote deficits for one locus in the 2008 data set (Pmv9; $P = 0.0328$) after correcting for multiple tests. Using the binomial likelihood approach for multiple tests (Chapman et al. 1999), any locus that was found to have significant heterozygote deficits in 2 or more of the 6 sites was considered to have significant heterozygote deficits overall and thus possible null alleles. We also detected significant heterozygote deficits for this locus when testing across all sites simultaneously ($P = 0.0213 \pm 0.0102$ standard error). However, in the 2006 data set, heterozygote deficits were not detected for any of the currently used loci (Crispo and Chapman 2008). A possible reason for deviations from HWE in 2 sites (Rwebakwata and Kahunge) in the 2008 data set is the presence of first-generation dispersers. It remains curious, however, why only one locus would show reductions in heterozygosity. Because this locus was used in the 2006 analysis, we also used it in the present analysis so that between-year comparisons could be made, but we also estimated F_{ST} values without Pmv9 and for Pmv9 only. We excluded Pmv9 to determine the robustness of the results, and we used Pmv9 only to observe whether this locus behaves differently than the other loci. We refer only to results obtained using all 10 loci unless otherwise specified.

We detected significant departures from LE for 3 pairs of loci in the 2008 data after correcting for multiple tests: Pmv4 and Pmv17 ($P < 0.0001$), Pmv3 and Pmv13 ($P = 0.0328$), and Pmv3 and Pmv17 ($P = 0.0328$). When LE was tested across all sites simultaneously, only 2 of these 3 pairs were in LD (Pmv4 and Pmv17; Pmv3 and Pmv13; $P < 0.001$ for both). The only pair of loci that showed LD in the 2006 data set was Pmv4 and Pmv17, but only when tested across sites simultaneously (Crispo and Chapman 2008). In the 2008 data set, however, we detected LD in all 6 sites for these loci. It thus remains dubious whether these loci are

actually physically linked or whether observed patterns are due to the sampling of first-generation dispersers; that is, allele combinations common to one site might have been introduced into another site in which different allele combinations are common. Because both of these loci were used in the 2006 data set, we also used them in the present analysis so that between-year comparisons could be made.

Both allelic richness and observed and expected heterozygosities decreased in 2008 relative to 2006 (Table 1; Crispo and Chapman 2008). There were 2 exceptions, where values were higher in 2008: allelic richness for Kahunge and observed heterozygosity for Kamwenge (but "expected" heterozygosity was lower in 2008 for all sites). However, when considering loci individually, not all loci showed decreases in allelic richness and heterozygosity in 2008 (Table 1). Genetic diversity showed some directionality—the most downstream river site (Kamwenge) had the highest allelic richness and heterozygosity and the most upstream river site (Bunoga) had the lowest (Table 1). The pattern of increasing diversity in the downstream direction was more pronounced in the 2008 data set than in the 2006 data set (Table 1; Crispo and Chapman 2008). In the swamp, allelic richness and heterozygosity were higher in the site most distant from the river (Kanyantale; Table 1; Figure 2). This pattern for the swamp was also evident in the 2006 data set (Table 1; Crispo and Chapman 2008). F_{IS} values were low, providing no evidence for local inbreeding (Table 1). Instead, negative F_{IS} values at many sites (i.e., higher than expected heterozygosities) might reflect outbreeding.

Very large confidence intervals were obtained for estimates of N_e and N_b, often with upper bounds at infinity (Table 2). Using the LD method, the estimated N_e tended to increase with decreasing P_{crit}, the allele frequency below which alleles were excluded from the analysis (data not shown). Because of the high number of alleles (Table 1), and thus low allele frequencies, in our data sets, we present only results from the analyses using the lowest P_{crit}, that is, 0.01 (Table 2). In addition, confidence intervals for N_e calculated using the jackknife procedure were always larger than those calculated using the parametric procedure (data not shown). Because confidence intervals for N_e estimates were large in general, we present only those calculated using the parametric procedure (Table 2). Overall, we observed no trends in N_e or N_b across methods of estimation (Table 2). The LD method revealed only a significant decrease in N_e between years for Bwera, whereas the heterozygote-excess method revealed only a significant decrease in N_b between years for Kamwenge and significant increases for Bunoga and Kahunge (and only when using the parametric method; Table 2). A possible reason for these inconsistent results is that the methods used for estimation assume that populations are closed (i.e., no gene flow) and that cohorts are discreet (i.e., no overlapping generations) (Pudovkin et al. 1996; Waples and Do 2008). Neither of these assumptions is realistic in the present system. Another problem with estimating N_e is the difficulty in defining a population. Sampling sites probably do not reflect discreet populations—instead, populations are probably continuous along the stream and throughout the swamp.

