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COLOR ASSORTATIVE MATING CONTRIBUTES TO SYMPATRIC DIVERGENCE OF NEOTROPICAL CICHLID FISH

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It is still debated vigorously whether sexual selection can result in speciation without physical barriers to gene flow. In this study, we used field data and molecular methods to investigate the gold–normal color polymorphism in two endemic cichlid fish species of crater lake Xiloá, Nicaragua. We found significant assortative mating by color in both *Amphilophus xiloaensis* and *A. sagittae*. Focusing on *A. xiloaensis*, microsatellite allele frequencies, an assignment test, and model-based cluster analysis demonstrates significant and clear genetic differentiation ($F_{ST} = 0.03$) between gold and normal individuals in sympatry. In addition, we find genetic differentiation between all three sympatric and ecologically distinct Midas cichlid species of Lake Xiloá, *A. amarillo*, *A. sagittae*, and *A. xiloaensis* ($F_{ST} = 0.03 - 0.19$), and clear genetic isolation of these species from their closest relative (*A. citrinellus*) in the neighboring great lake Managua. The *A. xiloaensis* gold morph is genetically more distinct from the lake's other two Midas cichlid species than is *A. xiloaensis*-normal. Thus, we have identified sexual isolation based on color that is evident in population genetics and mate choice. Our results suggest that sexual selection through color assortative mating may play an important role in incipient sympatric speciation in Midas cichlids of Nicaragua.

KEY WORDS: Color polymorphism, crater lake, Midas cichlid, sexual isolation, sexual selection, species complex.

During the four decades since John Maynard Smith's influential paper on sympatric speciation (Maynard Smith 1966), numerous additional theoretical insights on this subject have been gained (e.g., Dieckmann and Doebeli 1999; Higashi et al. 1999), however, empirical evidence for speciation in the face of continuous potential for gene flow remains scant (Bolnick and Fitzpatrick 2007). Theory suggests, however, that rapid diversification could occur if a phenotypic trait simultaneously affected both assortative mating and natural selection rather than ecological traits alone (Gavrilets 2004). It has been argued that isolated habitats such as islands and crater lakes are the most promising locations to identify diversification in sympatry (Coyne 2007). In corroboration, some of the most compelling cases of speciation without geographically restricted gene flow have occurred in crater lake cichlid fish driven by disruptive natural selection for ecological traits (Schliewen et al. 1994; Barluenga et al. 2006; Gavrilets et al. 2007).

Disruptive selection based on color polymorphism might contribute to the diversification of cichlids of African great lakes (e.g., Allender et al. 2003; Seehausen et al. 2008; see Gray and McKinnon 2007 for review). Furthermore, it has been postulated (McKaye 1980; Wilson et al. 2000), but so far not convincingly demonstrated, that selection for color acts under sympatric conditions for polymorphic species of crater lake cichlids. Assortative mating, fluctuations in selection regimes, and frequency-dependent selection by predation or intrasexual competition have all been found to contribute to the persistence of color morphs within a population (Roulin 2004; Bond 2007; Gray and McKinnon 2007; Puebla et al. 2007). It has also been suggested

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that within-population sexual selection can generate negative frequency dependence, initiating reproductive isolation, and hence allowing color morphs to speciate even in sympatry (Bolnick and Fitzpatrick 2007; Gray and McKinnon 2007).

Midas cichlids are so named because of a vibrant gold morph that is present in both sexes and several species of this species complex in the two great lakes (lakes Managua and Nicaragua) and some crater lakes of Nicaragua. However, most individuals have a cryptic gray–black coloration ("normal" morph). All fish begin life with the normal coloration and then some lose their melanophores and turn bright gold irrevocably (Barlow 1983; Dickman et al. 1988). Being gold is almost certainly controlled by a single dominant locus (Barlow 1983) (and demonstrated by 3G:1N F2 broods raised in our laboratory, S. Fukamachi and A. Meyer, unpubl. ms.). Midas cichlids are nonterritorial most of the year and school in roving groups until the beginning of the breeding season when they form monogamous pairs, acquire, and defend a territory (McKaye and Barlow 1976). Laboratory and field studies on the species complex in general indicate that they usually, but not completely, mate assortatively based on color (Barlow et al. 1977; McKaye 1980; Barlow 1992). The color polymorphism is thought to be maintained by an aggressive competitive advantage of golds (which requires rarity relative to normal) and color assortative mating, but might come at an increased cost in terms of natural selection (Barlow 1983).

