SHORT COMMUNICATION

Hybrid origin of a cichlid population in Lake Malawi: implications for genetic variation and species diversity

PETER F. SMITH,*§ AD KONINGS‡ and IRV KORNFIELD†
*Department of Biological Sciences, and †School of Marine Sciences, University of Maine, 5751 Murray Hall Orono, ME 04469–5751, USA, ‡Cichlid Press; PO Box 13608 El Paso, Texas 79913 USA

Abstract

The importance of species recognition to taxonomic diversity among Lake Malawi cichlids has been frequently discussed. Hybridization — the apparent breakdown of species recognition — has been observed sporadically among cichlids and has been viewed as both a constructive and a destructive force with respect to species diversity. Here we provide genetic evidence of a natural hybrid cichlid population with a unique colour phenotype and elevated levels of genetic variation. We discuss the potential evolutionary consequences of interspecific hybridization in Lake Malawi cichlids and propose that the role of hybridization in generating both genetic variability and species diversity of Lake Malawi cichlids warrants further consideration.

Keywords: Cichlidae, hybridization, *Maylandia*, *Metriaclima*, microsatellites, *Pseudotropheus zebra*, speciation

Received 6 February 2003; revision received 3 June 2003; accepted 3 June 2003

Introduction

Several hundred species of endemic cichlids have arisen in Lake Malawi since its origin less than 1 million years ago. The mechanisms of the evolution and maintenance of this spectacular species diversity are still largely unknown (Kornfield & Smith 2000). Prezygotic reproductive isolation is thought to be critical in the early stages of species divergence among African cichlids (Danley & Kocher 2001). Sexual selection on male colour has been repeatedly proposed as a mechanism that generates cichlid species diversity (Dominey 1984; McKaye 1991; McKaye et al. 1993; Seehausen et al. 1997, 1998; Seehausen 1999; Danley et al. 2000), and coloration is important to maintaining the separation between divergent cichlid populations after secondary contact (Seehausen et al. 1998). Mate choice has been demonstrated as a means of species recognition and species-specific mating in cichlids (Knight et al. 1998; Knight & Turner 1999). An intriguing corollary to the recognized importance of mate choice is that the lack of species-specific

mating, or hybridization, could also have important evolutionary consequences among these fishes. Though the evolutionary outcomes of hybridization are unpredictable, populations that experience introgression might possess increased genetic diversity over true-bred lineages. Moreover, if stable populations with unique phenotypes result, new species could be formed.

Previous studies have revealed that a great deal of genetic variation exists within species of African cichlids (Klein et al. 1993; Moran & Kornfield 1993, 1995; Kornfield & Parker 1997; Parker & Kornfield 1997; Van Oppen et al. 1997a; Arnegard et al. 1999; Markert et al. 1999, 2001; Danley et al. 2000; Ruber et al. 2001; Rico & Turner 2002; Smith & Kornfield 2002). Ancestral mitochondrial DNA polymorphisms are present among divergent genera as a result of incomplete lineage sorting (Moran & Kornfield 1993; Parker & Kornfield 1997), and the allelic diversity observed at microsatellite loci is greater than expected based on the age and population sizes of the examined taxa (Markert et al. 2001; Van Oppen et al. 1997b). These data are difficult to reconcile with the known history of Lake Malawi (Johnson 1996) and its biota (Meyer 1993); either population sizes must have been dramatically larger than presently conceived, or time since divergence of these taxa must have been longer than all other evidence suggests. Intergeneric hybridization

Correspondence: Irv Kornfield, 5751 Murray Hall, Orono, ME 04469–5751, USA. Fax: 207–581–2537; E-mail: irvk@maine.edu
§Present address: Maine Medical Center Research Institute; 81 Research Drive, Scarborough, Maine 04074, USA

was advanced as an alternative explanation for shared polymorphism among divergent genera, but further study has suggested that this is probably not a general phenomenon (Albertson *et al.* 1999). However, among congeners, if secondary contact of distinct allopatric populations or newly derived species often results in gene flow, genetic effective population sizes will be much higher than expected from census sizes. Thus, the paradox presented by excessively high levels of polymorphism within species might be explained by hybridization among closely related taxa.

Hybrids have been observed in nature among the cichlids of the three African Great Lakes. Putative hybrids were discovered in Lake Victoria, where species-specific mating was compromised by reduction in water clarity (Seehausen *et al.* 1997; Verschuren *et al.* 2002). In Lake Tanganyika, introgression is believed to have occurred upon secondary contact between a pair of currently sympatric species (Ruber *et al.* 2001), and a separate Lake Tanganyika hybridization event resulted in speciation (Salzburger *et al.* 2002). In Lake Malawi, some specimens of intergeneric hybrids have been discovered at one translocation site (Stauffer *et al.* 1996), and hybridization was postulated for translocated populations of *Pseudotropheus callainos* (Stauffer & Hert 1992). Greater understanding of the potential for natural hybridization, especially in undisturbed settings, is important to evaluating the role of hybridization as an evolutionary mechanism in African cichlids.

