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

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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; Fave and Turgeon 2008; Sonstebo 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; Gonza´lez-Wangu¨ emert 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; Masi 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
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).
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):

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

where $n$ is the total number of tests, $r$ is the number of significant tests, $\alpha$ 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 $\alpha$, 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 (\(F_{est}\)) 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 (\(P = 0.0102\) 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 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). FIS values were low, providing no evidence for local inbreeding (Table 1). Instead, negative FIS 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 \( N_h \), often with upper bounds at infinity (Table 2). Using the LD method, the estimated \( N_e \) tended to increase with decreasing \( P_{\text{dm}} \), 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 those calculated using the parametric procedure (Table 2). Overall, we observed no trends in \( N_e \) or \( N_h \) 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 \( N_h \) 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 (\( D_{sb} \)) 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

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

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Swamp</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kanyantale</td>
<td>34</td>
<td>136</td>
<td>112.5</td>
<td>3.1</td>
<td>0.9202</td>
<td>0.8697</td>
<td>0.0580</td>
</tr>
<tr>
<td>Bwera</td>
<td>29 (27)</td>
<td>130 (147)</td>
<td>104.4 (120.2)/6</td>
<td>3.4 (4.5)/7</td>
<td>0.8605 (0.9193)/6</td>
<td>0.8411 (0.8754)/9</td>
<td>0.0231 (−0.0502)</td>
</tr>
<tr>
<td>River</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bunoga</td>
<td>30 (31)</td>
<td>136 (150)</td>
<td>109.0 (112.6)/5</td>
<td>5.4 (5.5)/5</td>
<td>0.8249 (0.8742)/7</td>
<td>0.8337 (0.8415)/6</td>
<td>0.0106 (−0.0389)</td>
</tr>
<tr>
<td>Rwebakwata</td>
<td>30 (31)</td>
<td>148 (144)</td>
<td>115.0 (117.7)/5</td>
<td>5.3 (2.3)/1</td>
<td>0.8778 (0.9026)/7</td>
<td>0.8602 (0.8794)/8</td>
<td>0.0205 (−0.0264)</td>
</tr>
<tr>
<td>Kahunge</td>
<td>30 (30)</td>
<td>152 (144)</td>
<td>119.6 (116.9)/4</td>
<td>7.2 (5.0)/3</td>
<td>0.8900 (0.8917)/5</td>
<td>0.8587 (0.8687)/5</td>
<td>0.0365 (−0.0265)</td>
</tr>
<tr>
<td>Kamwenge</td>
<td>30 (18)</td>
<td>165 (143)</td>
<td>126.1 (133.9)/7</td>
<td>15.9 (30.2)/9</td>
<td>0.9185 (0.8874)/5</td>
<td>0.8795 (0.8879)/7</td>
<td>0.0443 (0.0006)</td>
</tr>
</tbody>
</table>

\( n \) is the sample size, \( H_{E} \) is observed heterozygosity, \( H_{C} \) is expected heterozygosity, \( F_{IS} \) is \( 1 − (H_{E}/H_{C}) \). The italicized values are the number of loci showing higher values in 2006 than in 2008.
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). Our estimates of $N_e$ and $N_b$, however, did not reveal any general trends in changes in population sizes between years. The estimated variation in $N_e$ among sites (Craw and Kimura 1970) and thus these population sizes have been linked to changes in allele frequencies (see Table 4). As noted above, a possible reason for this is high variation in $N_e$ among sampled sites, violating the assumptions of the tests 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. In the swamp, we suggest that increased directional gene flow has occurred between years (Table 4; Crispo and Chapman 2008; Table 1). Because $P_{mv9}$ cannot be caught in abundance at sites that were explored upstream of our sampling sites (Aliganyira E, Omeja P, Twinomugisha D, personal communications), it is not likely that significant gene flow is occurring from upstream nonsampled sites—it is more probable that these sites that were caught in abundance at our sampling sites were sampled at higher frequencies than the upstream sites.
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. Rwabakwata); that is, $F_{ST}$ was lower for downstream sites (Kahunge and Kamwenge; Table 3). As well, the 2 site-pairs for which $F_{ST}$ values were significant in 2006 but not in 2008 (Bunoga vs. Kahunge and Rwabakwata 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 3.** $F_{ST}$ and $D_{ps}$ values between sites within years

