

Population genetic structure across dissolved oxygen regimes in an African cichlid fish

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Abstract

Ecological isolation is a process whereby gene flow between selective environments is reduced due to selection against maladapted dispersers, migrant alleles, or hybrids. Although ecological isolation has been documented in several systems, gene flow can often be high among selective regimes, and more studies are thus needed to better understand the conditions under which ecological gradients or divergent selective regimes should influence population structure. We test for ecological isolation in a system in which high plasticity occurs with respect to traits that are adaptive in alternate forms under different environmental conditions. *Pseudocrenilabrus multicolor victoriae* is a widespread haplochromine cichlid fish in East Africa that exploits both normoxic (normal oxygen) rivers/lakes and hypoxic (low oxygen) swamps. Here, we examine population structure, using mitochondrial DNA and microsatellites, to determine if genetic divergence is significantly increased between dissolved oxygen regimes relative to within them, while controlling for geographical structure. Our results indicate that geographical separation influences population structure, while no effects of divergent selection with respect to oxygen regimes were detected. Specifically, we document (i) genetic clustering according to geographical region, but no clustering according to oxygen regime; (ii) higher genetic variation among than within regions, but no effect of oxygen regime on genetic variation; (iii) isolation by distance within one region; and (iv) decreasing genetic variability with increasing geographical distance from Lake Victoria. We speculate that plasticity may be facilitating gene flow between oxygen regimes in this system.

Keywords: divergent natural selection, ecological isolation, ecological speciation, hypoxia, isolation by distance, phenotypic plasticity

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Introduction

What is the importance of ecological variation in the structuring of natural populations? The potential effects of geographical isolation are obvious: physical barriers, such as mountain ranges and water bodies for terrestrial organisms (e.g. Parmakelis *et al.* 2005; Fritz *et al.* 2006), or waterfalls and expanses of land for aquatic organisms (e.g. Poissant *et al.* 2005; Crispo *et al.* 2006), can prevent the movement of individuals between populations, thus diminishing gene flow. Large physical distances can have a similar effect through isolation by distance (e.g. Pope *et al.*

2006; Mock *et al.* 2007). In these cases, geographically separated populations may diverge from one another via drift, and may be capable of adapting to local conditions without the constraining force of maladaptive gene flow. On the other hand, ecological gradients or divides can also influence population structure through the action of divergent natural selection. If divergent selection is sufficiently strong, it may lead to ecological isolation via reduced fitness of dispersers between selective environments in relation to well-adapted residents (Hendry 2004; Nosil 2004; Nosil *et al.* 2005), and/or reduced fitness of hybrids between individuals adapted to alternate environmental conditions (Via *et al.* 2000; Rundle 2002). The latter may lead to the evolution of reinforcement, that is, sexual isolation between individuals adapted to different environmental conditions

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(Boughman *et al.* 2005; Rundle *et al.* 2005), and together, these phenomena constitute the process of ecological speciation (Schluter 2000, 2001; Rundle & Nosil 2005). An effect of divergent selection on population structure will be apparent if genetic divergence is markedly higher between selective environments than within them, after controlling for geographical distance and physical barriers to dispersal (e.g. Smith *et al.* 2001; Ogden & Thorpe 2002; Saint-Laurent *et al.* 2003; Rocha *et al.* 2005; Smith *et al.* 2005; Crispo *et al.* 2006).

Several studies have clearly documented that divergent selection can influence genetic structure in natural systems. These systems include as examples: coral reef fishes adapted to cold- or warm-water habitats (Rocha *et al.* 2005), salt marsh beetles adapted to ponds or canals (Dhuyvetter *et al.* 2007), dwarf and normal whitefish ecotypes adapted to different trophic niches (Lu & Bernatchez 1999), and corn borers adapted to different host plants (Bethenod *et al.* 2005). However, genetic structuring in relation to ecological variables is not ubiquitous among systems in which phenotypically divergent populations occur under different selection pressures (e.g. Stanton *et al.* 1997; Crispo *et al.* 2006; Blank *et al.* 2007). That is, fitness of dispersers and their hybrids may not be sufficiently reduced among selective environments to detect a reduction in neutral gene flow. Linkage disequilibrium between selected and neutral loci that occurs in the second generation, due to recombination, could allow for high neutral gene flow despite genetic trait divergence among populations (i.e. the porous genome phenomenon; Barton & Bengtsson 1986; Wu 2001). Speculations as to why high gene flow occurs among selective environments include benefits of reduced inbreeding depression, enhanced adaptability to environmental change because of increased genetic variability, or the spread of beneficial alleles among populations (reviewed in Garant *et al.* 2007). These benefits of gene flow, however, should generally occur only for small populations and when temporal variation in selection occurs (Garant *et al.* 2007). Another explanation for high gene flow is that traits are highly plastic, so that individuals can alter their phenotypes to adapt to alternate selective environments, thus diminishing the effects of divergent natural selection (reviewed in Schlichting & Pigliucci 1998, pp. 66–67; West-Eberhard 2003, p. 178).

Our goal is to explore population structure within a system in which high levels of plasticity occur in traits that are adaptive in alternate forms under different environmental conditions. We test whether populations are structured more strongly by geography (i.e. physical separation, geographical distance) or by ecological contrasts by (i) examining patterns of genetic clustering, (ii) comparing genetic variation within and among regions, and within and among environment types, and (iii) testing for isolation by distance. We use natural populations of *Pseudocrenilabrus multicolor victoriae*, an East African haplochromine cichlid

fish, adapted to alternate oxygen regimes. This species is common throughout the Lake Victoria basin and Nile River system (Greenwood 1965; Schierwater & Mrowka 1987). It is the only haplochromine cichlid found in high abundance in the dense interior of the hypoxic (low oxygen) wetlands that were explored in the present study, and is also abundant in normoxic (normal oxygen) lakes and rivers (Fig. 1; Chapman *et al.* 1996a, b; Rosenberger & Chapman 1999; Chapman *et al.* 2002a).

Pseudocrenilabrus multicolor victoriae uses a variety of behavioural, morphological, and physiological strategies for exploiting hypoxic waters, for example, aquatic surface respiration, high haemoglobin concentration and haematocrit, a large gill surface area, and a relatively low critical oxygen tension (Chapman *et al.* 2000a; Rosenberger & Chapman 2000; Chapman *et al.* 2002a, b). In the wild, fish from hypoxic areas tend to have larger gills than fish from normoxic areas, which is probably an adaptive response (Chapman *et al.* 2000a, 2002a; L. Chapman & L. Piché, unpublished data), and trade-offs between gills and surrounding structural elements may contribute to the maintenance of this variation (Chapman *et al.* 2000a). Although plasticity in suites of traits (such as gill and trophic structures, and body shape) has been documented in *P. multicolor victoriae*, some of these traits differed between populations from normoxic and hypoxic waters when F_1 s were raised in a common garden environment, indicating the presence of both genetic and environmental components of trait variation (Chapman *et al.* 2000a; L. Chapman, F. Galis, & J. Albert, unpublished data). Plasticity may facilitate gene flow between oxygen regimes; however, because genetic components of trait divergence occur, one would predict that divergent selection may act to reduce dispersal or hybridization between oxygen regimes. Other factors contributing to divergent selection between oxygen regimes may include trophic differences, predation, and water flow, and these factors have been shown to influence phenotypic diversity in other fish species (e.g. Robinson & Wilson 1994; Langerhans *et al.* 2003; Langerhans & DeWitt 2004; Bhagat *et al.* 2006). Evidence suggests that *P. multicolor victoriae* may be morphologically adapted to differences in at least some of these conditions (Chapman *et al.* 2000a); thus, these factors, in addition to oxygen concentration, may contribute to ecological isolation. However, if plasticity in the above-mentioned traits is strong, divergent selection against dispersers and their offspring may be diminished.

Materials and methods

Sampling

Fish were collected using minnow traps from four regions in Uganda (Fig. 1a), including the Lake Nabugabo region (June 2003), the Mpanga River region (June 2006), Lake