Within the *Metriaclima* (= *Pseudotropheus*) *zebra* species complex in Lake Malawi there are multiple populations that differ in dorsal fin coloration (Stauffer *et al.* 1997). 'Red top' (RT) and 'blue-black' or 'blue with bars' (BB) forms have a mutually exclusive, checkerboard distribution, and

uniform dorsal fin phenotype within each population (A. Konings, I. Kornfield and P. Smith, personal observations). Areas of suitable *M. zebra* habitat are effectively islands; sandy bottom and deep water separating rocky areas may be strong barriers to dispersal (Markert *et al.* 1999). No obvious zones of contact or clines have previously been observed between the two colour forms, even when they inhabit adjacent rocky habitat. Because of the contemporary absence of sympatry, prezygotic reproductive isolation among allopatric variants of the *M. zebra* complex has not been tested in nature, but aquarium studies reveal no postzygotic barriers (Kornfield 1991). Thus, periods of sympatry could potentially lead to hybridization between RT and BB populations.

During fieldwork in 1998, we encountered a population of *Metriaclima* on the eastern shoreline of Lake Malawi exhibiting a mosaic of phenotypes intermediate between RT and BB. This population, located at Makanjila (13°43.906′S, 34°52.061′E), is situated midway between the RT population at Eccles Reef (13°45.991′S, 34°57.687′E), and the BB population at Chiofu Bay (13°31.733′S, 34°51.962′E). The RT at Eccles Reef is assigned to the nominal taxon, *Metriaclima thapsinogen*, and is thus specifically distinct from the *M. zebra* at Chiofu. Because these two species exist only in allopatry, nothing is known about their potential reproductive isolation; maintenance of distinct phenotypes and neutral genetic differentiation (Smith & Kornfield 2002) suggest the absence of gene flow between these populations. Territorial males in the Makanjila population exhibited a range of dorsal fin colour phenotypes, but none could be characterized as the typical RT or BB (see Fig. 1 for image). The intermediate coloration of this population and its
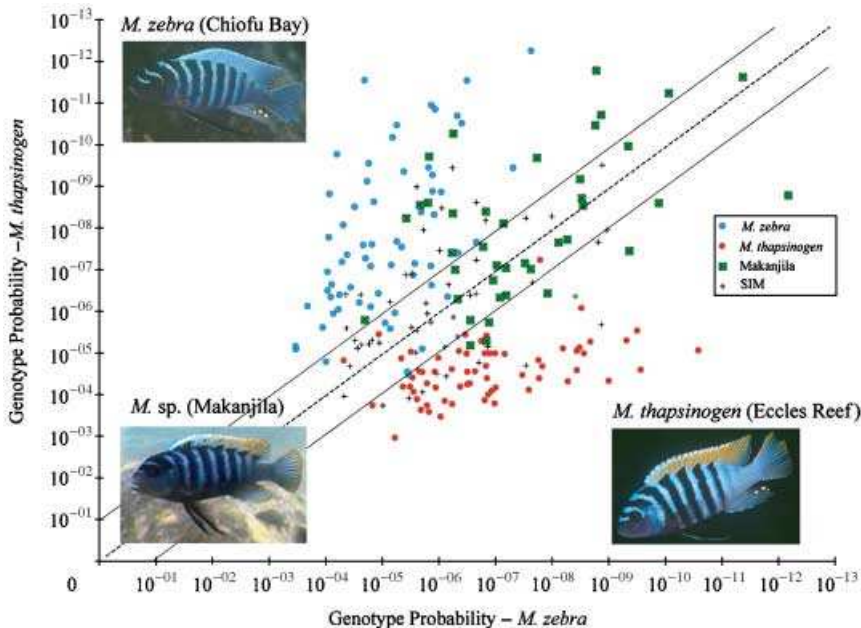

Fig. 1 Genotype assignment test to putative parental baselines (*Metriaclima thapsinogen* and *M. zebra*). All individuals were assigned a genotype probability for each baseline and plotted on a log scale. The dashed line indicates the region of the graph where the probability of a genotype in the two baseline samples is equal; samples falling above the line are 'assigned' to the *M. zebra* (Chiofu Bay) population and samples below the line are 'assigned' to the *M. thapsinogen* (Eccles Reef) population. The solid lines deviate from equality by a factor of 10. *Metriaclina thapsinogen* and *M. zebra* samples assigned to their own baseline with a few minor deviations. SIM samples assigned indiscriminately to the two baselines, as did the Makanjila samples. Some Makanjila samples had very low genotype probability values (points farthest from the origin on both axes), probably caused by unique alleles in that population.

geographical location between *M. thapsinogen* at Eccles Reef and *M. zebra* at Chiofu Bay are suggestive of a hybrid origin.