Changes in genetic structure were documented between years. First, F_{ST} values tended to be lower in 2008, with only one pair of sites showing a significant F_{ST} value in 2008 but not in 2006 (Bwera and Rwebakwata; Table 3). Second, 2 F_{ST} values "between" years "within" sites were significant, and these values were higher than some within-year F_{ST} values (Kahunge, Kamwenge; Table 4). These results were similar when Pmv9 (i.e., the locus out of HWE) was excluded from the analyses (Tables 3 and 4). Third, Mantel tests did not detect a significant correlation in F_{ST} values between years ($r^2 = 0.2587$; $P = 0.1290$). Fourth, isolation by distance was detected in 2006 (Crispo and Chapman 2008) but not in 2008 ($r^2 < 0.0001$; $P = 0.9944$; Figure 3). Fifth, AMOVA detected significant partitioning of genetic variation between years when averaged over all loci and for 7 of the 10 loci when tested individually (Table 5). Sixth, although the proportion of shared alleles (D_{ps}) was high between sites within years, there was a slight tendency for this proportion to be lower in 2008 (Table 3).

Pmv9 produced some results that differed from those produced using the other loci. In addition to the presence of heterozygote deficits for this locus (above), some patterns of F_{ST} differed (Tables 3 and 4). The most striking result was an

Table 1. Microsatellite variability for sites sampled in 2008 (2006 values in brackets, from Crispo and Chapman 2008)

Site	*N* 2008 (2006)	No. of alleles 2008 (2006)	Allelic richness 2008 (2006)	Private allelic richness 2008 (2006)	H_O: 2008 (2006)	H_E: 2008 (2006)	F_{IS}: 2008 (2006)
Swamp							
Kanyantale	34	136	112.5	3.1	0.9202	0.8697	−0.0580
Bwera	29 (27)	130 (147)	104.4 (120.2)/*6*	3.4 (4.5)/*7*	0.8605 (0.9193)/*6*	0.8411 (0.8754)/*9*	−0.0231 (−0.0502)
River							
Bunoga	30 (31)	136 (150)	109.0 (112.6)/*5*	5.4 (5.5)/*5*	0.8249 (0.8742)/*7*	0.8337 (0.8415)/*6*	0.0106 (−0.0389)
Rwebakwata	30 (31)	148 (144)	115.0 (117.7)/*5*	5.3 (2.3)/*1*	0.8778 (0.9026)/*7*	0.8602 (0.8794)/*8*	−0.0205 (−0.0264)
Kahunge	30 (30)	152 (144)	119.6 (116.9)/*4*	7.2 (5.0)/*3*	0.8900 (0.8917)/*5*	0.8587 (0.8687)/*5*	−0.0365 (−0.0265)
Kamwenge	30 (18)	165 (143)	126.1 (133.9)/*7*	15.9 (30.2)/*9*	0.9185 (0.8874)/*5*	0.8795 (0.8879)/*7*	−0.0443 (0.0006)

n is the sample size, H_O is observed heterozygosity, H_E is expected heterozygosity, F_{IS} is $1 - (H_O/H_E)$. The italicized values are the number of loci showing higher values in 2006 than in 2008.

Table 2. Effective population sizes (N_e) estimated using the LD method (Waples and Do 2008) and the number of breeders (N_b) estimated using the heterozygote-excess method (HetEx; Zhdanova and Pudovkin 2008)

	LD parametric		HetEx parametric		HetEx bootstrap	
Site	2006	2008	2006	2008	2006	2008
Swamp						
Bwera	145.7/466.8/infinity	38.7/49.8/67.9	22.5/37.8/125.0	48.6/82.3/272.9	11.4/18.7/55.4	13.1/26.7/infinity
River						
Bunoga	84.0/130.1/269.8	126.5/287.5/infinity	33.9/57.4/190.0	Infinity/infinity/infinity	13.8/25.0/164.3	23.1/infinity/infinity
Rwebakwata	101.7/176.6/574.8	135.7/292.7/infinity	Infinity/infinity/infinity	Infinity/infinity/infinity	20.9/131.1/infinity	19.7/80.8/infinity
Kahunge	186.8/1088.9/infinity	147.1/338.9/infinity	17.1/28.8/94.8	Infinity/infinity/infinity	11.4/17.6/41.4	15.3/41.2/infinity
Kamwenge	93.5/394.2/infinity	78.6/112.2/679.6	Infinity/infinity/infinity	104.0/176.3/583.2	20.8/infinity/infinity	16.0/35.9/infinity