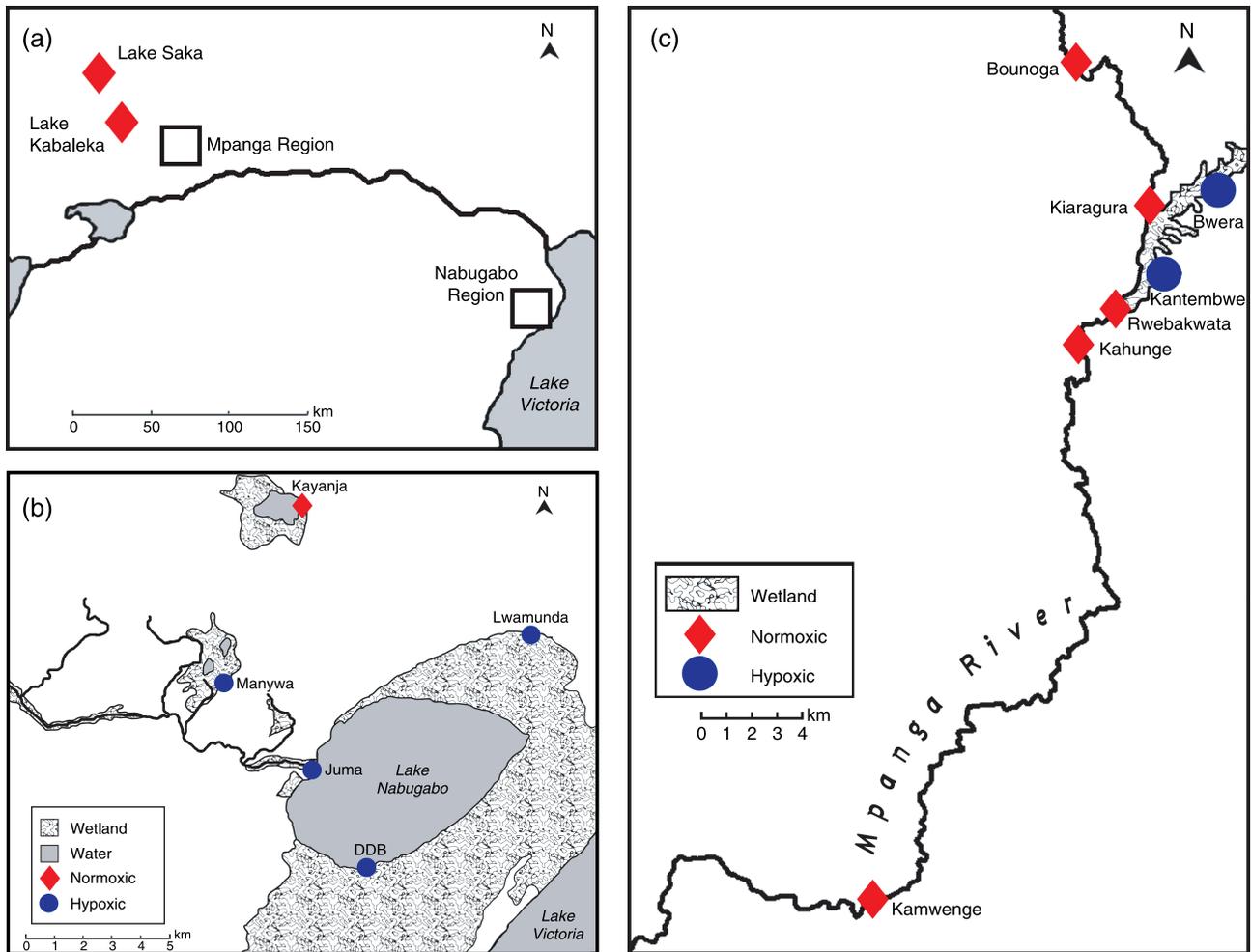


Fig. 1 Regions within Uganda from which fish were sampled (a), including sites within the Nabugabo region (b) and the Mpanga region (c).

Saka (June 2006 to January 2007), and Lake Kabaleka (June 2003). Five sites were sampled in the Lake Nabugabo region (Fig. 1b), and seven sites were sampled in the Mpanga River region (Fig. 1c). Lakes Kabaleka and Saka (Fig. 1a) were each treated as single sites because fish were sampled over relatively small areas. Sample sizes and environment type (hypoxic or normoxic) are indicated in Table 1. Fin clips were stored in 95% ethanol, and DNA was extracted using DNeasy Tissue Kits (QIAGEN). Dissolved oxygen concentration (mg/L) and water temperature (to convert to percentage oxygen saturation) were measured at 6–33 locations throughout the area from which fish were sampled in the upper 20 cm of the water column (Fig. 2). To explore the degree to which point-in-time measures represent intersite variation in dissolve oxygen, we also sampled a subset of sites once per month for 1 year. These sites included Kahunge, Kantembwe, Lwamunda, Kayanja, and Saka (Fig. 3).

Within the Mpanga region, two swamp sites were sampled that remain hypoxic year round (Kantembwe and

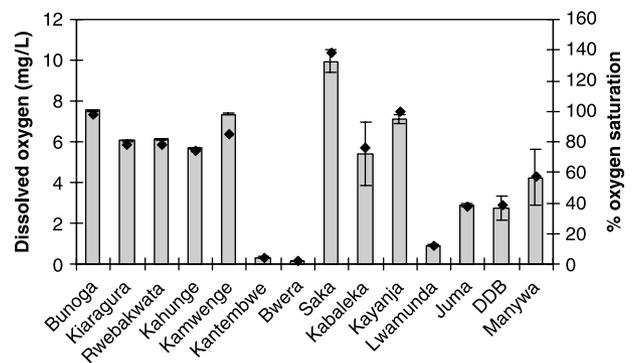


Fig. 2 Point-in-time mean (\pm standard error) dissolved oxygen concentration (bars) and the mean percent oxygen saturation (points) at each site. Measurements were taken in June during the year of fish sampling, with the exception of Kabaleka (August 1997 and May 1998), Kayanja (June 2000), and Manywa (June 2005).

Table 1 Sampling information, and measures of variability for mtDNA sequences and microsatellites. All fish were sampled in June of the year of sampling, with the exception of Saka (June 2006–January 2007)

Site	Region	Environment	Sampling year	n_{mtDNA}	No. of haplotypes	h	π	n_{msat}	No. of alleles	Allelic richness	H_O	H_E
Bun	Mpanga	N	2006	20	2	0.1895	0.0005	31	150	112.6	0.8742	0.8415
Kah	Mpanga	N	2006	20	2	0.5053	0.0013	30	144	116.9	0.8917	0.8687
Kia	Mpanga	N	2006	0	NA	NA	NA	30	140	112.9	0.8850	0.8607
Rwe	Mpanga	N	2006	0	NA	NA	NA	31	144	117.7	0.9026	0.8794
Kam	Mpanga	N	2006	18	4	0.7582	0.0034	18	143	133.9	0.8874	0.8879
Bwe	Mpanga	H	2006	20	2	0.5211	0.0013	27	147	120.2	0.9193	0.8754
Kant	Mpanga	H	2006	21	2	0.4667	0.0012	29	143	112.8	0.8759	0.8583
Kay	Nabugabo	N	2003	27	5	0.6268	0.0022	29	181	150.1	0.8745	0.9070
Man	Nabugabo	H	2003	20	3	0.4842	0.0016	23	164	136.6	0.9277	0.8923
DDB	Nabugabo	H	2003	15	4	0.7143	0.0026	18	162	149.6	0.9297	0.9016
Lwa	Nabugabo	H	2003	22	5	0.6797	0.0024	24	183	152.6	0.9227	0.9094
Juma	Nabugabo	H	2003	21	4	0.6143	0.0020	21	157	139.2	0.9043	0.8989
Saka	Lake Saka	N	2006–2007	12	1	0	0	30	64	57.5	0.6963	0.6997
Kab	Lake Kabaleka	N	2003	7	1	0	0	0	NA	NA	NA	NA

Bun, Bunoga; Bwe, Bwera; Kah, Kahunge; Kant, Kantembwe; Kia, Kiaragura; Kam, Kamwenge; Kay, Kayanja; Lwa, Lwamunda; Man, Manywa; N, normoxic; H, hypoxic; Rwe, Rwebakwata; DDB, Dead Duck Bay.

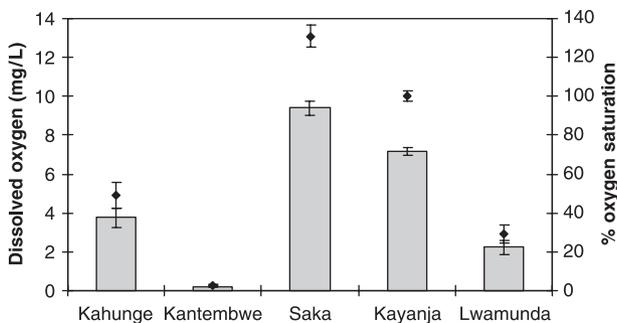


Fig. 3 Yearly oxygen measurements for five sites. Mean monthly values were recorded for one year and averaged over 12 months. Bars represent the mean (\pm standard error) dissolved oxygen concentration and points represent the mean (\pm standard error) percent oxygen saturation. Recordings were made during the following time periods: Kahunge and Kantembwe, June 2006–May 2007; Saka, June 2005–May 2006; Kayanja, October 1999–September 2000; Lwamunda, June 2000–May 2001.

Bwera; Figs 2 and 3). At least two sites within the Mpanga River (Bunoga and Kamwenge) are characterized by flowing open waters that remain normoxic year round; a similar site, located upstream of Bunoga, is described in Chapman *et al.* (2004), and this site was normoxic throughout the year. At least one river site (Kahunge) experiences dramatic seasonal fluctuations in dissolved oxygen (Fig. 3). This likely reflects the influx of decomposing debris into the river from the swamp during the rainy seasons due to flooding (Chapman *et al.* 2000b). Although seasonal measures of dissolved oxygen have not been obtained at Rwebakwata

and Kiaragura, these sites are likely to experience some seasonal fluctuations, because of their proximity to the swamp; nearby villagers have noted flooding into adjacent wetlands at these two sites during peak flood conditions. However, at any given time, oxygen in these sites probably never approaches levels as low as those in the dense interior of the swamp, and we thus refer to these river sites as 'normoxic' for analysis purposes (Table 1).

Lake Saka is a hyper-eutrophic lake with super-saturated oxygen conditions during most of the year (we label this site as 'normoxic' in Table 1 for simplicity; Figs 2 and 3). Dissolved oxygen measures (Figs 2 and 3) were taken in both in the morning and in the afternoon and averaged to capture diurnal variation. There is no evidence for a contemporary connection between Lake Saka and the Mpanga drainage. Lake Kabaleka is a shallow lake surrounded by papyrus swamp. A vertical gradient of dissolved oxygen exists in the open waters of Lake Kabaleka, with a well-oxygenated upper stratum, where fish were captured and dissolved oxygen measured (Fig. 2), and a moderately oxygenated lower stratum (Melnichuk & Chapman 2002). Contemporary connections between Lake Kabaleka and the Mpanga River probably exist via Lake George, although a large waterfall (Mpanga Falls) would serve as barrier to upstream movement between fish from lakes Kabaleka/George to the sites sampled in the Mpanga region.

