

**SIMILAR LEVELS OF DIVERSITY AND POPULATION STRUCTURE
IN SUPERFLOCK AND NON-SUPERFLOCK CICHLID FISHES FROM
LAKE VICTORIA, AFRICA**

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ABSTRACT

*The haplochromine cichlids of the African Great Lakes have evolved into a spectacularly ecologically diverse 1 800 species. However, even within the same lake, not all cichlid lineages have radiated equally and many lineages have remained relatively species depauperate. Multiple biological factors particular to superflock species have been proposed, one of which is extreme environmental sensitivity (stenotopy) resulting in restricted dispersal, which ultimately fosters microallopatric speciation. We use DNA microsatellite loci to estimate neutral genetic diversity and the level of gene flow among populations of two cichlid species from southern Lake Victoria, Africa: the endemic and stenotopic superflock species *Pundamilia nyererei* and the non-endemic, widespread species *Astatoreochromis alluaudi*. The two species have similar levels of overall gene diversity (*A. alluaudi* $H = 0.689$; *P. nyererei* $H = 0.737$). Both species show limited spatial-genetic structure among individuals. Our findings suggest that contemporary gene flow at small spatial scales may not be very different between these two cichlid species with different ecologies. We suggest that the level of contemporary gene flow at fine spatial scales may not be related to the historical rate of speciation.*

Key words: cichlid fishes; gene flow; spatial-genetic autocorrelation; population genetics; microsatellites; Lake Victoria

INTRODUCTION

The haplochromine superflock of cichlid fishes in Lake Victoria is a renowned adaptive radiation of 500 endemic species in the youngest of Africa's Great Lakes (Salzburger & Meyer, 2004; Elmer *et al.*, 2009). Across East Africa there are an estimated 1 800 species, which makes the haplochromine cichlid lineage by far the most species-rich lineage of cichlids. However, despite the rapid radiation of the haplochromines, many other lineages of cichlids have remained relatively species depauperate (e.g. the Tilapiini cichlid *Oreochromis*). Even within the haplochromines, not all genera are equally diverse (e.g. compare the species-rich *Haplochromis* with the species-poor genus *Astatoreochromis*). Ecological opportunity is thought to play a major role in the evolutionary success of haplochromine cichlids: only in heterogeneous lake environments have cichlids radiated spectacularly to form the famous species flocks, while in rivers there is no consistent pattern of conspicuous diversification. Non-endemic haplochromine lineages are always less species rich than the endemic lineages (Salzburger & Meyer, 2004), suggesting that dispersal ability may be a strong indicator of speciation potential.

Potential factors that may make some taxonomic lineages prone to radiate have been discussed extensively (e.g. van Alphen *et al.*, 2004; Salzburger *et al.*, 2005; Seehausen, 2006). While a variety of extrinsic environmental effects thought to influence diversification affect all local biota equally (for example lake level fluctuations, (Sturmbauer *et al.*, 2001), other extrinsic factors will affect species differently depending on their biology, such as the availability of suitable habitats, which can influence the potential for dispersal. At the same time, intrinsic biological factors such as trophic differentiation and disruptive sexual selection may also contribute to rapid speciation (Seehausen, 2000).

Fine-scale geographic subdivision and genetic drift have been frequently suggested as drivers of intralacustrine allopatric speciation in African superflock cichlids (e.g. Fryer, 1959; Meyer *et al.*, 1996; van Oppen *et al.*, 1997b; Rico & Turner, 2002). The combination of niche specificity and habitat heterogeneity has been proposed to reduce dispersal ability and result in pronounced philopatry and significant isolation of specialist populations of cichlids that might subsequently diverge by either selection or drift (Sturmbauer & Meyer, 1992; Meyer, 1993; Danley & Kocher, 2001). However, there is conflicting evidence as to whether extreme environmental sensitivity (stenotopy, in contrast to eurytopy for environmental tolerance) limits dispersal, with some molecular studies identifying restricted dispersal at fine spatial scales (e.g. van Oppen *et al.*, 1997b; Danley *et al.*, 2000; Rico & Turner, 2002; Koblmüller *et al.*, 2007) while other ecological (e.g. McKaye & Gray, 1984) and molecular (e.g. Danley *et al.*, 2000) studies suggest that gene flow is not greatly limited by habitat availability. Very few studies have explicitly tested this hypothesis by comparing the relationship between low interpopulation gene flow and high lineage species richness (Arnegard *et al.*, 1999; Shaw *et al.*, 2000; Taylor & Verheyen, 2001; Taylor *et al.*, 2001).