Values include the lower bound of the 95% confidence interval/actual estimate/upper bound of the 95% confidence interval. Estimates from the bootstrap analysis are the median values.

increase in F_{ST} between years for the Bwera site (Table 4)—a significant F_{ST} value for this site was obtained using Pmv9 but not using all loci combined. In addition, in the AMOVA, Pmv9 was the locus with the lowest percentage of variation among sites and had a high percentage of variation between years (Table 5). We predict that this locus might be weakly linked to a locus under selection between years, perhaps most strongly influenced by selection in the swamp.

Discussion

Our results show changes in genetic variability and genetic structure on a time scale of 2 years or approximately 4 generations. Most striking, strong isolation by distance had been detected in 2006 (Crispo and Chapman 2008) but was not evident in 2008 (Figure 3). We can envision 4 possible ways in which these genetic changes could have accrued: 1) fluctuations in population sizes, 2) increased gene flow among sites, 3) altered patterns of natural selection, and/or 4) genetic drift. We will discuss each of these possibilities in turn and how they are supported by our results.

Population Sizes

We observed slightly lower allelic richness and heterozygosities in 2008 than in 2006, although this pattern was not consistent among loci at all sites (Table 1). Lower genetic diversity has been linked to lower effective population sizes (Crow and Kimura 1970) and thus these results provide evidence that effective sizes might have declined between 2006 and 2008. This decline could be due to changes in abiotic or biotic conditions between years (see below) or through the physical movement of fish (e.g., Fausch et al. 2001; Grether et al. 2001; Barson et al. 2009). Our estimates of N_e and N_b, however, did not reveal any general trends in changes in population sizes between years (Table 2). As noted above, a possible reason for the high variation in these estimates is that gene flow is high among sampled sites, violating the assumptions of the tests that we used.

Gene Flow

Some evidence suggests that increased directional gene flow might have occurred between years. In the river, there was a slight tendency for genetic diversity to increase in the downstream direction, and this pattern was more pronounced in the 2008 data set than in the 2006 data set, suggesting higher than usual downstream gene flow between sampling years (Table 1; Crispo and Chapman 2008). In the swamp, allelic richness and heterozygosity were higher in the site most distant from the river (Kanyantale; Table 1; Figure 2). Because *P. multicolor* could not be caught in abundance at sites that were explored upstream (or "upswamp") of our sampling sites (Aliganyira E, Omeja P, Twinomugisha D, personal communications; Chapman LJ, personal observations), it is not likely that significant gene flow is occurring from upstream nonsampled sites—it is more probable that

Table 3. F_{ST} and D_{ps} values between sites within years

		F_{ST}		F_{ST}		F_{ST}		D_{ps}	
		All loci		No Pmv9		Only Pmv9		All loci	
Site 1	Site 2	2006	2008	2006	2008	2006	2008	2006	2008
Kanyantale	Bwera	—	**0.0127**	—	**0.0121**	—	**0.0177**	—	0.31766
Kanyantale	Bunoga	—	**0.0176**	—	**0.0173**	—	**0.0201**	—	0.33872
Kanyantale	Rwebakwata	—	**0.0067**	—	**0.0064**	—	0.0097	—	0.29777
Kanyantale	Kahunge	—	**0.0042**	—	**0.0043**	—	0.0037	—	0.26601
Kanyantale	Kamwenge	—	0.0002	—	0.0007	—	−0.0039	—	0.27925
Bwera	Bunoga	**0.0104**	**0.0111**	**0.0117**	**0.0107**	*−0.0030*	***0.0144***	0.33683	0.31436
Bwera	Rwebakwata	*−0.0054*	***0.0077***	*−0.0057*	***0.0065***	−0.0027	0.0178	0.23696	0.30721
Bwera	Kahunge	−0.0038	0.0036	−0.0043	0.0023	*0.0033*	***0.0142***	0.26927	0.30029
Bwera	Kamwenge	**0.0122**	**0.0061**	**0.0106**	**0.0045**	*0.0120*	***0.0193***	0.45206	0.32049
Bunoga	Rwebakwata	**0.0080**	**0.0066**	**0.0085**	**0.0065**	−0.0009	0.0072	0.33426	0.29417
Bunoga	Kahunge	***0.0086***	*0.0039*	***0.0084***	*0.0046*	0.0043	−0.0015	0.32195	0.30615
Bunoga	Kamwenge	**0.0327**	**0.0144**	**0.0308**	**0.0147**	***0.0195***	*0.0123*	0.49562	0.34420
Rwebakwata	Kahunge	−0.0040	−0.0004	−0.0017	−0.0042	0.0093	−0.0021	0.26403	0.24266
Rwebakwata	Kamwenge	***0.0139***	*0.0030*	***0.0103***	*0.0033*	0.0087	0.0009	0.42919	0.29133
Kahunge	Kamwenge	**0.0224**	**0.0038**	**0.0199**	**0.0045**	***0.0224***	*−0.0014*	0.48489	0.30229