Lake Nabugabo is a satellite lake, isolated from Lake Victoria since approximately 5000 years ago (Stager *et al.* 2005). The predatory Nile perch (*Lates niloticus*) was introduced into Lake Nabugabo in the 1960s, and its upsurge was followed by a dramatic decline in native fishes within

the main lake, but not in the surrounding wetlands or nearby satellite systems (Ogutu-Ohwayo 1993); thus, *Pseudocrenilabrus multicolor victoriae* could be collected from surrounding areas but not from the open waters. Because of predation pressures imposed by Nile perch, dispersal through open water is probably reduced; thus, we expect physical dispersal to be low in this region, relative to the Mpanga region. In the areas surrounding Lake Nabugabo, we collected fish from three hypoxic sites: the swamp ecotone of a small bay (Dead Duck Bay), the dense *Miscanthidium*-dominated Lwamunda swamp, and the papyrus-choked Juma River. In addition, we sampled fish from the hypoxic papyrus/*Miscanthidium* swamp surrounding a small satellite lake, Lake Manywa. This swamp is connected to Lake Nabugabo via the Juma River. Finally, we sampled fish from the normoxic waters of Lake Kayanja, a small satellite lake with no Nile perch. Although we have no direct evidence that Lake Kayanja is currently connected to other sites within the Nabugabo region, a local field assistant (J. Mutebi) has suggested that a small swampy contemporary connection exists.

Although considerable overlap in dissolved oxygen occurs between the sampled 'normoxic' and 'hypoxic' sites among regions (e.g. Kahunge and Lwamunda; Fig. 3), little or no overlap occurs between 'normoxic' and 'hypoxic' sites within region (Figs 2 and 3). Moreover, waters with dissolved oxygen that drops below 2.8 mg/L (Diaz & Rosenberg 1995; Wu 2002) or 2.0 mg/L (Chesney *et al.* 2000) have been considered hypoxic in previous studies. While the oxygen at the Manywa, Juma, and Dead Duck Bay sites often rose above these values (Fig. 2), the gill morphologies of *P. multicolor victoriae* collected at Manywa and Juma are clearly divergent from those collected at sites with higher dissolved oxygen (Chapman *et al.* 2000a, 2002b; L. Chapman, F. Galis, & J. Albert, unpublished data; gill metrics from fish from Dead Duck Bay were not measured). Thus, divergent selection is probably acting among populations within regions, and should reflect our 'hypoxic-normoxic' dichotomy (Table 1).

Mitochondrial DNA

We sequenced the first 395 bp of the mitochondrial control region, using the following primers: forward, 5'-AACTCTC ACCCCTAGCTCCCAAAG-3' (Meyer *et al.* 1994); reverse, 5'-CCTGAAGTAGGAACCAGATG-3' (Meyer *et al.* 1990; Lee *et al.* 1995). This region is the most variable in cichlids and has been used successfully in other studies of cichlid population structure (e.g. Sturmbauer *et al.* 2001; Abila *et al.* 2004; Katongo *et al.* 2005; Duftner *et al.* 2006). Polymerase chain reaction (PCR) was conducted using a 9700 Applied Biosystems thermocycler using the following programme: one cycle of 96 °C for 15 min, 35 cycles of 96 °C for 30 s, 58 °C for 30 s, 72 °C for 1 min, and one cycle of 72 °C for

10 min. Sequencing was conducted at the Génome Québec Innovation Centre using an Applied Biosystems 3730xl sequencer. Sequences were edited using CHROMAS LITE version 2.01 (Technelysium Pty. Ltd). Initial alignments were performed using CLUSTAL W version 1.82 (Chenna *et al.* 2003), and subsequent alignments were made by eye.

First, we used DNASP version 4.10 (Rozas *et al.* 2003) to calculate the number of haplotypes, and both within-site and overall estimates of haplotype diversity (h) and nucleotide diversity (π) (Table 1). Estimates of π with and without the Jukes & Cantor (1969) correction were nearly identical (the greatest difference between the two measures within a site was 0.00001), and thus, we only report the noncorrected estimates (Table 1). Second, to analyse phylogenetic relationships among haplotypes, we created a haplotype network based on maximum parsimony using TCS version 1.21 (Clement *et al.* 2000). Third, we used the HIERFSTAT version 0.04-4 (Goudet 2005) package in R version 2.4.1 (R Development Core Team 2006) to estimate hierarchical F -statistics. Here, genetic variability was compared (i) among the four regions (Mpanga, Nabugabo, Kabaleka, Saka), (ii) between environments (normoxic or hypoxic) within region, and (iii) among sites within region (i.e. a nested design). Comparing F -statistics at these levels allows us to infer whether geographical separation or ecological divergence is the strongest force driving genetic divergence. We conducted permutation analysis (10 000 permutations) to determine the significance of each F -statistic. For (i), populations were permuted among regions, for (ii), populations were permuted among hypoxic and normoxic environments within region, and for (iii) individuals were permuted among populations within region.

Microsatellites

Twelve tetranucleotide microsatellites loci were genotyped. These included seven loci isolated from *P. multicolor victoriae* (Pmv1, Pmv3, Pmv4, Pmv9, Pmv13, Pmv15, Pmv17; Crispo *et al.* 2007), and an additional five loci that were originally isolated from *Pundamilia pundamilia* (Ppun5, Ppun17) or a hybrid between *Pun. pundamilia* and *Pundamilia nyererei* (Ppun2, Ppun4, Ppun12; Taylor *et al.* 2002). Primers for the Ppun loci had not previously been designed for any species (see Table 2 for PCR details and GenBank Accession numbers for Ppun loci). Genotyping was performed using the methods outlined in Crispo *et al.* (2007). We tested for Hardy-Weinberg equilibrium (HWE) and linkage equilibrium (LE) within sites and across all sites using GENEPOP version 3.4 (Raymond & Rousset 1995) (1000 batches, 10 000 iterations per batch). Ppun2 and Ppun12 showed excesses of homozygotes (see Results), and they were thus excluded from further analyses, because of the possible presence of null alleles. Observed and expected (unbiased) heterozygosities were estimated using TFPGA version 1.3 (Miller

Table 2 GenBank Accession numbers and PCR information for five microsatellite loci isolated from *Pundamilia pundamilia* (Ppun5, Ppun17) or a *P. pundamilia* × *Pundamilia nyererei* hybrid (Ppun2, Ppun4, Ppun12) (Taylor *et al.* 2002), including the annealing temperature (T_a), the final concentration of $MgCl_2$, and the fluorescent label attached to the forward primer. Additional PCR information is identical to that in Crispo *et al.* (2007)

Locus	GenBank Accession no.	T_a (°C)	[$MgCl_2$] (mM)	Label
Ppun2	AY101471	55.5	1.5	FAM
Ppun4	AY101473	53.0	4.0	VIC
Ppun5	AF491646	55.5	2.5	PET
Ppun12	AY101478	55.5	1.5	FAM
Ppun17	AF491649	50.0	4.0	VIC

1997). Allelic richness was estimated using the rarefaction procedure in HP-RARE version 8-16-2004 (Kalinowski 2005), assuming 30 genes per site. We estimated pairwise F_{ST} , and used permutation analysis to detect significance levels, using ARLEQUIN version 3.01 (Excoffier *et al.* 2005; default settings).

First, we used STRUCTURE version 2.2 (Prichard *et al.* 2000) to estimate the number of genetic clusters in our data set. We used the admixture model, and specified a burn-in period of 10 000, and 10 000 iterations. We performed three analyses: (i) using the entire data set (values of K , the number of genetic clusters, from 1 to 13), (ii) using Mpanga samples only (K from 1 to 7), and (iii) using Nabugabo samples only (K from 1 to 5). Five independent runs of each were performed, and we used the correction of Evanno *et al.* (2005) to determine the most probable value of K . This correction detects the true value of K , which corresponds to the value of K for which the change in the posterior probability between successive values of K is greatest (i.e. ΔK), averaging across runs. Second, we used HIERFSTAT to partition genetic variation (as above, but with only three regions because we did not obtain microsatellite data for Kabaleka). Third, we used FSTAT version 2.9.3.2 (Goudet 2001) to conduct Mantel tests (Mantel 1967; 20 000 permutations) to compare F_{ST} (i) within and among regions, (ii) within and between environments within the Nabugabo region, and (iii) within and between environments within the Mpanga region while controlling for geographical distance among sites. For this analysis, F_{ST} was estimated among all pairwise site combinations using GENEPOP, which implements the methodology of Weir & Cockerham (1984). For (i), we used binary coding where a '0' represented sites in the same region and a '1' represented sites in different regions. A similar procedure was used for (ii) and (iii) but with respect to environment (hypoxic vs. normoxic). For (iii), we used a partial Mantel test (Smouse *et al.* 1986),

because two predictor matrices were included (i.e. environment and geographical distance). Geographical distances were included for the Mpanga region only, because dispersal distances within the Nabugabo region were not straightforward to estimate (because of the nonlinearity of the Nabugabo system, and the presence of predatory Nile perch that may restrict dispersal). Thus, isolation by distance was not tested for in the Nabugabo region. Geographical distances were estimated as the distance along the waterway, using ARCGIS version 9.1 (Environmental Systems Research Institute, Inc.).