Dispersal can be tracked by the observing individuals (e.g. mark-recapture) but dispersal relevant to evolution (i.e. gene flow) can only be determined using molecular tools (Clobert *et al.*, 2001). Genetic tools based on rapidly evolving molecular markers such as DNA microsatellites are able to resolve genetic structure and dispersal at fine spatial and temporal scales. In this study, we use molecular markers to examine gene flow and diversity among individuals and populations of endemic and non-endemic cichlids. The focal species live in the same geographical area of the rocky shores of southern Lake Victoria, Africa. *Pundamilia nyererei*, part of the ‘modern haplochromines’ lineage, (Salzburger *et al.*, 2005) is an endemic member of the rapidly radiating haplochromines while *Astatoreochromis alluaudi* is a member of a broadly-distributed and species-poor haplochromine lineage that is not part of the Lake Victoria cichlid superflock (Salzburger *et al.*, 2005; Seehausen, 2006). Species in the genus *Pundamilia* have been a model system for the ecology and evolution of Lake Victoria rock-dwelling cichlids (eg. Seehausen & van Alphen, 1998; Verzijden & ten Cate, 2007; Seehausen *et al.*, 2008). Though *P. nyererei* is stenotopic and *A. alluaudi* is eurytopic in ecology, they typically inhabit similar habitats of shallow, interrupted rocky-bottomed shorelines of the mainland and islands (Seehausen, 1996). We predicted that gene flow would be more restricted and limited by geographic distance in the environmentally sensitive *P. nyererei* relative to the more environmentally tolerant *A. alluaudi*. Proximally, differences in genetic diversity and patterns of gene flow between species-poor and species-rich cichlid species could provide an explanation for the observed differences in the species diversity of these lineages. The role of species’ contemporary ecologies and demographies may ultimately play important roles in rates of speciation and propensity for diversification. Why some lineages diversify rapidly and extensively while others remain species depauperate is a question of evolutionary potential relevant for cichlids specifically and vertebrate evolution generally. Intra- and interspecific population genetic analyses and comparisons across ecologically distinct but broadly sympatric taxa contribute to our understanding of differences in evolutionary processes.

MATERIAL AND METHODS

Fish were collected by Ole Seehausen from Lake Victoria in 1995 (Tables 1 and 2, Figure 1). Efforts were made to collect both species from each locality. Complete paired sampling was not possible because the two species are not always found in the same habitat. Straight-line kilometres between localities while avoiding land and reducing turns were georeferenced (Google Corporation, 2007)

Eight dinucleotide microsatellite loci (GT8/Pzeb3, GT9/Pzeb2, GT29A/Pzeb4 van Oppen *et al.*, 1997a; OSU09d, OSU19t, and OSU20d Wu *et al.*, 1999; and TmoM5 and TmoM11 Zardoya *et al.*, 1996) were amplified by PCR using standard protocols. Fragments were sized on an Applied Biosystems PRISM 377 or 3100 relative to 500 ROX Size Standard.

Hardy-Weinberg equilibrium and linkage disequilibrium were tested using Genepop on the Web (Raymond & Rousset, 1995). Genetic diversity was characterized in GenAIEx v6.1 (Peakall & Smouse, 2006). F_{ST} (comparable to θ) (Weir & Cockerham, 1984) and an analysis of molecular variance were both calculated in GenAIEx and significance assessed by permutation testing (999 permutations).

A two-dimensional factorial correspondence analysis was conducted on the multilocus genotypes of each individual using GENETIX v 4.02 (Belkhir *et al.*, 1996). Inter-individual analyses of spatial genetic structure were calculated in GenAIEx. Statistical significance of r was assessed with 999 permutations and bootstrap replicates (null distribution and error bars). This individual-based method is more sensitive than population-based tests (e.g., Mantel's) and is robust against heterogeneous interpopulation distances and sample sizes (Peakall *et al.*, 2003). Singleton populations were excluded from spatial autocorrelation and interpopulation analyses but included in overall diversity calculations.