Significant F_{ST} values ($\alpha = 0.05$) are in bold face, and values for which the significance differs between years are in italics. F_{ST} values for 2006 are from Crispo and Chapman (2008).

gene flow occurs from the river up through the swamp, which is plausible because water flow in the swamp is low. This pattern for the swamp was also evident in the 2006 data set, suggesting that gene flow is generally higher in this direction within the swamp (Table 1; Crispo and Chapman 2008).

Some other results suggest increased downstream gene flow between years. First, the 2 F_{ST} values that were higher in 2008 were for site pairs located upstream (Bunoga vs. Bwera and Bwera vs. Rwebakwata); that is, F_{ST} was lower for downstream sites (Kahunge and Kamwenge; Table 3; Figure 2). As well, the 2 site-pairs for which F_{ST} values were significant in 2006 but not in 2008 (Bunoga vs. Kahunge and Rwebakwata vs. Kamwenge) each contain one site located downstream (Table 3; Figure 2). Second, the 2 sites that had significant F_{ST} values between years within sites were the sites located farthest downstream in the river (Kahunge and Kamwenge) (Table 4; Figure 2).

We observed that the proportion of shared alleles among sites decreased in 2008 relative to 2006, and this decrease could be due to slight decreases in allelic richness in 2008 relative to 2006. Low private allelic richness in both years (Table 1) and a high proportion of shared alleles among sites (Table 3) indicate that gene flow is high overall. Even though the proportion of shared alleles was high "among" sites, there was a tendency for it to be even higher between years within sites (Table 4). Similarly, the AMOVA revealed a greater proportion of variation among sites than between years.

Table 4. F_{ST} and D_{ps} values within sites between years

	F_{ST}			D_{ps}
Site	All loci	No Pmv9	Only Pmv9	All loci
Bwera	0.0007	−0.0011	**0.0153**	0.51947
Bunoga	0.0010	0.0005	0.0051	0.47174
Rwebakwata	−0.0018	−0.0017	−0.0025	0.49913
Kahunge	**0.0040**	**0.0038**	0.0062	0.51603
Kamwenge	**0.0072**	**0.0072**	0.0097	0.62334

Significant F_{ST} values ($\alpha = 0.05$) are in bold face.

Natural Selection

A possible reason for variation in neutral genetic diversity between years is that patterns of natural selection had

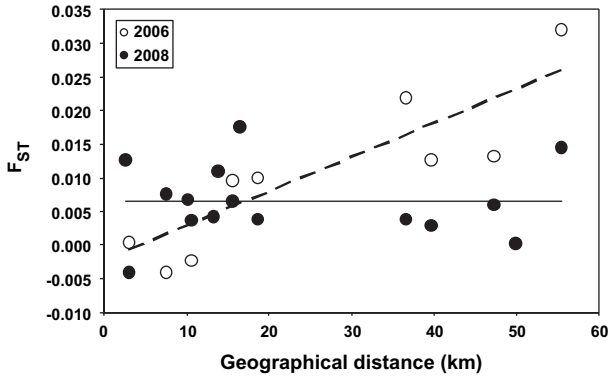

Figure 3. Relationship between F_{ST} and geographical distance in 2008 (black points and solid line) and 2006 (open points and dashed line). The 2006 data are only presented for sites common between the 2 years and have been modified from Figure 7 in Crispo and Chapman (2008). Isolation by distance was present in 2006 (common sites only: $r^2 = 0.7299$, $P = 0.0010$) but not in 2008 (all sites: $r^2 < 0.0001$, $P = 0.9944$; common sites only: $r^2 = 0.1956$, $P = 0.2040$).