Results

Mitochondrial DNA

Nine haplotypes were present in our data set (Fig. 4). The overall haplotype diversity (h) was 0.7122, and the overall nucleotide diversity (π) was 0.0027. Within-site h ranged from 0 to 0.7582, and π ranged from 0 to 0.0034 (Table 1). It is important to note that our sample sizes differed among the sites (Table 1), which could affect within-site variability estimates (number of haplotypes and h). Indeed, regression analysis revealed that the number of haplotypes ($r = 0.630$, $P = 0.028$) and h ($r = 0.646$, $P = 0.023$) were dependent on the sample size. However, this effect was due to two outlier sites with very low sample size, Kabaleka and Saka (Table 1). When these two sites were removed from the regression analysis, both the number of haplotypes ($r = 0.288$, $P = 0.421$) and h ($r = 0.109$, $P = 0.765$) were no longer dependent on sample size. The Nabugabo region was the most diverse (average $h = 0.6239 \pm 0.0879$; average $\pi = 0.0022 \pm 0.0004$; Fig. 5a, b). Diversity within the Mpanga region tended to be lower than within the Nabugabo region (average $h = 0.4882 \pm 0.2025$; average $\pi = 0.0015 \pm 0.0011$; Fig. 5a, b). Within regions, there appeared to be no obvious difference in diversity between environments (hypoxic or normoxic; Fig. 5a, b). Larger standard errors for h and π for normoxic sites within the Mpanga region were driven primarily by the high level of diversity within the Kamwenge sample in relation to other Mpanga river sites (Table 1; Figs 4 and 5a, b).

The maximum difference between any two haplotypes was five mutational steps. The most common haplotype was A, which occurred in 104 of 219 individuals (or 47.5%; Fig. 4). All sites, with the exception of Saka, contain this haplotype. This suggests that all fish sampled may have originated from one source population, in which this haplotype was common. Haplotypes C, D, and F–I are all present in the Nabugabo region, whereas haplotype B is only found in the Mpanga region. Kamwenge, the site lowest downstream in the Mpanga River, contains not only haplotypes A and B, but also D and F, which are most characteristic of Nabugabo sites. This suggests that fish

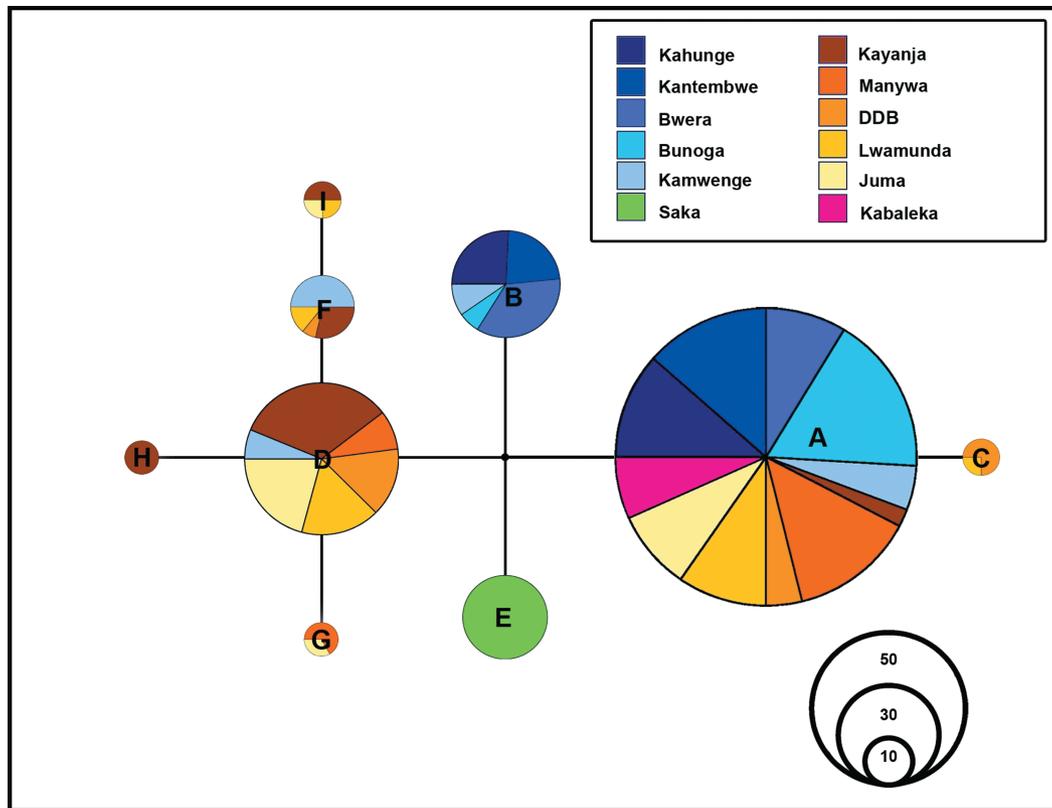


Fig. 4 Haplotype network, created using tcs.

Table 3 HIERSTAT results for mtDNA and microsatellites, including the significance level of F -statistics (10 000 permutations)

	mtDNA		Microsatellite	
	F -statistic	P value	F -statistic	P value
$F_{\text{region/total}}$	0.2955	< 0.0001	0.0617	< 0.0001
$F_{\text{environment/region}}$	0.0412	0.2794	-0.0009	0.9183
$F_{\text{site/region}}$	0.1572	< 0.0001	0.0058	< 0.0001

may have dispersed westward from the Nabugabo region and/or Lake Victoria to the lower reaches of the Mpanga River (see below). Only one haplotype is found in Saka, and this haplotype is unique to that lake, which is currently isolated from other sites in this study.

Using HIERFSTAT, key F -statistics are $F_{\text{region/total}}$ and $F_{\text{environment/region}}$ (Table 3); the former describes variation that is attributable to geographical separation, and the latter describes variation that is attributable to environmental variation (hypoxic vs. normoxic), nested within region. Results indicated that geographical separation ($P < 0.0001$) but not environmental divergence ($P = 0.2794$) had a

significant influence on genetic divergence. Significant site structuring also occurred within region ($F_{\text{site/region}}$; $P < 0.0001$).

Microsatellites

Three loci showed deviations from HWE after applying the binomial likelihood correction (Chapman *et al.* 1999) (Ppun2, Ppun4, and Ppun12; $P < 0.001$). This deviation was due to heterozygote deficiencies in Ppun2 and Ppun12, and as noted above, these loci were excluded from further analyses due to the potential for null alleles. Ppun4 had a heterozygote excess, and thus we kept this locus, as null alleles were not likely. Only one pair of loci (Pmv4 and Pmv17) showed evidence for linkage disequilibrium across all sites ($\chi^2 = \text{infinity}$, d.f. = 24, $P < 0.001$). However, because these loci were in LE in all sites when sites were considered separately (all $P > 0.617$), we infer that linkage disequilibrium is not strong and included both loci in the analyses. Genetic diversity (H_E and allelic richness; Table 1; Fig. 5c, d) was highest in the Nabugabo region (H_E : 0.8923–0.9094; allelic richness, 136.6–152.6) and lowest in Lake Saka (H_E : 0.6997; allelic richness, 57.7), although there was no obvious difference in diversity between environments within a region (Fig. 5).

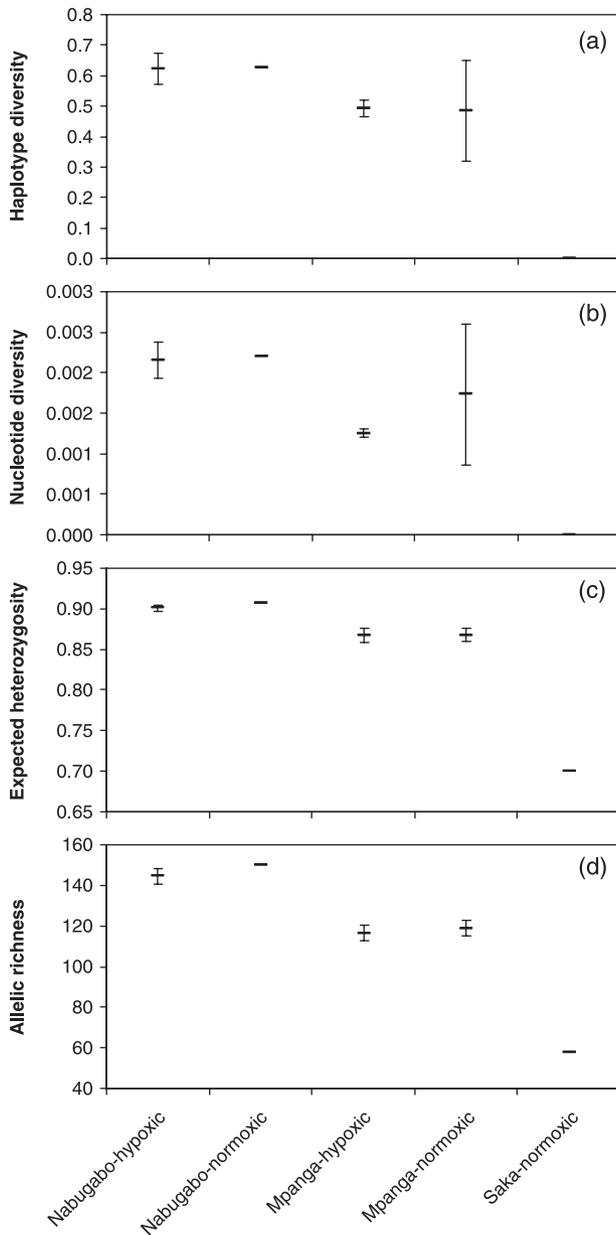


Fig. 5 Estimates of genetic diversity for mtDNA (a and b) and microsatellites (c and d) for sites within hypoxic areas of the Nabugabo region ($n = 4$), the site (Kayanja) within a normoxic area of the Nabugabo region ($n = 1$), sites within hypoxic areas of the Mpanga region ($n = 2$), sites within normoxic areas of the Mpanga region ($n = 5$), and Lake Saka ($n = 1$). Means \pm standard errors are presented.