In this study we use microsatellite markers to infer the history of the phenotypically intermediate Makanjila *Metriaclima* population, and examine its relationship to the adjacent RT (*M. thapsinogen*) and BB (*M. zebra*) populations. We compare the Makanjila population to the putative parental populations and to a simulated population of F_1 hybrids.

Materials and methods

Fish were collected with monofilament gill nets using SCUBA. Only males were sampled to ensure unambiguous species identification. Small portions of the caudal fin were clipped from individual fish and preserved in 95% ethanol; ethanol was changed after 48 h. DNA was extracted using the QiAmp tissue kit (Qiagen) following the manufacturer's instructions.

Four microsatellite loci were examined: Pzeb3, Pzeb4, Pzeb5 [GenBank accession numbers X99784, X99786, X99787 (Van Oppen *et al.* 1997b)] and UME003 [U14397 (Parker & Kornfield 1996)]. Polymerase chain reaction (PCR) and genotyping followed the technique of Smith & Kornfield (2002).

In the absence of fixed diagnostic alleles in parental populations, genetic relationships were examined via comparison to a simulated hybrid population. The simulated F_1 hybrid population (SIM) was generated by combining alleles randomly drawn from the frequency distribution of each putative parental population at each locus to create a sample of 50 F_1 multilocus genotypes. This simulation models origination of an F_1 hybrid population by extensive hybridization between the putative parental populations, with equal contributions from each population. Though allele frequencies might change during successive generations within a true hybrid population, the SIM sample provides a simple snapshot of allele frequency expectations for an F_1 population created by extensive hybridization. The SIM population was subsequently treated identically to the other populations during analysis.

Tests of Hardy–Weinberg equilibrium, linkage equilibrium, and population differentiation were performed with GENEPOP (Raymond & Rousset 1995). Allele frequencies, heterozygosities and mean number of alleles were calculated using the microsatellite toolkit macro for MICROSOFT EXCEL™ (Park 2001). Assignment tests were performed with WHICHRUN (Banks & Eichert 1999). Factorial correspondence analysis (FCA) was performed on the data to examine genetic structuring among the three populations. The four locus genotypes of each individual were used as variables and FCA was performed as implemented in the computer program GENETIX (Belkhir 1999)

Sequencing of microsatellite alleles was performed by cloning PCR products from individuals typed for the allele of interest. PCR cloning was performed with the TA cloning kit (Qiagen, Valencia, CA). Cycle sequencing was performed as previously described (Haye *et al.* 2002).

Results

Examination of the relationships among populations revealed a general trend of intermediacy of the Makanjila population, which was similar to the SIM population. An exact test of population differentiation was significant for Eccles Reef *Metriaclima thapsinogen* and Chiofu Bay *M. zebra* populations at all four loci, with less complete (fewer loci significant) differentiation among all other population comparisons (data not shown). A genotype assignment test of individuals to the *M. thapsinogen* and *M. zebra* baseline populations (Fig. 1) showed accurate assignment of individuals from each of those populations with intermediate assignment values of the SIM samples, which displayed nonpreferential assignment between the two tested baseline population samples. Makanjila samples were also assigned nonpreferentially.

The FCA was used to represent graphically the partitioning of genetic variance among populations (Fig. 2). Again, clusters of samples from the Eccles Reef *M. thapsinogen* and Chiofu Bay *M. zebra* populations were well separated by axis 1 of the FCA plots, with minimal overlap around the origin of the graph. The placement of SIM and Makanjila samples was contained within the ranges of the two putative parental populations on axis 1, clustering principally in the area where *M. thapsinogen* and *M. zebra* samples overlapped. However, the second FCA axis strongly separated samples of the Makanjila population from the others (axes 3 and 4 contribute no additional separation). The intermediacy of Makanjila samples relative to the putative parental populations, and the similarity of Makanjila samples to the SIM samples were supportive of the hypothesis of hybrid origin. The separation provided by axis 2 of the FCA plots, like the assignment tests, was driven largely by the contribution of the unique Makanjila alleles (below).

The Makanjila population had greater genetic diversity (Nei's unbiased gene diversity, 0.727) than the Eccles Reef *M. thapsinogen* (0.682) and Chiofu Bay *M. zebra* (0.696) populations, but was nearly identical to the simulated F_1 hybrid (SIM) population (0.725). However, significant overall heterozygote deficiency ($P < 0.01$) was observed only in the Makanjila population as well as a positive inbreeding coefficient. Because each population was phenotypically distinct and the samples of putative parental populations were more than 25 km apart with no parental-types observed at the Makanjila location, the heterozygote deficiency was probably not the result of a Whalund effect, but might better be explained by inbreeding.