Table 5. Percentage of variation in each grouping obtained from AMOVA

	% Variation		
Locus	Among sites	Between years within sites	Within sites within years
Pmv1	**0.6758**	−0.1112	**99.4354**
Pmv3	0.5892	**0.3753**	**99.0355**
Pmv4	0.4537	**0.3311**	**99.2152**
Pmv9	0.0872	**0.6478**	**99.2650**
Pmv13	0.3492	0.0155	99.6353
Pmv15	**0.8086**	−0.4753	99.6667
Pmv17	0.4390	**0.4233**	**99.1377**
Ppun4	**1.4324**	−0.8953	**99.4628**
Ppun5	0.3393	**0.4989**	**99.1618**
Ppun17	0.4886	**0.8032**	**98.7082**
All	**0.5241**	**0.2148**	**99.2611**

Values for all loci are based on the weighted averages. Bold values indicate significant ($\alpha = 0.05$) variance components (1000 permutations).

changed. For example, unusual patterns of flooding might have altered local abiotic and/or biotic conditions between years, thus influencing which genotypes were best adapted for the prevailing conditions. Observable effects of natural selection on genetic structure within and among populations would only be expected if microsatellite markers were linked to some degree with loci that are under divergent selection between years (e.g., Nielsen et al. 2006; see also Storz 2005; Nosil et al. 2009). If different neutral alleles are associated with different selected alleles, changes in neutral allele frequencies might accrue between years. We observed some significant changes in Pmv9 between years, particularly in the swamp (Tables 3 and 4; above) and thus this locus might be linked to a locus (or loci) that was (or were) under divergent selection between years.

The developmental stage at which selection occurs might affect population structure, and thus information on the ages of sampled fish might have provided an insight into whether observed genetic changes were due to selection. For example, genetic changes in brown trout (*Salmo trutta*) in Sweden were greater among cohorts than among years overall (Palm et al. 2003), suggesting that different patterns of selection might have acted on juveniles from different cohorts. We do not have information on the ages of our sampled individuals, however, and samples probably consist of a mixture of cohorts, each of which might have experienced different patterns of selection during development.

Genetic Drift

In systems characterized by low genetic differentiation at neutral markers, small fluctuations in allele frequencies can greatly influence the accuracy and precision of F_{ST} estimates (Waples 1998). The F_{ST} values estimated in our study are much lower than the average estimates for freshwater fishes (Table 3; Ward et al. 1994; Waples 1998), indicating very low genetic differentiation in this system. Small demographic changes from one year to the next, or slight deviations from random sampling, could have influenced the results.

Effects of Floods?

Genetic structure can be strongly influenced by flooding in riverine systems (e.g., Congdon 1995; Jowers et al. 2008; Masci et al. 2008; Barson et al. 2009). Because patterns of rainfall in the study area remained relatively constant over a number of years (Figure 1), such striking changes in genetic structure are surprising. However, rainfall measures might not directly correlate with water flow, and thus undetected differences in water flow among seasons might have influenced fish population structure between years. Indeed, over a 2-year period, we noted a nonsignificant relationship between monthly water depth readings at Kahunge (Figure 2) and monthly rainfall at this site and at locations upstream of this site (data not shown). We noted higher water levels during the second sampling season than the first (Crispo E, personal observations), even though both sets of samples were collected in May–June, indicating that water discharge might have increased between years. The effects of water flow could influence population structure directly, or population structure could be influenced by other biotic or abiotic properties that affect fish movement or survival (e.g., Congdon 1995; Fausch et al. 2001; Grether et al. 2001).

If the observed genetic patterns in 2008 were, in fact, influenced by a flooding event that occurred between 2006 and 2008, how much evolutionary time would be required for the build-up of genetic differences among sites similar to those observed before the flood? That is, given the F_{ST} values observed in 2008, how much time is needed until genetic differences among sites are similar to those observed in 2006? The answer to this question would shed light on how often large floods, or similar events, occur in this system to produce genetic change. We consider the following equation for the relationship between F_{ST} at 2 points in time (Hartl 2000, p. 65):

$$1 - F_{ST} = (1 - F'_{ST})(1 - (1/(2N)))^t,$$

where F_{ST} is the value before the flood (2006), F'_{ST} is the value after the flood (2008), N is the population size, and t is the number of generations between F'_{ST} and F_{ST}. We use this equation to estimate the number of years since the last flooding event, prior to 2006, which resulted in F_{ST} values similar to those observed in 2008. We assume 2 generations per year and use 100, 500, and 1000 as values of N. Implicit in this equation is the assumption that no gene flow occurs among sites during periods of nonflooding. We estimate that anywhere from a fraction of a year to nearly 19 years would be required for the build-up of F_{ST} between sites for which F_{ST} was lower in 2008 (Table 6), depending on the estimated value of N and the site pair considered. Notably, the time required generally increases with geographical distance between sites, reflecting a pattern of isolation by distance (Table 6). It is therefore likely that gene flow occurs continuously in this system, with higher gene flow between sites that are geographically close to each other. Therefore, the above equation might not be valid for this system, and these estimates should be interpreted with caution.