<table>
<thead>
<tr>
<th>Site 1</th>
<th>Site 2</th>
<th>All loci 2006</th>
<th>All loci 2008</th>
<th>No Pmv9 2006</th>
<th>No Pmv9 2008</th>
<th>Only Pmv9 2006</th>
<th>Only Pmv9 2008</th>
<th>All loci 2006</th>
<th>All loci 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kanyantale</td>
<td>Bwera</td>
<td>—</td>
<td>0.0127</td>
<td>—</td>
<td>0.0121</td>
<td>—</td>
<td>0.0177</td>
<td>—</td>
<td>0.31766</td>
</tr>
<tr>
<td>Kanyantale</td>
<td>Bunoga</td>
<td>—</td>
<td>0.0176</td>
<td>—</td>
<td>0.0173</td>
<td>—</td>
<td>0.0201</td>
<td>—</td>
<td>0.33872</td>
</tr>
<tr>
<td>Kanyantale</td>
<td>Rwabakwata</td>
<td>—</td>
<td>0.0067</td>
<td>—</td>
<td>0.0064</td>
<td>—</td>
<td>0.0097</td>
<td>—</td>
<td>0.29777</td>
</tr>
<tr>
<td>Kanyantale</td>
<td>Kahunge</td>
<td>0.0042</td>
<td>—</td>
<td>0.0043</td>
<td>—</td>
<td>0.0007</td>
<td>—</td>
<td>0.0037</td>
<td>0.26601</td>
</tr>
<tr>
<td>Kanyantale</td>
<td>Kamwenge</td>
<td>—</td>
<td>0.0002</td>
<td>—</td>
<td>0.0007</td>
<td>—</td>
<td>0.0039</td>
<td>—</td>
<td>0.27925</td>
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<td>0.0104</td>
<td>0.0111</td>
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<td>—</td>
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</tr>
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<td>0.0061</td>
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<td>0.0023</td>
<td>0.0033</td>
<td>0.00142</td>
<td>0.26927</td>
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<tr>
<td>Bwera</td>
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<td>0.0120</td>
<td>0.0193</td>
<td>0.45206</td>
<td></td>
</tr>
<tr>
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<td>Rwabakwata</td>
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<td>—</td>
<td>0.0009</td>
<td>0.0072</td>
<td></td>
</tr>
<tr>
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<td>Kahunge</td>
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<td>0.0039</td>
<td>0.0084</td>
<td>0.0046</td>
<td>0.0043</td>
<td>0.0015</td>
<td>0.32195</td>
<td></td>
</tr>
<tr>
<td>Bunoga</td>
<td>Kamwenge</td>
<td>0.0327</td>
<td>0.0144</td>
<td>0.0308</td>
<td>0.0147</td>
<td>0.0195</td>
<td>0.0123</td>
<td>0.49562</td>
<td></td>
</tr>
<tr>
<td>Rwabakwata</td>
<td>Kahunge</td>
<td>—</td>
<td>0.0040</td>
<td>0.0004</td>
<td>0.0017</td>
<td>0.0042</td>
<td>0.0095</td>
<td>0.0021</td>
<td></td>
</tr>
<tr>
<td>Rwabakwata</td>
<td>Kamwenge</td>
<td>0.0139</td>
<td>0.0039</td>
<td>0.0103</td>
<td>0.0033</td>
<td>0.0087</td>
<td>0.0009</td>
<td>0.42919</td>
<td></td>
</tr>
<tr>
<td>Kahunge</td>
<td>Kamwenge</td>
<td>0.0224</td>
<td>0.0038</td>
<td>0.0199</td>
<td>0.0045</td>
<td>0.0224</td>
<td>0.0014</td>
<td>0.48489</td>
<td></td>
</tr>
</tbody>
</table>

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).

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

<table>
<thead>
<tr>
<th>Site</th>
<th>All loci</th>
<th>No Pmv9</th>
<th>Only Pmv9</th>
<th>All loci</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bwera</td>
<td>0.0007</td>
<td>—0.0011</td>
<td>0.0153</td>
<td>0.51947</td>
</tr>
<tr>
<td>Bunoga</td>
<td>0.0010</td>
<td>0.0005</td>
<td>0.0051</td>
<td>0.47174</td>
</tr>
<tr>
<td>Rwabakwata</td>
<td>—0.0018</td>
<td>—0.0017</td>
<td>—0.0025</td>
<td>0.49913</td>
</tr>
<tr>
<td>Kahunge</td>
<td>0.0040</td>
<td>0.0038</td>
<td>0.0062</td>
<td>0.51603</td>
</tr>
<tr>
<td>Kamwenge</td>
<td>0.0072</td>
<td>0.0072</td>
<td>0.0097</td>
<td>0.62334</td>
</tr>
</tbody>
</table>