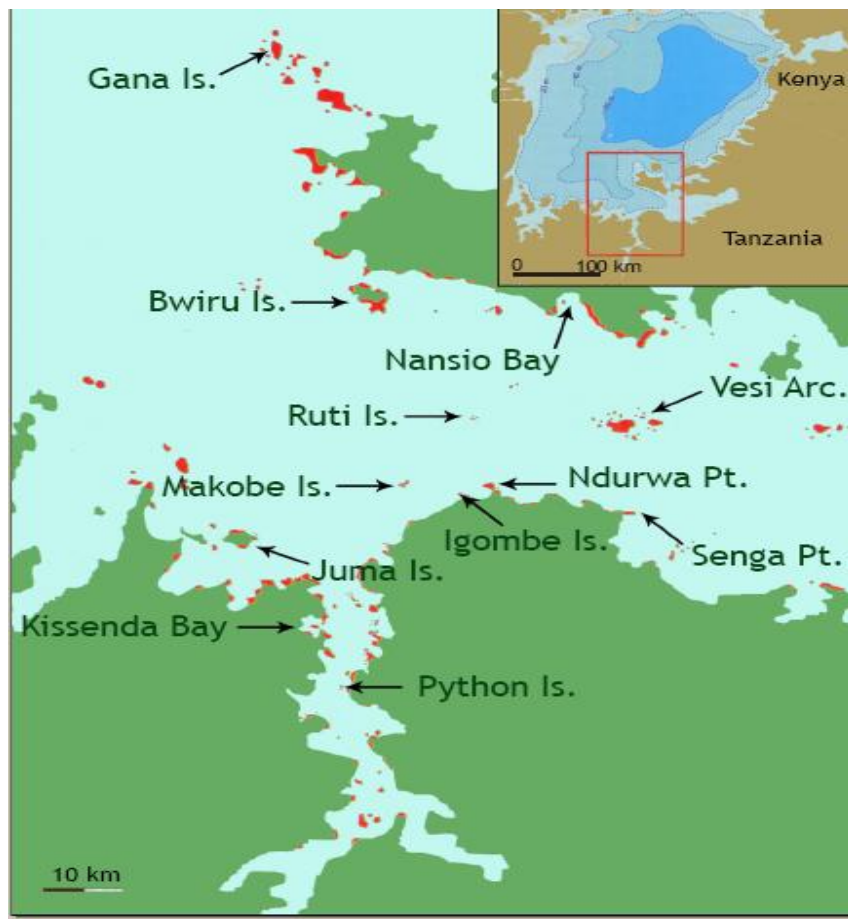


Fig. 1: Sample localities in this study (adapted from Seehausen, 1996). Red shading indicates rocky shore habitat.

RESULTS

Sample localities of both species are essentially biological populations: no localities of *A. alluaudi* deviate significantly from Hardy-Weinberg equilibrium for eight microsatellite loci, and only a single population of *P. nyererei* does (Makobe Island, which has a significant heterozygote deficiency: $H_o=0.657$, $H_E=0.746$: $\chi^2 = 37.04$, $df = 16$, $P = 0.002$) (Tables 1 and 2). No linkage disequilibrium was found, indicating that microsatellite loci segregate independently. Unbiased expected heterozygosity (the most suitable estimator when sample sizes are small and heterogeneous, Pruett & Winker, 2008) does not differ significantly between *A. alluaudi* and *P. nyererei* (Wilcoxon unbiased two-sample test: $Z = -1.670$, $P = 0.867$) (Tables 1 and 2).

An analysis of molecular variance indicates that a similarly low proportion of genetic variation can be explained by between population differences in both species: 4% in *A. alluaudi* and 3% in *P. nyererei*.