A microsatellite saltine distribution (Kornfield & Parker 1997) was identified at locus UME003 in a lake-wide
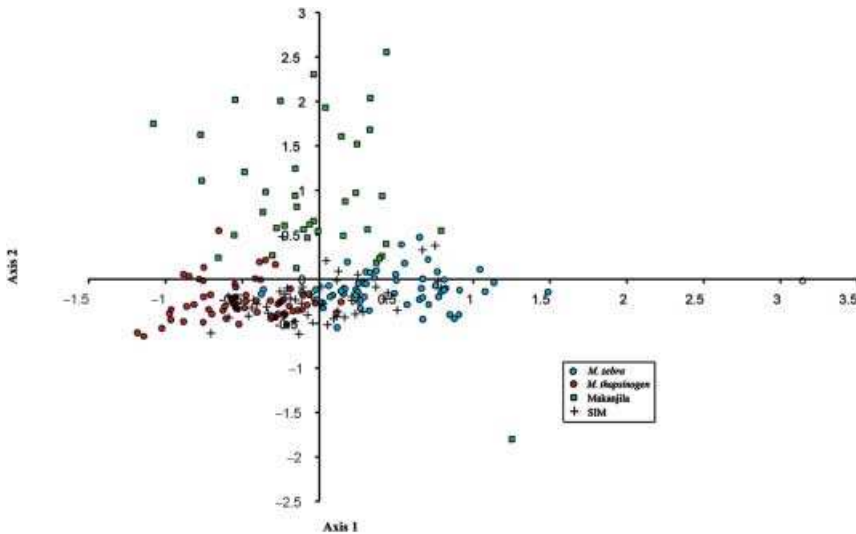
Fig. 2 Representation of genetic variance among samples by factorial correspondence analysis (FCA). Axis 1 explains 3.46% of the variance and separates the *Metriaclima thapsinogen* (Eccles Reef) and *M. zebra* (Chiofu Bay) samples. The SIM and Makanjila samples cluster more centrally, and are completely contained within the range set by the parental taxa on this axis. Axis 2 explains an additional 3.37% of the variance, and separates most Makanjila individuals from all others in all populations. The alleles predominantly contributing to axis 2 are all unique to the Makanjila population.

population survey of RT and BB *Metriaclima* populations; a large proportion of the alleles observed in RT populations throughout the lake occurred in the lower portion of this disjunct allele frequency distribution (allele size < 177 base pairs), while BB populations had low frequencies of these alleles (P. Smith & I. Kornfield, unpublished results). The cumulative frequency of alleles within this saltine distribution in the Makanjila population (16%) was intermediate between the high frequency (> 23%) in the Eccles Reef (*M. thapsinogen*) population and the minimal (< 2%) frequency in the Chiofu Bay (*P. zebra*) population. This intermediacy was also suggestive of a hybrid origin. Indeed, examination of the allele frequency distributions for the other loci (Table 1) revealed intermediate frequencies for the Makanjila population (and similarity to SIM); this was most evident for alleles with relatively high frequency in one of the putative parental populations. Thus, the allele frequencies generally fitted the pattern consistent with the creation of a hybrid taxon via an episode of extensive hybridization between RT *M. thapsinogen* and BB *M. zebra* populations. However, there were exceptions to this pattern, which hinted at a more complex history for the Makanjila population.

For three of the loci in this study (Pzeb3, Pzeb4, and UME003), a total of 12 alleles were detected only in the Makanjila population. Some of these alleles had very low frequencies, and may not have truly been unique, so we performed a simulation of population sampling as an evaluation of our empirical allele frequency distribution. For its frequency to be considered 'significantly unique', an allele must be sampled with greater than 95% confidence in our simulation. Four alleles (indicated by ** in Table 1) were significantly unique to the Makanjila population based on this simulation; if these alleles existed in equal frequencies in the flanking populations, they would be sampled with > 95% confidence.

In order to test whether alleles may have arisen as new mutations, we sequenced allele '126' from locus Pzeb4. This allele, which had a frequency of over 30% in the Makanjila population, was absent from the other two samples. Locus Pzeb4 was previously examined in Lake Malawi cichlid populations (Van Oppen *et al.* 2000). That study revealed that alleles with the same gel mobility often have different sequences. If allele '126' originated by mutation in the Makanjila population, it may have a different sequence than the same sized allele in other *Pseudotropheus* populations. Alleles in the '126' size class were also sequenced from *P. zebra* (BB) populations at Nkhata Bay and Namalenji Island, two distant localities representing northern and southern portions of the *M. zebra* range, respectively. All '126' alleles sequenced were identical and matched the sequence reported by Van Oppen *et al.* (2000). Our data did not indicate that this allele arose by mutation in the Makanjila population, but that remains a possibility.

Discussion

Genetic analysis of the phenotypically intermediate Makanjila *Metriaclima* sample indicates that this population has arisen as a result of extensive hybridization. The geographical juxtaposition of the Makanjila population suggests that *M. thapsinogen* from Eccles Reef and *M. zebra* from Chiofu Bay may be the parental populations of the extant Makanjila hybrid population. Our microsatellite analyses provide some support for this hypothesis, but indicate that the *Metriaclima* population at Makanjila may have a more complex history.