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

**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: $r^2 = 0.1956$, $P = 0.2040$).
In systems characterized by low genetic differentiation at
enced different patterns of selection during development.
of a mixture of cohorts, each of which might have experi-
cohorts. We do not have information on the ages of our
of selection might have acted on juveniles from different
overall (Palm et al. 2003), suggesting that different patterns
Sweden were greater among cohorts than among years
changes from one year to the next, or slight deviations from
levels during the second sampling season than the first
observed before the flood? That is, given the
for the build-up of genetic differences among sites similar to
those observed before the flood? That is, given the
FST values observed in 2008, how much evolutiona
tected differences in water flow among seasons might
have influenced fish population structure between years.
Indeed, over a 2-year period, we noted a nonsignificant rela-
tionship 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
abiotic properties that affect fish movement or survival (e.g.,
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.
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 in 2008. We assume 2 generations
between sites, reflecting a pattern of isolation by distance
required generally increases with geographical distance
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 genetic differences among
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 5. Percentage of variation in each grouping obtained from AMOVA

<table>
<thead>
<tr>
<th>Locus</th>
<th>Among sites</th>
<th>Between years within sites</th>
<th>Within sites within years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pmv1</td>
<td>0.6758</td>
<td>-0.1112</td>
<td>99.4354</td>
</tr>
<tr>
<td>Pmv3</td>
<td>0.5892</td>
<td>0.3753</td>
<td>99.0355</td>
</tr>
<tr>
<td>Pmv4</td>
<td>0.4537</td>
<td>0.3311</td>
<td>99.2152</td>
</tr>
<tr>
<td>Pmv9</td>
<td>0.0872</td>
<td>0.6478</td>
<td>99.2650</td>
</tr>
<tr>
<td>Pmv13</td>
<td>0.3492</td>
<td>0.0155</td>
<td>99.6353</td>
</tr>
<tr>
<td>Pmv15</td>
<td>0.8086</td>
<td>-0.4753</td>
<td>99.6667</td>
</tr>
<tr>
<td>Pmv17</td>
<td>0.4389</td>
<td>0.4233</td>
<td>99.1377</td>
</tr>
<tr>
<td>Ppun4</td>
<td>1.4324</td>
<td>-0.8953</td>
<td>99.4628</td>
</tr>
<tr>
<td>Ppun5</td>
<td>0.3393</td>
<td>0.4989</td>
<td>99.1618</td>
</tr>
<tr>
<td>Ppun17</td>
<td>0.4886</td>
<td>0.8032</td>
<td>98.7082</td>
</tr>
<tr>
<td>All</td>
<td>0.5241</td>
<td>0.2148</td>
<td>99.2611</td>
</tr>
</tbody>
</table>

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

 inflicted. 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
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
samples individually, however, and samples probably consist
of a mixture of cohorts, each of which might have experi-
enced 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.
Table 6. Time required (in years) for $F_{ST}$ values after a putative flood (2008 values) to build up to values observed in 2006

<table>
<thead>
<tr>
<th>Site 1</th>
<th>Site 2</th>
<th>$N = 100$</th>
<th>$N = 500$</th>
<th>$N = 1000$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bunoga</td>
<td>Rwebakwata</td>
<td>0.14</td>
<td>0.70</td>
<td>1.41</td>
</tr>
<tr>
<td>Bunoga</td>
<td>Kahunge</td>
<td>0.47</td>
<td>2.36</td>
<td>4.73</td>
</tr>
<tr>
<td>Bunoga</td>
<td>Kamwenge</td>
<td>1.87</td>
<td>9.37</td>
<td>18.74</td>
</tr>
<tr>
<td>Bwera</td>
<td>Kamwenge</td>
<td>0.61</td>
<td>3.08</td>
<td>6.15</td>
</tr>
<tr>
<td>Rwebakwata</td>
<td>Kamwenge</td>
<td>1.10</td>
<td>5.49</td>
<td>10.99</td>
</tr>
<tr>
<td>Kahunge</td>
<td>Kamwenge</td>
<td>1.88</td>
<td>9.42</td>
<td>18.84</td>
</tr>
</tbody>
</table>

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 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.

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