F_{ST} values between Saka and other sites were considerably greater than F_{ST} values within a region or between Nabugabo and Mpanga sites (Table 4). Within the Nabugabo region, only one F_{ST} value was significant (between Dead Duck Bay and Manywa) although this value was low ($F_{ST} = 0.0097$; Table 4). Within the Mpanga region, a considerably larger

number of sites were significantly differentiated from one another (11/21 pairwise F_{ST} values were significant; Table 4). Significant F_{ST} values included those between the Kamwenge site and all other sites, two additional hypoxic-normoxic site comparisons, and three additional normoxic-normoxic site comparisons (Table 4).

Using STRUCTURE, the highest value of ΔK for the entire data set, estimated over five runs according to Evanno *et al.* (2005), was located at $K = 3$, with a value of 77.3. The second highest value of ΔK was located at $K = 2$, with a value of 60.8, and the third highest value of ΔK was located at $K = 4$, with a value of 2.1. We thus conclude that $K = 3$ is the most probable number of genetic clusters. Genotypes within the Mpanga region tended to form one genetic cluster, genotypes within the Nabugabo region tended to form one genetic cluster, and genotypes within Lake Saka tended to form one genetic cluster. One notable exception is that most of the genotypes within Kamwenge (Fig. 6) tended to group primarily with genotypes from the Nabugabo region, rather than with the other genotypes from within the Mpanga region. This may be due to historical or ongoing gene flow from the Nabugabo region upstream through the Katonga River (see below). We did not use the correction of Evanno *et al.* (2005) when evaluating the Mpanga and Nabugabo samples separately, because this correction method does not allow for the estimation of ΔK for $K = 1$. In the Mpanga region, the most probable number of clusters was one (\ln posterior probability ranged from -9770.6 to -9775.4 over five runs); the next highest \ln posterior probability was -9808.8 (for $K = 2$). Within the Nabugabo region, the most probable number of clusters was also one (\ln posterior probability ranged from -6164.5 to -6171.7 over five runs); only one other \ln posterior probability value fell within this range (-6166.8 for $K = 2$) and all others fell below it. Thus, based on these analyses, samples within the Mpanga region tend to fall into one genetic cluster and samples within the Nabugabo region tend to fall into one genetic cluster.

HIERFSTAT results from microsatellites were similar to those from mtDNA (Table 3). A significant proportion of the total variability was attributable to region ($P < 0.0001$), yet no significant proportion of the variability within regions could be attributed to environment ($P = 0.9183$) (Table 3). Site structuring was significant within region ($P < 0.0001$) (Table 3). Similarly, Mantel tests detected an effect of region (same or different) on F_{ST} ($r^2 = 0.117$, $P = 0.002$). Considering only sites within the Nabugabo region in the Mantel test, environment (same or different) had no effect on F_{ST} ($r^2 = 0.218$, $P = 0.162$). Considering only sites within the Mpanga region in the partial Mantel test, environment (same or different) also had no effect on F_{ST} ($P = 0.628$), but the effect of geographical distance was highly significant (Fig. 7; $r^2 = 0.810$, $P < 0.001$). This was also the case when the most geographically distant

Table 4 F_{ST} values from ARLEQUIN. Bold-face values are significant at the $\alpha = 0.05$ level. Site abbreviations are as in Table 1. Comparisons within the Mpanga region, within the Nabugabo region, and between these two regions, have been enclosed in three respective boxes, for ease of visual comparison

	Bun	Bwe	Kah	Kant	Kia	Kam	Rwe	Saka	DDB	Juma	Kay	Lwa
Bwe	0.0104											
Kah	0.0086	-0.0038										
Kant	0.0037	-0.0012	0.0040									
Kia	0.0061	-0.0056	-0.0015	-0.0018								
Kam	0.0327	0.0122	0.0224	0.0212	0.0188							
Rwe	0.0080	-0.0054	-0.0004	0.0033	-0.0002	0.0139						
Saka	0.1350	0.1401	0.1401	0.1342	0.1348	0.1441	0.1403					
DDB	0.0471	0.0324	0.0373	0.0415	0.0409	0.0255	0.0327	0.1382				
Juma	0.0498	0.0390	0.0421	0.0492	0.0473	0.0263	0.0345	0.1353	-0.0003			
Kay	0.0300	0.0057	0.0140	0.0154	0.0151	-0.0055	0.0033	0.1370	-0.0177	-0.0184		
Lwa	0.0465	0.0333	0.0379	0.0415	0.0382	0.0203	0.0279	0.1386	-0.0007	-0.0022	-0.0094	
Man	0.0560	0.0446	0.0492	0.0543	0.0518	0.0317	0.0455	0.1342	0.0097	0.0017	-0.0180	-0.0015

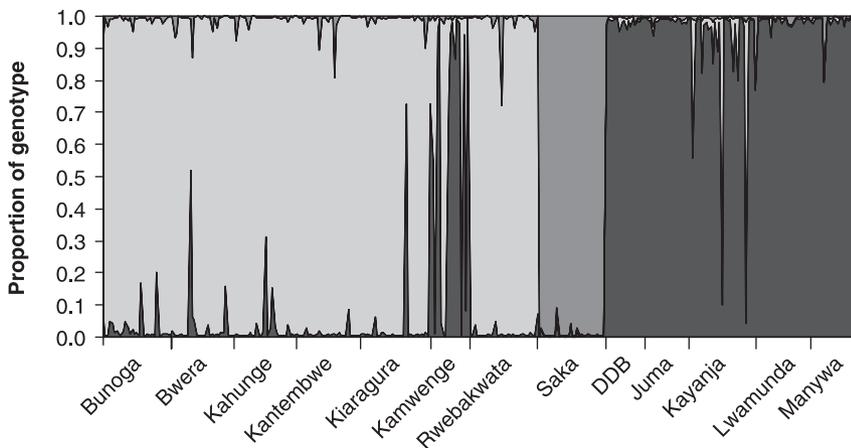


Fig. 6 Apportionment of genotypes within populations into three genetic clusters, as identified by STRUCTURE. Results are from the run with the highest posterior probability of the data out of the five runs.

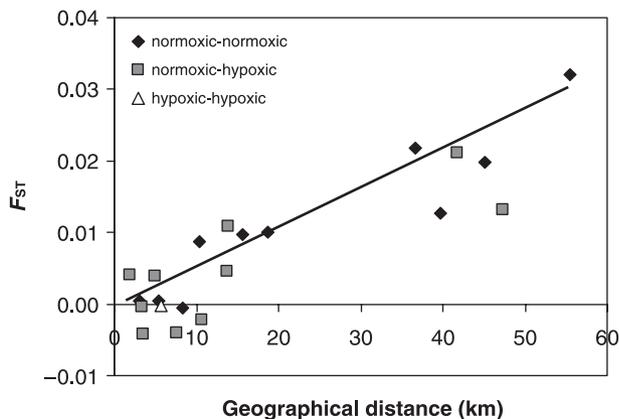


Fig. 7 F_{ST} vs. geographical distance along the waterway in the Mpanga region.

site (Kamwenge) was excluded ($r^2 = 0.464$, $P_{\text{environment}} = 0.681$, $P_{\text{geographical distance}} = 0.014$).

Discussion

Effects of geography

Our results indicate that geography affects genetic structure in *Pseudocrenilabrus multicolor victoriae*. We draw this conclusion based on (i) higher genetic variability within the Nabugabo region than within the Mpanga region and Lake Saka (Figs 4 and 5), (ii) genetic clustering according to region (Fig. 6), (iii) F -statistics that were higher among regions than within regions (Table 3), and (iv) the presence of isolation by distance (Fig. 7). This conclusion is congruent with studies of other systems in suggesting that allopatry (e.g. Kidd & Friesen 1998; Parmakelis *et al.* 2005; Whitaker 2006) and geographical distance (e.g. Irwin *et al.* 2005; Pope *et al.* 2006; Mock *et al.* 2007) play large roles in population structure and speciation in natural systems. Our finding of

isolation by distance within the Mpanga region suggests that sufficient time has occurred for the establishment of equilibrium conditions (Hutchison & Templeton 1999), at least within this region for microsatellites. The presence of equilibrium conditions suggests that the present patterns of observed genetic structuring are due to current gene flow, rather than historical patterns of colonization.

Evidence suggests that Lake Victoria was dry until approximately 15 000 years ago (Johnson *et al.* 1996, 2000; Talbot & Lærdal 2000), during which time fish survived in surrounding lakes/ rivers and/ or in small pockets that remained within the lake basin (Kaufman *et al.* 1997). It is possible that the sampled sites were colonized by Lake Victorian fish (or fish from surrounding satellite lakes/ rivers), a conclusion that is based on the higher genetic variability found within the Nabugabo region sites, which are geographically close to Lake Victoria, than found in the more westerly samples (Figs 1, 4, and 5). Lake Nabugabo was recently connected to Lake Victoria (5000 years ago; Stager *et al.* 2005), which is large and probably contains a larger effective population of *P. multicolor victoriae* than sites sampled in the present study. As well, periodical connections between the lakes Nabugabo and Victoria may be present (L. Kaufman, personal communication), which may account for high genetic diversity within the Nabugabo sites.

Kaufman *et al.* (1997) suggest that during the time of maximum flooding of the Victoria basin (8000 years ago), connections may have occurred between Lake Victoria and more westerly lakes and rivers, during which time these western regions were colonized. The Mpanga River may currently be connected to Lake Victoria via the Katonga River, but the geographical distance between these regions is large (> 150 km) and fish would have to swim upstream during the journey. Nevertheless, colonization of the Mpanga region may have occurred via this route, evidenced by the presence of both haplotypes and microsatellite genotypes in the lower reaches of the Mpanga River that are characteristic of fish from the Nabugabo region (Figs 4 and 6; lower genetic diversity in the Mpanga than in the Nabugabo region suggests that the reverse route is not likely). Furthermore, the presence of a haplotype that is common to all sampled sites (with the exception of Lake Saka) is suggestive of a common ancestral population (Fig. 4).