The small sample sizes of several populations of *A. alluaudi* restrict the possibility of detailed population-level genetic comparisons. Therefore, we employ inter-individual analyses of spatial genetic structure that are robust against unmatched sample localities and uneven sample sizes (Peakall *et al.*, 2003). This is a more powerful approach to assess gene flow, which is inherently more informative when based on individual-level information than population-level averages (for more discussion and explanation, see for example, Smouse & Peakall, 1999; Barbujani, 2000; Manel *et al.*, 2003; Piggott *et al.*, 2006; Elmer *et al.*, 2007).

Significant spatial genetic structure was identified in *A. alluaudi* from 0-5 km and there is a weak indication of significant positive spatial structure at 40-50 km (r is greater than the null distribution but the more conservative error bar crosses zero) (Figure 2). There is no significant spatial genetic structure in *P. nyererei*. A visual survey of the FCA on individual multilocus genotypes demonstrates that, although sample sizes differ, the inter-individual spread of genetic variance is very similar for the both species (Figure 3).

TABLE 1
Population level genetic diversity statistics and sample sizes for *A. alluaudi*.

		Number of alleles	Observed heterozygosity	Unbiased expected heterozygosity	Sample size
<i>A. alluaudi</i>					
Bwiru Island	Mean	3.875	0.771	0.813	3
	±SE	0.350	0.094	0.062	
Gana Island	Mean	3.500	0.667	0.708	3
	±SE	0.598	0.109	0.120	
Juma Island	Mean	3.500	0.667	0.617	3
	±SE	0.655	0.141	0.129	
Kissenda Bay	Mean	1.875	0.438	0.417	2
	±SE	0.581	0.175	0.167	
Makobe Island	Mean	10.750	0.674	0.732	15
	±SE	2.136	0.105	0.102	
Nansio Bay	Mean	6.125	0.692	0.741	6
	±SE	1.109	0.108	0.099	
Ndurwa Point	Mean	3.750	0.688	0.754	3
	±SE	0.491	0.080	0.077	
Ruti Island	Mean	6.625	0.732	0.767	7
	±SE	1.117	0.095	0.082	
Vesi Archipelago	Mean	5.000	0.638	0.651	6
	±SE	0.802	0.122	0.119	
Total	Mean	5.000	0.663	0.689	
	±SE	0.430	0.038	0.037	

TABLE 2

Population level genetic diversity statistics and sample sizes for *P. nyererei*.

		Number of alleles	Observed heterozygosity	Unbiased expected heterozygosity	Sample size
<i>P. "red nyererei"</i>					
Igombe Island	Mean	3.375	0.688	0.754	3
	± SE	0.420	0.135	0.071	
Kissenda Bay	Mean	4.500	0.677	0.716	4
	± SE	0.802	0.091	0.106	
Makobe Island	Mean	10.375	0.657	0.746	13
	± SE	2.228	0.093	0.095	
Python Islands	Mean	11.250	0.725	0.763	14
	± SE	2.589	0.088	0.098	
Ruti Island	Mean	5.625	0.652	0.742	6
	± SE	1.133	0.124	0.119	
Senga Point	Mean	4.500	0.656	0.701	4
	± SE	0.906	0.145	0.135	
Total	Mean	6.604	0.676	0.737	
	± SE	0.749	0.044	0.041	