The gold–normal color polymorphism is found in Nicaragua's great lakes and is therefore most likely ancestral to all the crater lake populations in the *A. citrinellus* complex. However, color has not yet been convincingly shown to be associated with intraspecific genetic divergence. Two previous studies on Midas cichlids suggest a degree of genetic differentiation by color morph in Lake Apoyo (Wilson et al. 2000) and some, but not other, great lake localities (Barluenga and Meyer 2004). However, given the recent revisions to the species complex with the description of several new species (Stauffer and McKaye 2002; Stauffer et al. 2008) and the restricted ability of mtDNA to reliably discern species or lake of origin within the complex (Barluenga and Meyer 2004; Barluenga et al. 2006), these conclusions need to be readdressed with more sensitive genetic markers and in light of the new taxonomy of this species complex.

We studied the color assortative mate choice of two ecologically differentiated, endemic, sympatric sister species of Midas cichlid fish in crater lake Xiloá: *Amphilophus sagittae* is an elongated limnetic form and *A. xiloaensis* is a deeper bodied benthic species that breeds in deeper water than *A. sagittae* (McKaye et al. 2002; Stauffer and McKaye 2002). Both species have gold and normal morphs and are found only in Lake Xiloá, Nicaragua (Fig. 1), a very young (<6000 years old [Kutterolf et al. 2007]), small (approximately 3.7 km^2) and deep (96 m) volcanic crater lake (INETER 2008) that is not connected to any other water systems and does not contain any identifiable intralake barriers. Additionally, we use population genetic approaches to infer the level of differentiation between all three sympatric species of Midas cichlid in Lake Xiloa (*A. amarillo*, *A. sagittae*, and *A. xiloaensis*) and color morphs of *A. xiloaensis*. Both our lines of inquiry suggest incipient sympatric divergence within species based on color and clear differentiation between the established species.

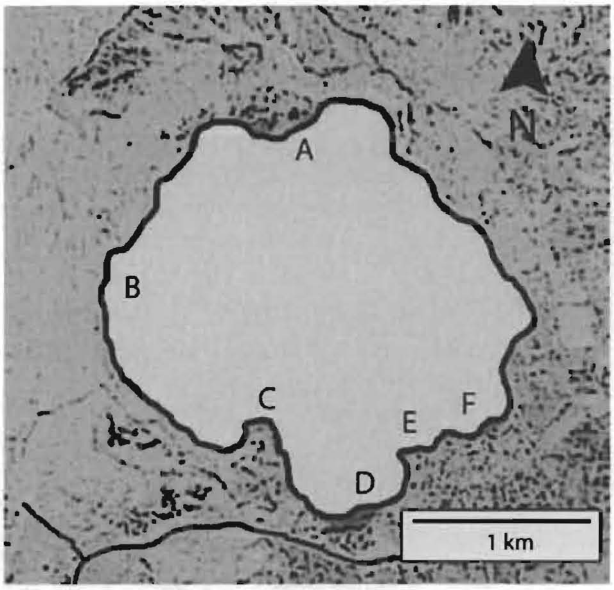

Figure 1. Lake Xiloá is an extinct volcano crater lake in western Nicaragua. The north and northwest side of the lake is made up of steep crater slope and to the east lies the great Lake Managua. Sample collections were made at all sites. Transect observations were conducted at all sites except site D.

Methods

FIELD STUDY

Field investigations were conducted between December 2007 and early February 2008 in Lake Xiloá, Nicaragua (12°13′N, 86°19′W). The study period coincided with the peak breeding season of both *A. sagittae* and *A. xiloaensis*. A SCUBA diver (TKL) swam along 2-m interval depth contours (ranging 4–16 m) and recorded breeding pairs of both species within 1.5 m of him (1 m at vertical rocky cliffs). Depth reading for each breeding territory was done with a "Suunto Air Lux" diving computer. A similar counting effort at each depth was ensured by using a 50 m reference line or the combination of swimming time, the number of fin kicks, and bottom typography. In total, a stretch of approximately 200 m at each depth was assessed at sites B, C, E, and F (Fig. 1) and 100 m at site A, covering approximately 18,000 m^2 (with total transect length of 6.3 km). Taxon identification was based on color patterns, head morphology (shape), and body height, in that order (as per McKaye et al. 2002; Stauffer and McKaye 2002). The gold morph is extreme rare or absent in *A. amarillo* (Stauffer and McKaye 2002; TKL pers. obs.), so this species was included only for population genetic analyses.