The low genetic variability in Lake Saka is suggestive of a small number of founding individuals, and/ or recent genetic bottlenecks due to the introduction of predatory Nile perch, followed by population resurgence after intense fishing of Nile perch (L. Chapman, personal observation). This result would be intuitive if colonization occurred in a step-wise fashion by individuals from Lake Victoria; Lake Saka is the most geographically distant site from Lake Victoria, and genetic diversity is expected to decrease with distance from the source, due to cumulative founder effects (Le Corre & Kremer 1998). The large genetic divergence

between Saka and other sites in this study may suggest that Lake Saka has been genetically isolated from Lake Victoria for a longer period of time. An alternate explanation is that Lake Saka has been colonized via a different source population of *P. multicolor*, and that the Lake Saka form evolved independently from those in Lake Victoria, the Nabugabo region, and the Mpanga region. Because only seven samples could be obtained for Lake Kabaleka, we refrain from making predictions based on diversity for this site — low apparent diversity in Lake Kabaleka could be due to small sample size.

One notable result was that F_{ST} values tended to be lower within the Nabugabo region than within the Mpanga region, and a larger proportion of F_{ST} values were non-significant in the former (Table 4). This result contradicts what we would expect because sites within the Nabugabo region have less contemporary connectivity, and thus less predicted gene flow, than those within the Mpanga region (see above). It is probable that sites in the Nabugabo region were colonized more recently than more westerly sites, by Lake Victorian fish during the split of Lake Nabugabo and other satellite lakes from Lake Victoria (see above). That is, fish may have colonized the westerly regions before the formation of Lake Nabugabo. Thus, insufficient time may have occurred for the establishment of genetic equilibrium in the Nabugabo region, resulting in lower F_{ST} values among sites (e.g. see Austerlitz *et al.* 1997; Kinnison *et al.* 2002).

Effects of natural selection

We found no evidence that divergent selection is influencing genetic structure, based on the neutral genetic markers used in this study. We draw this conclusion based on (i) a lack of genetic clustering within environments (Fig. 6), and (ii) genetic variability not greater among environments than within them (Table 3). Yet, previous studies have indicated that genetic components of trait divergence exist between normoxic and hypoxic environments and that this trait divergence should have an adaptive basis (Chapman *et al.* 2000a; L. Chapman, F. Galis, & J. Albert, unpublished data). We here found a lack of genetic structuring among selective environments, indicating that evolutionary divergence in ecologically important traits can occur in the face of high gene flow, which is supported by other studies in natural systems (e.g. Smith *et al.* 2001; Nice *et al.* 2002; Emelianov *et al.* 2004; Smith *et al.* 2005). For example, populations of Trinidadian guppies (*Poecilia reticulata*) experiencing divergent predation regimes differ in body colour (Endler 1978, 1980), body shape and size (Hendry *et al.* 2006), life-history traits (Reznick & Endler 1982; Reznick *et al.* 1996), predator avoidance behaviour (Seghers 1974; Magurran *et al.* 1992), and mate preference (Endler & Houde 1995; Schwartz & Hendry 2007); however, gene flow was found to be *higher* between predation regimes

than within them (Crispo *et al.* 2006). As noted above, equilibrium conditions are likely present in our system, at least within one region, indicating that sufficient time since colonization has occurred for detection of ecological isolation, had it been occurring in this system. This suggests that the 'porous genome' phenomenon may be applicable to our study system, whereby linkage that occurs between neutral and selected genes in dispersers and their F_1 hybrids breaks down in the F_2 generation (Barton & Bengtsson 1986; Wu 2001). In this situation, genes that are disadvantageous in the new environment are selected against, yet migrant neutral alleles may be incorporated into the gene pool. Although theory predicts that divergent selection may act as a generalized barrier to gene flow if selection occurs against dispersers and their F_1 progeny (Gavrilets 2004, pp. 147–148; Grahame *et al.* 2006), substantial neutral gene flow will occur if selection against dispersers and F_1 s is not sufficiently strong to prevent it.

In the *P. multicolor victoriae* system from Uganda, why is divergent selection between oxygen regimes not sufficiently strong to act as a generalized barrier to gene flow? That is, why is selection against dispersers and F_1 hybrids not strong enough to produce signatures of reduced neutral gene flow between oxygen regimes? One explanation may be the high level of plasticity observed in this species with respect to traits that are advantageous in alternate forms under alternate oxygen regimes (Chapman *et al.* 2000a; L. Chapman, F. Galis, & J. Albert, unpublished data). Wright (1931) proposed that plasticity could dampen the effects of natural selection by allowing individuals to adapt to an array of environmental conditions. Plasticity may allow individuals to 'jump' from one fitness peak to another on an adaptive landscape, without traversing through low-fitness valleys (Price *et al.* 2003; Ghalambor *et al.* 2007). This effect could result in increased gene flow by permitting survival of dispersers between selective environments, even though the environmentally induced phenotype is suboptimal for maximized fitness in the alternate environment (see Fig. 8). Genetic components of trait divergence in *P. multicolor* could occur because of a discrepancy between the maximum phenotypic change that can be induced by the environment and the optimal difference in the phenotype between environments. Although it is not known whether adaptively plastic traits in *P. multicolor* are labile (i.e. reversible and can occur throughout life) or developmental (i.e. phenotypic trajectories are dictated during development and are irreversible), in either case we would expect that increased gene flow may be facilitated. The effects of labile plasticity are obvious: dispersers would be capable of altering their phenotype to increase adaptation to the new environment. The adaptive effects of developmental plasticity would be evident in the progeny of dispersers, assuming that divergent selection is not strong enough to substantially reduce fitness of

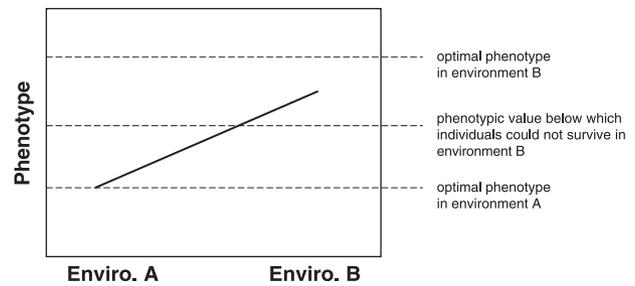


Fig. 8 Depiction of why plasticity could allow for increased gene flow between selective environments. Bold line represents the reaction norm (i.e. relationship between phenotype and environment for a given genotype) for hypothetical population A. Individuals are able to survive in environment B because of plasticity, but further evolutionary change would be required for attainment of maximal fitness in environment B.

dispersing individuals. Another possible explanation for gene flow is that it may be beneficial to (i) reduce kin competition, or, in the case of dispersal from normoxic to hypoxic environments, competition with other cichlid species that do not penetrate hypoxic habitats; (ii) increase fitness by moving to a less harsh environment, in the case of dispersal from hypoxic areas to normoxic areas; (iii) reduce inbreeding depression; and (iv) promote the spread of universally adaptive mutations that may arise in one population (reviewed in Garant *et al.* 2007). If gene flow occurs due to these benefits, then plasticity may be selected for (Sultan & Spencer 2002), and be the consequence, rather than the cause, of gene flow.

Summary

Our results indicate that geographical structure has played a role in gene flow and genetic structuring in our study system. We base this conclusion on greater divergence between geographically separated regions than within region and significant isolation by distance. Lake Victoria may be the source of the sampled populations, because greater genetic variability was found in the Nabugabo region (near Lake Victoria) relative to the other regions, and genetic diversity tends to decrease with increasing distance from Lake Victoria. Yet, lower F_{ST} values within the Nabugabo region than within the Mpanga region, despite putatively lower physical dispersal in the former, suggests that the former region was the most recently colonized. Low F_{ST} values between most population pairs in the Mpanga region probably reflect a leaky system with high gene flow, rather than a recent expansion, because Mpanga populations are in genetic equilibrium (evidenced by isolation by distance). Ecological divergence, with respect to oxygen regimes, does not seem to be influencing genetic structure and neutral gene flow.

In a system where plasticity is high with respect to traits that are adaptive in alternate forms under alternate environmental conditions, dispersal and gene flow may be facilitated among selective environments. If plasticity aids adaptation, the ultimate effects of divergent selection may be weakened. This would lead to enhanced survival of dispersers and their F_1 progeny, diminishing the effects of divergent selection as a generalized barrier to gene flow (see above; Gavrillets 2004, pp. 147–148; Grahame *et al.* 2006). Previous studies in *Pseudocrenilabrus multicolor victoriae* have shown that plasticity in gill size is high with respect to oxygen regimes, with larger gills manifest in hypoxic waters (Chapman *et al.* 2000a; L. Chapman, F. Galis, & J. Albert, unpublished data). Presumably, this increase in size would be adaptive for increased oxygen uptake in oxygen-scarce environments, and thus plasticity may be implicated in high gene flow between selective environments in this species. An alternate explanation is that high gene flow selects for increased plasticity (Sultan & Spencer 2002), and thus high gene flow is the cause of plasticity rather than vice versa. Future studies in this system will focus on relationships between plasticity, gene flow, and the environment to disentangle their potential relationships.