Private alleles exist at high frequency in the Makanjila population, an observation potentially inconsistent with a simple hybrid origin of this population. The presence of these unique alleles at Makanjila can be explained either by unique origin within the isolated hybrid population (e.g.

Table 1 Allele frequencies (as percent) for three populations and the simulated hybrid at four microsatellite loci

Locus	Allele	*M. zebra*	*M. thapsinogen*	Makanjila	SIM
PZeb3	310*	...	...	2.50	...
	312	44.44	64.06	52.50	58.00
	314	3.17	13.28	10.00	5.00
	316†	...	...	5.00	...
	318	26.19	...	21.25	10.00
	320	8.73	21.09	1.25	16.00
	322	1.59	0.78	1.25	2.00
	324	6.35	0.78	3.75	4.00
	326	2.38	...	1.25	4.00
	328	0.79	...	...	...
	330	1.59	...	...	...
	332	3.17	...	...	1.00
	336	1.59	...	...	...
	340*	...	...	1.25	...
PZeb4	112	3.97	...	...	4.00
	120	36.51	28.13	30.00	34.00
	124	9.52	44.53	16.25	21.00
	126†	...	...	32.50	...
	128	34.92	3.13	18.75	21.00
	130	3.97	...	1.25	2.00
	132	0.79	...	...	...
	134	0.79	0.78	1.25	2.00
	136	6.35	21.09	...	10.00
	138	3.17	...	...	3.00
	148	...	2.34	...	3.00
PZeb5	123	5.56	40.63	28.75	29.00
	129	76.98	51.56	62.50	63.00
	137	17.46	7.81	8.75	8.00
	N	63	64	40	50
	A	11.75	11.50	11.75	12.25
	H_E	0.696	0.682	0.727	0.725
	H_O	0.698	0.703	0.656‡	0.725
	F_{IS}	−0.004	−0.031	0.098	−0.0004

Locus	Allele	*M. zebra*	*M. thapsinogen*	Makanjila	SIM
UME003	111	...	0.78	...	1.00
	113*	...	...	1.25	...
	115*	...	...	2.50	...
	117	...	1.56	...	...
	121	...	0.78	2.50	...
	123	...	1.56	...	5.00
	125*	...	...	2.50	...
	127	...	4.69	...	4.00
	129	...	3.13	...	...
	131	0.79	1.56	1.25	1.00
	133	0.79	5.47	...	4.00
	135	...	1.56	...	...
	141	...	0.78	...	2.00
	143*	...	...	3.75	...
	147	...	0.78	...	...
	149	...	0.78	1.25	...
	151*	...	...	1.25	...
	177*	...	...	1.25	...
	181	...	3.13	...	3.00
	185	...	0.78	...	...
	189	2.38	...	6.25	...
	191†	...	...	12.50	...
	193	...	1.56	2.50	1.00
	195†	...		7.50	...
	197	3.17	16.41	1.25	7.00
	199	...	0.78	2.50	...
	201	...	3.13	6.25	1.00
	203	0.79	3.91	...	5.00
	205	0.79	1.56	6.25	1.00
	207	...	3.13	7.50	1.00
	209	1.59	0.78	5.00	1.00
	211	3.17	3.91	3.75	5.00
	213	7.94	7.81	3.75	7.00
	215	4.76	7.81	2.50	5.00
	217	6.35	4.69	...	5.00
	219	7.14	2.34	...	2.00
	221	8.73	5.47	3.75	7.00
	223	4.76	3.91	1.25	5.00
	225	6.35	3.91	2.50	6.00
	227	7.14	...	3.75	3.00
	229	9.52	0.78	...	6.00
	231	6.35	...	1.25	4.00
	233	5.56	...	2.50	3.00
	235	3.97	...	...	2.00
	237	3.17	0.78	...	2.00
	239	2.38	...	...	...
	241	1.59	...	...	...
	243	0.79	...	...	1.00

Genetic diversity statistics, *N*, number of samples; *A*, average number of alleles; H_E, Nei's unbiased gene diversity; H_O, observed heterozygosity; F_{IS}, method of moments estimate of inbreeding coefficient.
*Allele unique to Makanjila population but not significant based on simulation (see text).
†Allele unique to Makanjila and significant based on simulation (see text).
‡Significant global heterozygote deficiency ($P = 0.0095$).

by mutation), or by introgression from populations other than the flanking populations sampled. The origin by mutation of new allelic variants in hybrid populations, i.e. hybrizymes, is a recognized, though poorly understood, phenomenon (Hoffman & Brown 1995; Schilthuizen *et al.* 2001). Sequence analysis of one allele class (Pzeb4, 126) suggests that it is unlikely that this allele arose in the Makanjila population by mutation since the hybridization

event. One possibility concerning potential introgression is that the *M. thapsinogen* from Eccles Reef and *M. zebra* from Chiofu Bay are not the parental populations of the Makanjila hybrids. Instead, additional RT and/or BB population(s) from the region, either unsampled or now extinct, could have contributed novel alleles to the Makanjila population. Intriguingly, two other *Metriaclima* populations that have not yet been studied exist in the region of Makanjila and thus are potential extant contributors to this hybrid population.