Table 6. Time required (in years) for F_{ST} values after a putative flood (2008 values) to build up to values observed in 2006

Site 1	Site 2	$N = 100$	$N = 500$	$N = 1000$
Bunoga	Rwebakwata	0.14	0.70	1.41
Bunoga	Kahunge	0.47	2.36	4.73
Bunoga	Kamwenge	1.87	9.37	18.74
Bwera	Kamwenge	0.61	3.08	6.15
Rwebakwata	Kamwenge	1.10	5.49	10.99
Kahunge	Kamwenge	1.88	9.42	18.84

Assumes 2 generations per year.

Conclusions

Our results unequivocally show that changes in genetic structure accrued between years in this system. However, it is difficult to ascertain the causes of these changes. Our results provide some evidence that changes are due to increased gene flow and possibly changes in population sizes and patterns of natural selection. Because genetic structure is low overall, small fluctuations in allele frequencies, such as those due to drift or nonrandom sampling, could have had large impacts on the results. The most probable explanation is that a combination of these factors influenced genetic change in this system between years.

In conclusion, population genetic structure of riverine fishes can fluctuate over short time scales. Similar results have been found in some other freshwater systems (e.g., Congdon 1995; Beneteau et al. 2009), but they conflict with results from some other studies of aquatic systems, which showed genetic structure to be stable across years (e.g., Favé and Turgeon 2008; Gonzalez et al. 2008; Ungfors et al. 2009). More studies are needed to determine under what scenarios temporal variation is likely to occur. Systems characterized by low differentiation might be particularly prone to show variation in genetic patterns over time, which can be greatly influenced by slight variation in demographic parameters. Estimates based on samples taken at one point in time might thus not be valid across time. Understanding how population structure is expected to fluctuate on short time scales is imperative for knowing how populations will respond over longer periods of time and for establishing conservation policies. Future work on riverine organisms should examine temporal changes in genetic structure, rather than drawing conclusions based on point-in-time estimates.

Funding

Natural Sciences and Engineering Research Council of Canada in the form of a Canada Graduate Scholarship (to E.C.), a Discovery Grant (to L.J.C.), and Canada Research Chair funds (to L.J.C.).

Acknowledgments

Field work was completed with the help of Emmanuel Aliganyira, Patrick Omeja, Jaclyn Paterson, Diana Sharpe, and Dennis Twinomugisha. Genotyping was completed by Geneviève Geneau at Génome Québec Innovation Centre. The rainfall data for Kahunge were provided by Erin Reardon. Helpful comments were provided by Alison Derry, Marie-Julie Favé, Andrew Hendry, Adam Herman, Simon Joly, Daniel Schoen, Robin Waples, and 2 anonymous reviewers.

References

Barluenga M, Stölting KN, Salzburger W, Muschick M, Meyer A. 2006. Sympatric speciation in Nicaraguan crater lake cichlid fish. Nature. 439: 719–723.

Barson NJ, Cable J, van Oosterhout C. 2009. Population genetic analysis of microsatellite variation of guppies (*Poecilia reticulata*) in Trinidad and Tobago: evidence for a dynamic source–sink metapopulation structure, founder events and population bottlenecks. J Evol Biol. 22:485–497.

Beneteau CL, Mandrak NE, Heath DD. 2009. The effects of river barriers and range expansion of the population genetic structure and stability in Greenside Darter (*Etheostoma blennioides*) populations. Conserv Genet. 10:477–487.

Bernatchez L, Wilson CC. 1998. Comparative phylogeography of nearctic and palearctic fishes. Mol Ecol. 7:431–452.

Bowcock AM, Ruiz-Linares A, Tomfohrde J, Minch E, Kidd JR, Cavalli-Sforza LL. 1994. High resolution of human evolutionary trees with polymorphic microsatellites. Nature. 368:455–457.