This work has clear implications for fish conservation. Although hypoxia occurs naturally in systems characterized by low light and low mixing (e.g. heavily vegetated wetlands), the frequency of hypoxia is increasing globally through the influx of municipal waste and fertilizers that accelerate eutrophication (Chesney *et al.* 2000; Pollock *et al.* 2007). It has thus become increasingly important to understand the consequences of low-oxygen stress on fish populations. Our study suggests that lowered oxygen may not pose a great threat to some fishes, because the fitness of fish dispersing between oxygen regimes did not appear to be reduced enough to produce signatures of ecological isolation (i.e. genetic divergence was not reduced between environments). However, fish kills associated with upwellings of hypoxic waters in Lake Victoria (Balirwa *et al.* 2003) suggest that hypoxic stress imposes a high risk for some species. Future work on conservation efforts related to hypoxia will need to focus on direct fitness consequences of fish dispersing between selective environments, as well as differences among fish species, both with respect to plasticity and with respect to evolvability, in order to make inferences about how fishes will respond to changing conditions.

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References

- Abila R, Barluenga M, Engelken J, Meyer A, Salzburger W (2004) Population-structure and genetic diversity in a haplochromine fish cichlid of a satellite lake of Lake Victoria. *Molecular Ecology*, **13**, 2589–2602.
- Austerlitz F, Jung-Muller B, Godelle B, Gouyon P-H (1997) Evolution of coalescence time, genetic diversity and structure during colonization. *Theoretical Population Biology*, **51**, 148–164.
- Balirwa JS, Chapman CA, Chapman LJ *et al.* (2003) Biodiversity and fishery sustainability in the Lake Victoria basin: an unexpected marriage? *Bioscience*, **53**, 703–715.
- Barton N, Bengtsson BO (1986) The barrier to genetic exchange between hybridising populations. *Heredity*, **57**, 357–376.
- Bethenod M-T, Thomas Y, Rousset F *et al.* (2005) Genetic isolation between two sympatric host plant races of the European corn borer, *Ostrinia nubilalis* Hübner. II: assortative mating and host-plant preferences for oviposition. *Heredity*, **94**, 264–270.
- Bhagat Y, Fox MG, Ferreira MT (2006) Morphological differentiation in introduced pumpkinseed *Lepomis gibbosus* (L.) occupying different habitat zones in Portuguese reservoirs. *Journal of Fish Biology*, **69** (Suppl. C), 79–94.
- Blank J, Stauss MJ, Tomiuk J, Fietz J, Segelbacher G (2007) Habitat type does not affect population genetic structure in sympatric great tits (*Parus major*) and blue tits (*P. caeruleus*). *Journal of Negative Results*, **4**, 1–14.
- Boughman JW, Rundle HD, Schluter D (2005) Parallel evolution of sexual isolation in sticklebacks. *Evolution*, **59**, 361–373.
- Chapman LJ, Chapman CA, Chandler M (1996a) Wetland ecotones as refugia for endangered fishes. *Biological Conservation*, **78**, 263–270.
- Chapman LJ, Chapman CA, Ogutu-Ohwayo R, Chandler M, Kaufman L, Keiter AE (1996b) Refugia for endangered fishes from an introduced predator in Lake Nabugabo, Uganda. *Conservation Biology*, **10**, 554–561.
- Chapman RW, Sedberry GR, Koenig CC, Eleby BM (1999) Stock identification of gag, *Mycteroperca microlepis*, along the southeast coast of the United States. *Marine Biotechnology*, **1**, 137–146.
- Chapman LJ, Galis F, Shinn J (2000a) Phenotypic plasticity and the possible role of genetic assimilation: hypoxia-induced trade-offs in the morphological traits of an African cichlid. *Ecology Letters*, **3**, 387–393.
- Chapman LJ, Chapman CA, Crisman TL, Prenger J (2000b) Predictors of seasonal oxygen levels in a Ugandan swamp/river systems: a 3-year profile. *Verhandlungen Internationale Vereinigung Limnologie*, **27**, 3048–3053.
- Chapman LJ, Chapman CA, Nordlie FG, Rosenberger AE (2002a) Physiological refugia: swamps, hypoxia tolerance and maintenance of fish diversity in the Lake Victoria region. *Comparative Biochemistry and Physiology, Part A*, **133**, 421–437.
- Chapman LJ, Nordlie FG, Seifert A (2002b) Respiratory oxygen consumption among groups of *Pseudocrenilabrus multicolor victoriae* subjected to different oxygen concentrations during development. *Journal of Fish Biology*, **61**, 242–251.

- Chapman LJ, Schneider KR, Apodaca C, Chapman CA (2004) Respiratory ecology of macroinvertebrates in a swamp-river system of East Africa. *Biotropica*, **36**, 572–585.
- Chenna R, Sugawara H, Koike T *et al.* (2003) Multiple sequence alignment with the CLUSTAL series of programs. *Nucleic Acids Research*, **31**, 3497–3500.
- Chesney EJ, Baltz DM, Thomas RG (2000) Louisiana estuarine and coastal fisheries and habitats: perspectives from a fish's eye view. *Ecological Applications*, **10**, 350–366.
- Clement M, Posada D, Crandall KA (2000) rcs: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1660.
- Crispo E, Bentzen P, Reznick DN, Kinnison MT, Hendry AP (2006) The relative influence of natural selection and geography on gene flow in guppies. *Molecular Ecology*, **15**, 49–62.
- 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 victoriarum*) from Uganda. *Molecular Ecology Notes*, **7**, 1293–1295.
- Dhuyvetter H, Hendrickx F, Gaublomme E, Desender K (2007) Differentiation between two salt marsh beetle ecotypes: evidence for ongoing speciation. *Evolution*, **61**, 184–193.
- Diaz JR, Rosenberg R (1995) Marine benthic hypoxia: a review its ecological effects and the behavioural responses of benthic macrofauna. *Oceanography and Marine Biology Annual Review*, **33**, 245–303.
- Duftner N, Sefc KM, Kublmüller S *et al.* (2006) Distinct population structure in a phenotypically homogeneous rock-dwelling cichlid fish from Lake Tanganyika. *Molecular Ecology*, **15**, 2381–2395.
- Emelianov I, Marec F, Mallet J (2004) Genomic evidence for divergence with gene flow in host races of the larch budmoth. *Proceedings of the Royal Society B: Biological Sciences*, **271**, 97–105.
- Endler JA (1978) A predator's view of animal color patterns. *Evolutionary Biology*, **11**, 319–364.
- Endler JA (1980) Natural selection on color patterns in *Poecilia reticulata*. *Evolution*, **34**, 76–91.
- Endler JA, Houde AE (1995) Geographic variation in female preference for male traits in *Poecilia reticulata*. *Evolution*, **49**, 456–468.
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611–2620.
- Excoffier L, Laval G, Schneider S (2005) ARLEQUIN ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47–50.
- Fritz U, Barata M, Busack SD, Fritsch G, Castilho R (2006) Impact of mountain chains, sea straits and peripheral populations on genetic and taxonomic structure of a freshwater turtle, *Mauremys leprosa* (Reptilia, Testudines, Geoemydidae). *Zoologica Scripta*, **35**, 97–108.
- Garant D, Forde SE, Hendry AP (2007) The multifarious effects of dispersal and gene flow on contemporary adaptation. *Functional Ecology*, **21**, 434–443.
- Gavrilets S (2004) *Fitness Landscapes and the Origin of Species*. Princeton University Press, Princeton, New Jersey.
- Ghalambor CK, McKay JK, Carroll SP, Reznick DN (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, **21**, 394–407.
- 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/software/fstat.html>.
- Goudet J (2005) HIERFSTAT, a package for R to compute and test hierarchical F-statistics. *Molecular Ecology Notes*, **5**, 184–186.
- Grahame JW, Wilding CS, Butlin RK (2006) Adaptation to a steep environmental gradient and an associated barrier to gene exchange in *Littorina saxatilis*. *Evolution*, **60**, 268–278.
- Greenwood PH (1965) The cichlid fishes of Lake Nabugabo, Uganda. *Bulletin of the British Museum of Natural History (Zoology)*, **12**, 315–357.
- Hendry AP (2004) Selection against migrants contributes to the rapid-evolution of reproductive isolation. *Evolutionary Ecology Research*, **6**, 1219–1236.
- Hendry AP, Kelly ML, Kinnison MT, Reznick DN (2006) Parallel evolution of the sexes? Effects of predation and habitat features on the size and shape of wild guppies. *Journal of Evolutionary Biology*, **19**, 741–754.
- Hutchinson DW, Templeton AR (1999) Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution*, **53**, 1898–1914.
- Irwin DE, Staffan B, Irwin JH, Price TD (2005) Speciation by distance in a ring species. *Science*, **307**, 414–416.
- Johnson TC, Scholz CA, Talbot MR *et al.* (1996) Late Pleistocene desiccation of Lake Victoria and rapid evolution of cichlid fishes. *Science*, **273**, 1091–1093.
- Johnson TC, Kelts K, Odada E (2000) The Holocene history of Lake Victoria. *Ambio*, **29**, 2–11.
- Jukes TH, Cantor CR (1969) Evolution of protein molecules. In: *Mammalian Protein Metabolism* (ed. Munro HN), pp. 21–132. Academic Press, New York.
- Kalinowski ST (2005) HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. *Molecular Ecology Notes*, **5**, 187–189.
- Katongo C, Koblmüller S, Duftner N, Makasa L, Sturmbauer C (2005) Phylogeography and speciation in the *Pseudocrenilabrus philander* species complex in Zambian rivers. *Hydrobiologia*, **542**, 221–233.
- Kaufman LS, Chapman LJ, Chapman CA (1997) Evolution in fast forward: haplochromine fishes of the Lake Victoria region. *Endeavour*, **21**, 23–30.
- Kidd MG, Friesen VL (1998) Analysis of mechanisms of micro-evolutionary change in *Cepphus* guillemots using patterns of control region variation. *Evolution*, **52**, 1158–1168.
- Kinnison MT, Bentzen P, Unwin MJ, Quinn TP (2002) Reconstructing recent divergence: evaluating nonequilibrium population structure in New Zealand chinook salmon. *Molecular Ecology*, **11**, 739–754.
- Langerhans RB, DeWitt TJ (2004) Shared and unique features of evolutionary diversification. *American Naturalist*, **164**, 335–349.
- Langerhans RB, Layman CA, Langerhans AK, DeWitt TJ (2003) Habitat-associated morphological divergence in two Neotropical fish species. *Biological Journal of the Linnean Society*, **80**, 689–698.
- Le Corre V, Kremer A (1998) Cumulative effects of founding events during colonization on genetic diversity and differentiation in an island and stepping-stone model. *Journal of Evolutionary Biology*, **11**, 495–512.
- Lee W-J, Conroy J, Howell WH, Kocher TD (1995) Structure and evolution of teleost mitochondrial control regions. *Journal of Molecular Evolution*, **41**, 54–66.