Alternatively, Makanjila may have been colonized by hybrids produced between the Eccles Reef and Chiofu Bay populations studied here. Again, the origin of unique alleles could be explained by introgression from an additional, unidentified population subsequent to colonization of the Makanjila population. All four alleles significantly unique to the Makanjila population are found in *Metriaclima* populations throughout the lake at varying frequencies (Van Oppen *et al.* 2000; personal observations). FCA suggests that unique alleles are the principal factor generating the genetic distinctiveness of the Makanjila population. The common alleles at Pzeb3, Pzeb4, and Pzeb5 show a striking pattern of intermediacy with the exception of the unique alleles. In sum, these data suggest that the basis for the departure of the Makanjila genetic make-up from the expectations of intermediacy is a phenomenon created by the presence of unique genetic material in this population, and not pan-genomic differences. Thus, additional introgression may have come from a congener with a similar genetic background that possesses some genetic divergence.

The proposal of multiple hybridization events may not seem parsimonious given the data on low levels of gene flow among adjacent populations (Van Oppen *et al.* 1997a, 1998; Arnegard *et al.* 1999; Knight & Turner 1999; Markert *et al.* 1999; Danley *et al.* 2000; Rico & Turner 2002; Rico *et al.* 2003). However, colonization studies on Lake Malawi cichlids have shown rapid habitation of artificial reefs, suggesting extensive movement of many cichlid species (McKaye & Gray 1984). Thus, the low levels of gene flow inferred by population genetics may be the result of biotic factors, such as mate choice, rather than extrinsic limitations on movement. If mating tolerances and coloration are sufficiently perturbed within newly formed hybrid populations, gene flow from other populations might occur.

Hybridization between congeners, such as that discovered at Makanjila, may provide explanatory power for both the maintenance of high levels of genetic variation within populations and for the origin of new species. First, fluctuations in lake level continually isolate and merge areas of habitat, facilitating episodic sympatry between divergent allopatric populations, incipient species, or species (Sturmbauer 1998; Sturmbauer *et al.* 2001). If populations maintain reproductive barriers during sympatry, species are preserved or established. However, some gene exchange might occur at such times, particularly when densities become

high and breeding territories become limited (Kornfield & Smith 2000). With gene flow on secondary contact, previously isolated populations could acquire additional variation or could recover genetic diversity lost during periods of finite population size. Hybridization could thus explain the maintenance of high genetic variation within populations of Lake Malawi cichlids. Second, systematists frequently elevate phenotypically distinct populations, such as that at Makanjila, to specific status (e.g. Stauffer *et al.* 1997). While the presence of reproductive isolation cannot be easily evaluated, colour differentiation in *Metriaclima* and other lineages is the central component maintaining separation of gene pools. Hybridization has been characterized as an under-appreciated mechanism of speciation in animals (Dowling & Secor 1997; Arnold & Emms 1998). The existence of the unique Makanjila population suggests that hybridization could be a speciation mechanism in cichlids, contributing to the high species diversity in Lake Malawi and elsewhere. Finally, the hybrid *Metriaclima* population at Makanjila was initially recognized by its intermediate colour pattern. In the absence of such noticeable features, hybrids probably go undetected. Based on the current data set and recent published reports (Ruber *et al.* 2001; Salzburger *et al.* 2002; Smith & Kornfield 2002), we suggest that hybridization among cichlids may be more pervasive than currently appreciated, and the evolutionary impact of interspecific hybridization among these diverse fishes warrants additional consideration.

Acknowledgements

References

Albertson CR, Markert JA, Danley PD, Kocher TD (1999) Phylogeny of a rapidly evolving clade: the cichlid fishes of Lake Malawi, East Africa. *Proceedings of the National Academy of Sciences USA*, **96**, 5107–5110.

Arnegard ME, Markert JA, Danley PD, Stauffer JRJ, Ambali AJ, Kocher TD (1999) Population structure and colour variation of the cichlid fish *Labeotropheus fuelleborni* Ahl along a recently formed archipelago of rocky habitat patches in southern Lake Malawi. *Proceedings of the Royal Society of London B*, **266**, 119–130.

Arnold ML, Emms SK (1998) Paradigm Lost: natural hybridization and evolutionary innovations. In: *Endless Forms, Species and*

Speciation (eds Howard DJ, Berlocher SH), pp. 379–389. Oxford University Press, Oxford.

Banks M, Eichert W (1999) whichrun, Version 4.1. Available from http://www-bml.ucdavis.edu/whichrun.htm.

Belkhir K (1999) genetix version. 4.0. Laboratoire Genome, Populations, Interactions, CNRS UPR 9060, Montpellier, France.