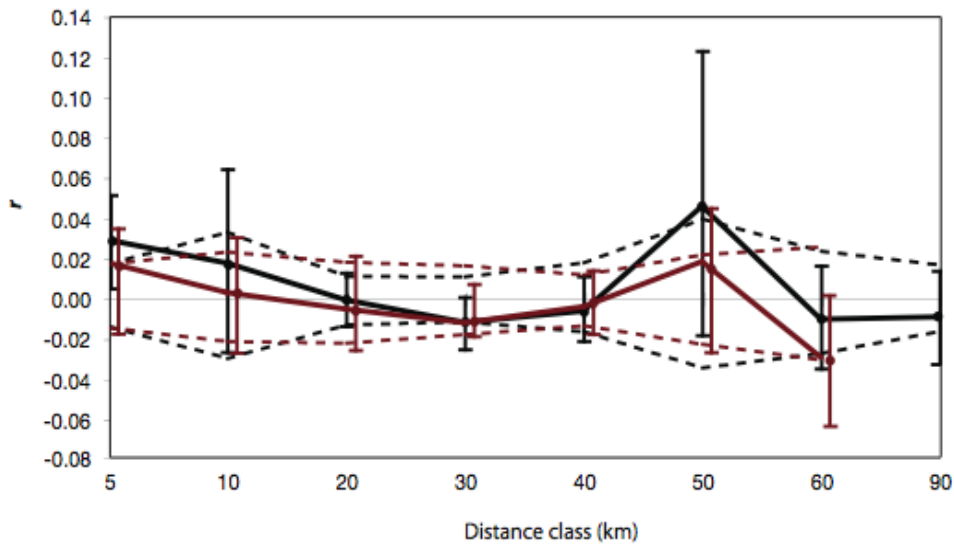


Fig. 2: Spatial genetic structure in *A. alluaudi* (black lines) and *P. nyererei* (red lines) as tested by inter-individual comparison of genetic and geographic distance matrices (N.B. no populations of *P. nyererei* are more distant than the 60 km class). Data points and error bars are offset for clearer visualization. *A. alluaudi* shows weak population genetic structuring in the first distance class (0-5 km). There is no significant spatial-genetic structure among *P. nyererei* individuals.

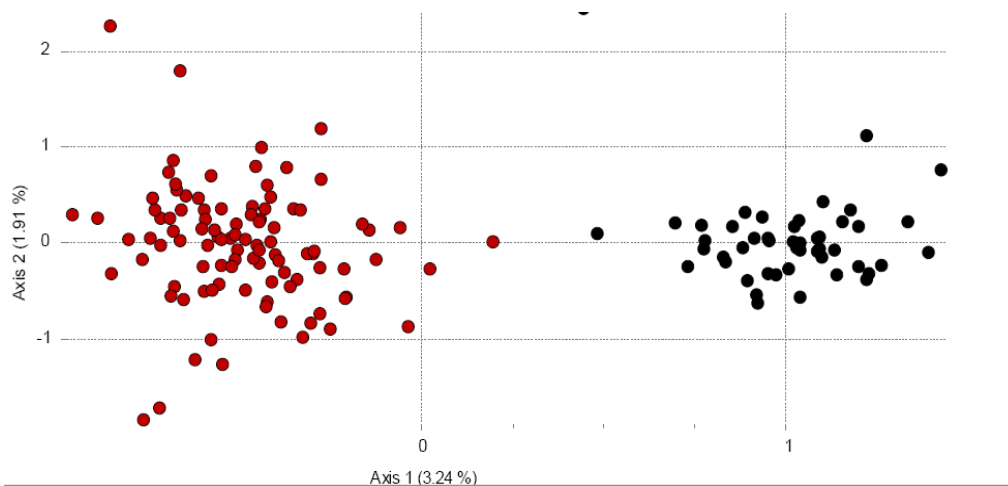


Fig. 3: A factorial correspondence analysis of multilocus genotypes for each individual of *A. alluaudi* (black dots) and *P. nyererei* (red dots). The relative explanatory power of each of the principal axes is indicated. There are similar levels of allelic variation in both species across the study area.

DISCUSSION

The eurytopic cichlid *A. alluaudi* and the stenotopic cichlid *P. nyererei* have similar patterns of low spatial-genetic structure despite heterogeneity of allele frequencies across populations. Overall levels of genetic diversity and heterozygosity are similarly high in both species. In contrast to high levels of genetic diversity within populations, isolation-by-distance does not appear to be a powerful influence in either species, as the statistical correlation between genetic and geographic distance is weak (Figure 2). The distribution of genetic variation is also similar in both species, as visualized in a correspondence analysis (Figure 3). The analytical approaches we employed here are not dependent on genetical “populations” but rather reflect individual-level differentiation based on global multilocus allele frequencies. Our findings, though preliminary, indicate that there may not be a difference in individual-level gene flow or population-level genetic diversity between these species of different ecologies and evolutionary histories: a Lake Victoria haplochromine superflock cichlid (*P. nyererei*) and a widespread non-superflock haplochromine cichlid fish (*A. alluaudi*).