- Lu G, Bernatchez L (1999) Correlated trophic specialization and genetic divergence in sympatric lake whitefish ecotypes (*Coregonus clupeaformis*): support for the ecological speciation hypothesis. *Evolution*, **53**, 1491–1505.
- Magurran AE, Seghers BH, Carvalho GR, Shaw PW (1992) Behavioural consequences of an artificial introduction of guppies (*Poecilia reticulata*) in N. Trinidad: evidence for the evolution of anti-predator behaviour in the wild. *Proceedings of the Royal Society B: Biological Sciences*, **248**, 117–122.
- Mantel NA (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.
- Melnchuk MC, Chapman LJ (2002) Hypoxia tolerance of two haplochromine cichlids: swamp leakage and potential for interlacustrine dispersal. *Environmental Biology of Fishes*, **65**, 99–110.
- Meyer A, Kocher TD, Basasibwaki P, Wilson AC (1990) Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature*, **347**, 551–553.
- Meyer A, Morrissey JM, Scharti M (1994) Recurrent origin of a sexually selected trait in *Xiphophorus* fishes inferred from a molecular phylogeny. *Nature*, **368**, 539–542.
- 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.
- Mock KE, Bentz BJ, O'Neill EM, Chong JP, Orwin J, Pfrender ME (2007) Landscape-scale genetic variation in a forest outbreak species, the mountain pine beetle (*Dendroctonus ponderosae*). *Molecular Ecology*, **16**, 553–568.
- Nice CC, Fordyce JA, Shapiro AM, Ffrench-Constant R (2002) Lack of evidence for reproductive isolation among ecologically specialized lycaenid butterflies. *Ecological Entomology*, **27**, 702–712.
- Nosil P (2004) Reproductive isolation caused by visual predation on migrants between divergent environments. *Proceedings of the Royal Society B: Biological Sciences*, **271**, 1521–1528.
- Nosil P, Vines TH, Funk DJ (2005) Reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution*, **59**, 705–719.
- Ogden R, Thorpe RS (2002) Molecular evidence for ecological speciation in tropical habitats. *Proceedings of the National Academy of Sciences, USA*, **99**, 13612–13615.
- Ogutu-Ohwayo R (1993) The effects of predation by Nile perch, *Lates niloticus* L., on the fish of Lake Nabugabo, with suggestions for conservation of endangered endemic cichlids. *Conservation Biology*, **7**, 701–711.
- Parmakelis A, Pfenninger M, Spanos L, Papagiannakis G, Louis C, Mylonas M (2005) Inference of a radiation in *Mastus* (Gastropoda, Pulmonata, Enidae) on the island of Crete. *Evolution*, **59**, 991–1005.
- Poissant J, Knight TW, Ferguson MM (2005) Nonequilibrium conditions following landscape rearrangement: the relative contribution of past and current hydrological landscape on the genetic structure of a stream-dwelling fish. *Molecular Ecology*, **14**, 1321–1331.
- Pollock MS, Clarke LMJ, Dubé MG (2007) The effects of hypoxia on fishes: from ecological relevance to physiological effects. *Environmental Reviews*, **15**, 1–14.
- Pope LC, Domingo-Roura X, Erven K, Burke T (2006) Isolation by distance and gene flow in the Eurasian badger (*Meles meles*) at both a local and broad scale. *Molecular Ecology*, **15**, 371–386.
- Price TD, Qvarnström A, Irwin DE (2003) The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society B: Biological Sciences*, **270**, 1433–1440.
- Prichard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.
- R Development Core Team (2006) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. Available from URL: <http://www.R-project.org>.
- Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.
- Reznick D, Endler JA (1982) The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution*, **36**, 160–177.
- Reznick DN, Rodd FH, Cardenas M (1996) Life-history evolution in guppies (*Poecilia reticulata*: Poeciliidae). IV. Parallelism in life-history phenotypes. *American Naturalist*, **147**, 319–338.
- Robinson BW, Wilson DS (1994) Character displacement in fishes: a neglected literature. *American Naturalist*, **144**, 596–627.
- Rocha LA, Robertson DR, Roman J, Bowen BW (2005) Ecological speciation in tropical reef fishes. *Proceedings of the Royal Society B: Biological Sciences*, **272**, 573–579.
- Rosenberger AE, Chapman LJ (1999) Hypoxic wetland tributaries as faunal refugia from an introduced predator. *Ecology of Freshwater Fish*, **8**, 22–34.
- Rosenberger AE, Chapman LJ (2000) Respiratory characters of three species of haplochromine cichlids: implications for use of wetland refugia. *Journal of Fish Biology*, **57**, 483–501.
- Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rosas R (2003) DNASP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, **19**, 2496–2497.
- Rundle HD (2002) A test of ecologically dependent postmating isolation between sympatric sticklebacks. *Evolution*, **56**, 322–329.
- Rundle HD, Nosil P (2005) Ecological speciation. *Ecology Letters*, **8**, 336–352.
- Rundle HD, Chenoweth SF, Doughty P, Blows MW (2005) Divergent selection and the evolution of signal traits and mating preferences. *PLoS Biology*, **3**, 1988–1995.
- Saint-Laurent R, Legault M, Bernatchez L (2003) Divergent selection maintains adaptive differentiation despite high gene flow between sympatric rainbow smelt ecotypes (*Osmerus mordax* Mitchell). *Molecular Ecology*, **12**, 315–330.
- Schierwater B, Mrowka W (1987) Factors influencing oxygen consumption in the cichlid fish *Pseudocrenilabrus multicolor*. *Zoologischer Anzeiger*, **219**, 305–312.
- Schlichting CD, Pigliucci M (1998) *Phenotypic Evolution: A Reaction Norm Perspective*. Sinauer & Associates, Sunderland, Massachusetts.
- Schluter D (2000) *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford, UK.
- Schluter D (2001) Ecology and the origin of species. *Trends in Ecology & Evolution*, **16**, 372–380.
- Schwartz AK, Hendry AP (2007) A test for the parallel co-evolution of male colour and female preference in Trinidadian guppies (*Poecilia reticulata*). *Evolutionary Ecology Research*, **9**, 71–90.
- Seghers BH (1974) Schooling behavior in the guppy (*Poecilia reticulata*): an evolutionary response to predation. *Evolution*, **28**, 486–489.
- Smith TB, Calsbeek R, Wayne RK, Holder KH, Pires D, Bardeleben C (2005) Testing alternative mechanisms of evolutionary divergence in an African rain forest passerine bird. *Journal of Evolutionary Biology*, **18**, 257–268.

- Smith TB, Schneider CJ, Holder K (2001) Refugial isolation versus ecological gradients. *Genetica*, **112–113**, 383–398.
- Smouse PE, Long JC, Sokal RR (1986) Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Systematic Zoology*, **35**, 627–632.
- Stager JC, Westwood J, Grzesik D, Cumming BF (2005) A 5500-year environmental history of Lake Nabugabo, Uganda. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **218**, 347–354.
- Stanton ML, Galen C, Shore J (1997) Population structure along a steep environmental gradient: consequences of flowering time and habitat variation in the snow buttercup, *Ranunculus adoneus*. *Evolution*, **51**, 71–94.
- Sturmbauer C, Baric S, Salzburger W, Rüber L, Verheyen E (2001) Lake level fluctuations synchronize genetic divergences of cichlid fishes in African lakes. *Molecular Biology and Evolution*, **18**, 144–154.
- Sultan SE, Spencer HG (2002) Metapopulation structure favors plasticity over local adaptation. *American Naturalist*, **160**, 271–283.
- Talbot MR, Lærdal T (2000) The Late Pleistocene–Holocene palaeolimnology of Lake Victoria, East Africa, based upon elemental and isotopic analyses of sedimentary organic matter. *Journal of Paleolimnology*, **23**, 141–164.
- Taylor MI, Meardon F, Turner G, Seehausen O, Mrossa HDJ, Rico C (2002) Characterization of tetranucleotide microsatellite loci in a Lake Victorian, haplochromine cichlid fish: a *Pundamilia pundamilia* × *Pundamilia nyererei* hybrid. *Molecular Ecology Notes*, **2**, 443–445.
- Via S, Bouck AC, Skillman S (2000) Reproductive isolation between divergent races of pea aphids on two hosts. II. Selection against migrants and hybrids in the parental environments. *Evolution*, **54**, 1626–1637.
- Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.
- West-Eberhard MJ (2003) *Developmental Plasticity and Evolution*. Oxford University Press, Oxford, UK.
- Whitaker RJ (2006) Allopatric origins of microbial species. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **361**, 1975–1984.
- Wright S (1931) Evolution in Mendelian populations. *Genetics*, **16**, 97–159.
- Wu C-I (2001) The genic view of the process of speciation. *Journal of Evolutionary Biology*, **14**, 851–865.
- Wu RSS (2002) Hypoxia: from molecular responses to ecosystem responses. *Marine Pollution Bulletin*, **45**, 35–45.

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