Danley PD, Kocher TD (2001) Speciation in rapidly diverging systems: lessons from Lake Malawi. *Molecular Ecology*, **10**, 1075–1086.

Danley PD, Markert JA, Arnegard ME, Kocher TD (2000) Divergence with gene flow in the rock-dwelling cichlids of Lake Malawi. *Evolution*, **54**, 1725–1737.

Dominey W (1984) Effects of sexual selection and life history on speciation: species flocks in African Cichlids and Hawaiian *Drosophila*. In: *Evolution of Fish Species Flocks* (eds Echelle A, Kornfield I), pp. 231–249. University of Maine Press, Orono, ME.

Dowling TE, Secor CL (1997) The role of hybridization and introgression in the diversification of animals. *Annual Review of Ecology and Systematics*, **28**, 593–619.

Haye P, Tam YK, Kornfield I (2002) Molecular phylogenetics of mole crabs (Hippidae: Emerita). *Journal of Crustacean Biology*, **22**, 903–915.

Hoffman SMG, Brown WM (1995) The molecular mechanism underlying the 'rare allele phenomenon' in a subspecific hybrid zone of the California field mouse, *Peromyscus californicus*. *Journal of Molecular Evolution*, **41**, 1165–1169.

Johnson TC (1996) Sedimentary processes and signals of past climatic change in the large lakes of the East African rift valley. In: *The Limnology, Climatology, and Paloclimatology of the East African Lakes* (eds Johnson TD, Odada EO), pp. 367–412. Gordon and Breach, Toronto.

Klein D, Ono H, O'hUigín C, *et al.* (1993) Extensive MHC variability in cichlid fishes of Lake Malawi. *Nature*, **364**, 330–334.

Knight ME, Turner GF (1999) Reproductive isolation among closely related Lake Malawi cichlids: can males recognize conspecific females by visual cues? *Animal Behaviour*, **58**, 761–768.

Knight ME, Turner GF, Rico C, Van Oppen MJH, Hewitt GM (1998) Microsatellite paternity analysis on captive Lake Malawi cichlids supports reproductive isolation by direct mate choice. *Molecular Ecology*, **7**, 1605–1610.

Kornfield I (1991) Genetics. In: *Cichlid Fishes Behaviour, Ecology, and Evolution* (ed. Keenlyside M), pp. 103–128. Chapman & Hall, New York.

Kornfield I, Parker A (1997) Molecular systematics of a rapidly evolving species flock: the mbuna of Lake Malawi and the search for phylogenetic signal. In: *Molecular Systematics of Fishes* (eds Kocher TD, Stephien CD), pp. 25–27. Academic Press, Boston.

Kornfield I, Smith PF (2000) African cichlid fishes: models systems for evolutionary biology. *Annual Review of Ecology and Systematics*, **31**, 163–196.

Markert JA, Arnegard ME, Danley PD, Kocher TD (1999) Biogeography and population genetics of the Lake Malawi cichlid *Melanochromis auratus*: habitat transience, philopatry and speciation. *Molecular Ecology*, **8**, 1013–1026.

Markert JA, Danley PD, Arnegard ME (2001) New markers for new species: microsatellite loci and the East African cichlids. *Trends in Ecology and Evolution*, **16**, 100–107.

McKaye KR (1991) Sexual selection and the evolution of the Cichlid fishes of Lake Malawi, Africa. In: *Cichlid Fishes Behaviour, Ecology, and Evolution* (ed. Keenlyside M), pp. 241–257. Chapman & Hall, New York.

McKaye K, Gray N (1984) Extrinsic barriers to gene flow in rock-dwelling cichlids of Lake Malawi: macrohabitat heterogeneity and reef colonization. In *Evolution of Fish Species Flocks* (eds Echelle A, Kornfield I), pp. 169–183. University of Maine Press, Orono, ME.

McKaye KR, Howard J, Stauffer JA, Morgan R, Shonhiwa F (1993) Sexual selection and genetic relationships of a sibling species complex of bower building cichlids in Lake Malawi, Africa. *Japan Journal of Ichthyology*, **40**, 15–21.

Meyer A (1993) Phylogenetic relationships and evolutionary processes in East African cichlid fishes. *Trends in Ecology and Evolution*, **8**, 279–285.

Moran P, Kornfield I (1993) Retention of an ancestral polymorphism in the mbuna species flock (Pices: Cichlidae) of Lake Malawi. *Molecular Biology and Evolution*, **10**, 1015–1029.

Moran P, Kornfield I (1995) Were population bottlenecks associated with the radiation of the mbuna species flock of Lake Malawi? *Molecular Biology and Evolution*, **12**, 1085–1093.

Park SDE (2001) Trypanotolerance in West African cattle and the population genetic effects of selection. PhD Thesis. University of Dublin.

Parker A, Kornfield I (1996) Polygynandry in *Pseudotropheus zebra*, a cichlid fish from Lake Malawi. *Environmental Biology of Fishes*, **47**, 345–352.