Chapman LJ. 1995. Seasonal dynamics of habitat use by an air-breathing catfish (*Clarias liocephalus*) in a papyrus swamp. Ecol Fresh Fish. 4: 113–123.

Chapman LJ, Albert J, Galis F. 2008. Developmental plasticity, genetic differentiation, and hypoxia-induced trade-offs in an African cichlid fish. Open Evol J. 2:75–88.

Chapman LJ, Liem KF. 1995. Papyrus swamps and the respiratory ecology of *Barbus neumayeri*. Env Biol Fish. 44:183–197.

Chapman LJ, Nordlie FG, Seifert A. 2002. Respiratory oxygen consumption among groups of *Pseudocrenilabrus multicolor victoriae* subjected to different oxygen concentrations during development. J Fish Biol. 61:242–251.

Chapman LJ, Schneider KM, Apodaca C, Chapman CA. 2004. Respiratory ecology of macroinvertebrates in a swamp–river system of East Africa. Biotropica. 36:572–585.

Chapman RW, Sedberry GR, Koenig CC, Eleby BM. 1999. Stock identification of gag, *Mycteroperca microlepis*, along the southeast coast of the United States. Mar Biotech. 1:137–146.

Congdon BC. 1995. Unidirectional gene flow and maintenance of genetic diversity in mosquitofish *Gambusia holbrooki* (Teleostei, Poeciliidae). Copeia. 1995:162–172.

Crispo E, Chapman LJ. 2008. Population genetic structure across dissolved oxygen regimes in an African cichlid fish. Mol Ecol. 17:2134–2148.

Crispo E, Hagen C, Glenn T, Geneau G, Chapman LJ. 2007. Isolation and characterization of tetranucleotide microsatellite markers in a mouth-brooding haplochromine cichlid fish (*Pseudocrenilabrus multicolor victoriae*) from Uganda. Mol Ecol Notes. 7:1293–1295.

Crow JF, Kimura M. 1970. Introduction to population genetics theory. New York: Harper and Row.

DeSalle R, Amato G. 2004. The expansion of conservation genetics. Nature Rev Genet. 5:702–712.

Dieringer D, Schlötterer C. 2003. MICROSATELLITE ANALYSER (MSA): a platform independent analysis tool for large microsatellite data sets. Mol Ecol Notes. 3:167–169.

Excoffier L, Laval G, Schneider S. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform Online. 1:47–50.

Fausch KD, Taniguchi Y, Nakano S, Grossman GD, Townsend CR. 2001. Flood disturbance regimes influence rainbow trout invasion success among five holarctic regions. Ecol Appl. 11:1438–1455.

Favé MJ, Turgeon J. 2008. Patterns of genetic diversity in Great Lakes bloaters (*Coregonus hoyi*) with a view to future reintroduction in Lake Ontario. Conserv Genet. 9:281–293.

Fraser DJ, Bernatchez L. 2005. Allopatric origins of sympatric brook charr populations: colonization history and admixture. Mol Ecol. 14:1497–1509.

Gonzalez EG, Beerli P, Zardoya R. 2008. Genetic structuring and migration patterns of Atlantic bigeye tuna, *Thunnus obesus* (Lowe, 1839). BMC Evol Biol. 8:252–266.

González-Wangüemert N, Pérez-Ruzafa Á, Cánovas F, García-Charton JA, Marcos C. 2007. Temporal genetic variation in populations of *Diplodus sargus* from the SW Mediterranean Sea. Mar Ecol Prog Ser. 334:237–244.

Goudet J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from URL. http://www.unil.ch/izea/softwares/fstat.html

Grether GF, Millie DF, Bryant MJ, Reznick DN, Mayea W. 2001. Rain forest canopy cover, resource availability, and life history evolution in guppies. Ecology. 82:1546–1559.

Hartl DL. 2000. A primer of population genetics. Sunderland (MA): Sinauer Associates, Inc.

Hernandez-Martich JD, Smith MH. 1997. Downstream gene flow and genetic structure of *Gambusia holbrooki* (eastern mosquitofish) populations. Heredity. 79:295–301.

Huey JA, Baker AM, Hughes JM. 2008. The effect of landscape processes upon gene flow and genetic diversity in an Australian freshwater fish, *Neosilurus hyrtlii*. Freshwater Biol. 53:1393–1408.