Gene flow may not be as restricted as the stenotopic and eurytopic species’ ecology might suggest. Some taxonomic uncertainty (Seehausen, 1996) and inter-population variation in ecology (Fermon & Cibert, 1998) makes it difficult to directly compare our findings with the currently known ecology and population genetics of the many geographic variants of *Pundamilia*; most local variants still need to be described (O. Seehausen, personal communication). Sexual selection is known to be important in diversification of *P. nyererei* in particular (Seehausen *et al.*, 1998, 2008) and many African cichlids in general (Salzburger, 2009). Conversely, phenotypic plasticity of feeding apparatus and greater environmental tolerance in the eurytopic *A. alluaudi* may reduce the tension to speciate at microallopatric scales (Greenwood, 1965; Turner *et al.*, 2001).

Many biologists have argued that the stenotopy of African rock-dwelling cichlids means that local variation in aquatic habitat presents a dispersal barrier at the microgeographic scale in Lake Malawi (e.g. van Oppen *et al.*, 1997b; Arnegard *et al.*, 1999; Markert *et al.*, 1999; Danley *et al.*, 2000; Rico & Turner, 2002; Pereyra *et al.*, 2004) and Lake Tanganyika (Rüber *et al.*, 2001; Taylor *et al.*, 2001; Duftner *et al.*, 2006; Koblmüller *et al.*, 2007; Sefc *et al.*, 2007). Cichlids that are not rocky habitat specialists tend to show much less interpopulation genetic structure (Shaw *et al.*, 2000; Taylor & Verheyen, 2001; Abila *et al.*, 2004; Pereyra *et al.*, 2004). However, not all studies have found restricted gene flow in superflock cichlids (Meyer *et al.*, 1996) or evidence that intervening habitats consistently influence

dispersal distance among rock-specialist species (Danley *et al.*, 2000). Restricted spatial genetic population structure can be found in rock-dwelling cichlids that are not part of the Lake Victoria superflock (e.g. Pereyra *et al.*, 2004), though the focus of this research on rapidly radiating lineages makes it difficult to determine whether there is a correlation between species richness and spatial-genetic structure, as some researchers have proposed (e.g. van Oppen *et al.*, 1997b). In a correlogram, restricted spatial genetic structure is reflected in high correlation coefficients at short inter-individual geographic distances, a pattern which is not evident in our data (Figure 2).

The results presented here, though preliminary, suggest that restricted gene flow is not based on a species' environmental sensitivity in Lake Victoria cichlids, at least in *A. alluaudi* and *P. nyererei*. Further, contemporary gene flow is high whether the species is a member of the rapidly radiating superflock or an evolutionarily more static lineage. Our findings are in contrast with the prevailing expectation of reduced gene flow in stenotopic cichlids and the prediction that there should be a correlation between species richness and fine-scale gene flow. The lack of differentiation in both cichlid species may be partly due to lake level fluctuations over geological and recent time (Nicholson, 1998), which could disrupt the establishment of local genetic structuring and migration-drift equilibrium. Alternatively, neutral markers such as microsatellites may be underestimating micro-scale adaptive and ecologically relevant differentiation (e.g. 'porous genome' Gavrillets & Vose, 2005), which is known to exist among *P. nyererei* populations in southern Lake Victoria (Terai *et al.*, 2006; Seehausen *et al.*, 2008).

Further comparative studies that control for range sampling, ecology, and phylogenetic history are needed to clarify the association between ecology and speciation rates. Such research should attempt to sample a wider number of stenotopic and eurytopic cichlids at similar spatial scales and, if possible, include larger population sizes, which would allow the use of traditional estimates of population genetic structure. If the results presented here are supported in a larger taxonomic sample, we will need to reconsider some models of cichlid evolution. As the conclusions of this work may also be relevant in other adaptive radiations, researchers working in other rapidly diversifying groups should use a similar comparative approach to determine the factors responsible for adaptive radiations. Such comparative studies will help to clarify the relationship between spatial-structure, ecology and population genetics, and the relative importance of these factors during the different phases of adaptive radiations (Streelman & Danley, 2002).

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