Parker A, Kornfield I (1997) Evolution of the mitochondrial DNA control region in the mbuna (Cichlidae) species flock of Lake Malawi, East Africa. *Journal of Molecular Evolution*, **45**, 70–83.

Raymond M, Rousset F (1995) genepop (Version 1.2): Population genetics software for exact test and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Rico C, Turner GF (2002) Extreme microallopatric divergence in a cichlid species from Lake Malawi. *Molecular Ecology*, **11**, 1585–1590.

Rico C, Bouteillon P, Van Oppen MJH, Knight ME, Hewitt GM, Turner GF (2003) No evidence for parallel sympatric speciation in cichlid species of the genus *Pseudotropheus* from North-Western Lake Malawi. *Journal of Evolutionary Biology*, **16**, 37–46.

Ruber L, Meyer A, Sturmbauer C, Verheyen E (2001) Population structure in two sympatric species of the Lake Tanganyika cichlid tribe Eretmodini: evidence for introgression. *Molecular Ecology*, **10**, 1207–1225.

Salzburger W, Baric S, Sturmbauer C (2002) Speciation via introgressive hybridization in African Cichlids? *Molecular Ecology*, **11**, 619–625.

Schilthuizen M, Hoekstra RF, Gittenberger E (2001) The 'rare allele phenomenon' in a ribosomal spacer. *Molecular Ecology*, **10**, 1341–1345.

Seehausen O (1999) Explosive speciation rates and unusual species richness in Haplochromine cichlids — effects of sexual selection. In: *Ancient Lakes: Biodiversity, Evolution and Ecology* (eds Rossiter A, Kwanabe H.), pp. 325–371. Academic Press, New York.

Seehausen O, van Alphen JJM, Witte F (1997) Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science*, **277**, 1808–1811.

Seehausen O, Witte F, van Alphen JJM, Bouton N (1998) Direct mate choice maintains diversity among sympatric Cichlids in Lake Victoria. *Journal of Fish Biology*, **53**, 37–55.

Smith PF, Kornfield I (2002) Phylogeography of Lake Malawi cichlids of the genus *Pseudotropheus*: significance of allopatric colour variation. *Proceedings of the Royal Society of London B*, **269**, 2495–2502.

Stauffer JR Jr, Hert E (1992) *Pseudotropheus callainos*, a new species of mbuna (Cichlidae), with analyses of changes associated with

two intra-lacustrine transplantations in Lake Malawi. *Ichthyo-logical Explorations of Freshwaters*, **3**, 253–264.

Stauffer JR, Bowers NJ, Kocher TD, McKaye KR (1996) Evidence of hybridization between *Cyanotilapia afra* and *Psuedotropheus Zebra* (Teleostei: Cichlidae) following an intralacustrine trans-location in Lake Malawi. *Copiea*, 203–208.

Stauffer JRJ, Bowers NJ, Kellogg KA, McKaye KR (1997) A revision of the blue-black *Pseudotropheus zebra* (Teleostei: Cichlidae) complex from Lake Malawi, Africa, with a description of a new genus and ten new species. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **148**, 189–230.

Sturmbauer C (1998) Explosive speciation in Cichlid fishes of the African Great Lakes: a dynamic model of adaptive radiation. *Journal of Fish Biology*, **53**, 18–36.

Sturmbauer C, Baric S, Salzburger W, Ruber L, Verheyen E (2001) Lake level fluctuations synchronize genetic divergences of cichlid fishes in African lakes. *Molecular Biology and Evolution*, **18**, 144–154.

Van Oppen MJH, Turner GF, Rico G, Deutsch JC, Ibrahim KM, Robinson RL, Hewitt GM (1997a) Unusually fine-scale genetic structuring found in rapidly speciating Malawi cichlid fishes. *Proceedings of the Royal Society of London B*, **264**, 1803–1812.

Van Oppen MJH, Rico C, Deutsch JC, Turner GF, Hewitt GM (1997b) Isolation and characterization of microsatellite loci in the cichlid fish *Pseudotropheus zebra*. *Molecular Ecology*, **6**, 387–388.

Van Oppen MJH, Turner GF, Rico C, Robinson RL, Deutsch JC, Genner MJ, Hewitt GM (1998) Assortative mating among rock-dwelling cichlid fishes supports high estimates of species richness from Lake Malawi. *Molecular Ecology*, **7**, 991–1001.

Van Oppen MJH, Rico C, Turner GF, Hewitt GM (2000) Extensive homoplasy, non-stepwise mutations and shared ancestral poly-morphism at a complex microsatellite locus in Lake Malawi Cichlids. *Molecular Biology and Evolution*, **17**, 489–498.

Verschuren D, Johnson TC, Kling HJ, Edgington DN, Leavitt PR, Brown ET, Talbot MR, Hecky RE (2002) History and timing of human impact on Lake Victoria, East Africa. *Proceedings of the Royal Society of London B*, **269**, 289–294.