Johnson LS, Taylor EB. 2004. The distribution of divergent mitochondrial DNA lineages of threespine stickleback (*Gasterosteus aculeatus*) in the northeastern Pacific Basin: post-glacial dispersal and lake accessibility. J Biogeog. 31:1073–1083.

Jowers MJ, Cohen BL, Downie JR. 2008. The cyprinodont fish *Rivulus* (Aplocheiloidei: Rivulidae) in Trinidad and Tobago: molecular evidence for marine dispersal, genetic isolation and local differentiation. J Zool Syst Evol Res. 46:48–55.

Kalinowski ST. 2005. HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. Mol Ecol Notes. 5:187–189.

Kotlik P, Markova S, Choleva L, Bogutskaya NG, Ekmekci FG, Ivanova PP. 2008. Divergence with gene flow between Ponto-Caspian refugia in an anadromous cyprinid *Rutilus frisii* revealed by multiple gene phylogeography. Mol Ecol. 17:1076–1088.

Kuhner MK. 2009. Coalescent genealogy samplers: windows into population history. Trends Ecol Evol. 24:86–92.

Lacson JM, Morizot DC. 1991. Temporal genetic variation in subpopulations of bicolor damselfish (*Stegastes partitus*) inhabiting coral reefs in the Florida Keys. Mar Biol. 110:353–357.

Manel S, Gaggiotti OE, Waples RS. 2005. Assignment methods: matching biological questions techniques with appropriate. Trends Ecol Evol. 20: 136–142.

Martin PR, McKay JK. 2004. Latitudinal variation in genetic divergence of populations and the potential for future speciation. Evolution. 58:938–945.

Masci KD, Ponniah M, Hughes JM. 2008. Patterns of connectivity between the Lake Eyre and Gulf drainages, Australia: a phylogeographic approach. Mar Freshwater Res. 59:751–760.

Meldgaard T, Nielsen EE, Loeschcke V. 2003. Fragmentation by weirs in a riverine system: a study of genetic variation in time and space among populations of European grayling (*Thymallus thymallus*) in a Danish river system. Conserv Genet. 4:735–747.

Miller MP. 1997. Tools for population genetic analysis (TFPGA) 1.3: a Windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by author.

Nielsen EE, Hansen MM, Meldrup D. 2006. Evidence of microsatellite hitch-hiking selection in Atlantic cod (*Gadus morhua* L.): implications for inferring population structure in nonmodel organisms. Mol Ecol. 15: 3219–3229.

Nosil P, Funk DJ, Ortiz-Barrientos D. 2009. Divergent selection and heterogeneous genomic divergence. Mol Ecol. 18:375–402.

Palm S, Laikre L, Jorde PE, Ryman N. 2003. Effective population size and temporal genetic change in stream resident brown trout (*Salmo trutta*, L.). Conserv Genet. 4:249–264.

Pearse DE, Crandall KA. 2004. Beyond F_{ST}: analysis of population genetic data for conservation. Conserv Genet. 5:585–602.

Pudovkin AI, Zaykin DV, Hedgecock D. 1996. On the potential for estimating the effective number of breeders from heterozygote-excess in progeny. Genetics. 144:383–387.

Raymond M, Rousset F. 1995. Genepop (version 1.2): population genetics software for exact tests and ecumenicism. J Hered. 86:248–249.

Sønstebø JH, Borgstrøm R, Heun M. 2008. Genetic structure in alpine brown trout *Salmo trutta* L. shows that indirect stocking affects native lake populations. J Fish Biol. 72:1990–2001.

Storz JF. 2005. Using genome scans of DNA polymorphism to infer adaptive population divergence. Mol Ecol. 14:671–688.

Ungfors A, McKeown NJ, Shaw PW, André C. 2009. Lack of spatial genetic variation in the edible crab (*Cancer pagurus*) in the Kattegat–Skagerrak area. ICES J Mar Sci. 66:462–469.

Waples RS. 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. J Hered. 89:438–450.

Waples RS. 2006. A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. Conserv Genet. 7:167–184.

Waples RS, Do C. 2008. LDNE: a program for estimating effective population size from data on linkage disequilibrium. Mol Ecol Res. 8:753–756.

Ward RD, Woodwark M, Skibinski DOF. 1994. A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. J Fish Biol. 44:213–232.

Zhdanova OL, Pudovkin AI. 2008. Nb_HetEx: a program to estimate the effective number of breeders. J Hered. 99:694–695.

Received March 17, 2009; Revised July 26, 2009;
Accepted August 12, 2009

Corresponding Editor: Robin Waples