We applied JMATING, a program that calculates several different measures of sexual isolation and deviation from random mating (Rolán-Alvarez and Caballero 2000; Carvajal-Rodriguez and Rolan-Alvarez 2006). The input data included the observed frequencies of the four possible mating types by color (N♂–N♀, N♂–G♀, G♂–N♀, and G♂–G♀). In addition, we tested for differences in depth of breeding territories between pairs in which the male and female belonged to the same color morph (N♂–N♀ and G♂–G♀), and mixed pairs (N♂–G♀ and G♂–N♀) with a Kruskal–Wallis test with Scheirer–Ray–Hare extension having "species" (*A. sagittae* or *A. xiloaensis*) and "pair type" (single colored pair or mixed) as the explanatory variables.

GENETIC ANALYSES

Adult fish were caught by harpooning or gill netting from six localities around Lake Xiloá (Fig. 1) (sample sizes: 30 *A. xiloaensis*–normal, 31 *A. xiloaensis*-gold, 38 *A. sagittae*-normal, 20 *A. amarillo*). As it is not possible to sample fish nondestructively, the fish we analyzed for genetics are not the same individuals that were observed in the noninvasive field study. Gold *A. sagittae* were very rare (30 fish in 446 observed *A. sagittae* and 18,000 m^2 of observation) and were not collected for ethical and practical reasons. *Amphilophus sagittae* are more common in the mostly shallower and sandier habitat around the southern half of the lake whereas *A. xiloaensis* are found more reliably at the north (see Table S2 for details). DNA was extracted from muscle tissue using standard high-salt extraction methods. DNA was amplified for 12 fluorescently labeled microsatellite loci by standard PCR: M1M(=Acit1), M2(=Acit2), M7(=Acit3), M12(=Acit4) (Noack et al. 2000), UNH002 (Kellogg et al. 1995), UNH012, UNH013 (McKaye et al. 2002); Abur28, Abur45, Abur82, Abur151 (Sanetra et al. 2009), Burtkit F 474/R672 (Salzburger et al. 2007). Fragments were analyzed on an ABI3130XL (Applied Biosystems) and sized according to Rox 500 internal standard in GeneMapper version 4.0 (Applied Biosystems, Darmstadt, Germany).

Partially Bayesian assignment of individuals by genotype to populations (i.e., color) was computed in Geneclass2 (Piry et al. 2004) using the Rannala and Mountain (1997) criterion and calculating the associated probability by Monte Carlo resampling (Paetkau et al. 2004) of 10,000 individuals ($\alpha = 0.01$, assignment threshold 0.05). Individual-level differentiation between all Midas cichlid species and possible introgression from the necessary source population, great lake Managua, was assessed with a slightly abridged dataset (all loci except Abur28 and Abur45) in Structure version 2.2 (Pritchard et al. 2000; Falush et al. 2003). We applied an admixture model to the Xiloá species and a population prior on *A. citrinellus* from Managua (gold and normal, $n = 47$), with correlated allele frequencies (Falush et al. 2003) and 200,000 generations of analysis after 50,000 generations of burn-in for five clusters. Individual membership coefficients were visualized in Distruct version 1.1 (Rosenberg 2004). F_{ST} for microsatellites (Michalakis and Excoffier 1996; ϕ_{ST} equivalent to Weir and Cockerham's [1984] θ,) was calculated in GenoDive version 2.0b11 (Meirmans and Van Tienderen 2004) using the reduced dataset and significance assessed with 999 permutations.

Results

All encountered pairs were between two members of the same species. *Amphilophus sagittae* was breeding in shallower water than *A. xiloaensis* (Kruskal–Wallis test with Scheirer–Ray–Hare extension, species effect, $H = 26.1$, df $= 1$, $P < 0.001$) (Table 1). An assignment test in Structure and analyses of genetic differentiation indicate that *A. amarillo*, *A. sagittae*, and *A. xiloaensis* in

Table 1. Assortativeness of mating by color and depth distributions of the different pair types in both species of Midas cichlid studied in Lake Xiloá. The overall observed frequency of each possible pairing by color can be compared to the expected frequency of the pair type within the species (as inferred from the overall proportion of each color morph in the study population). Territory depths are given as mean±standard error.

Species		Pair type				
		N♂ N♀	N♂ G♀	G♂ N♀	G♂ G♀	Combined
A. sagittae pair types	Total observed	202	3	9	9	223
	Freq. observed	91%	1.4%	4.0%	4.0%	100%
	Freq. expected	87%	6.3%	6.3%	0.45%	100%
A. xiloaensis pair types	Total observed	94	11	20	11	136
	Freq. observed	69%	8.1%	15%	8.1%	100%
	Freq. expected	65%	16%	16%	3.8%	100%
A. sagittae territory depth		9.3±0.2	9.8±0.9	11.8±0.6	9.6±0.7	9.4±0.2
A. xiloaensis territory depth		12.3±0.3	13.7±0.6	13.7±0.5	12.9±1.0	12.7±0.3

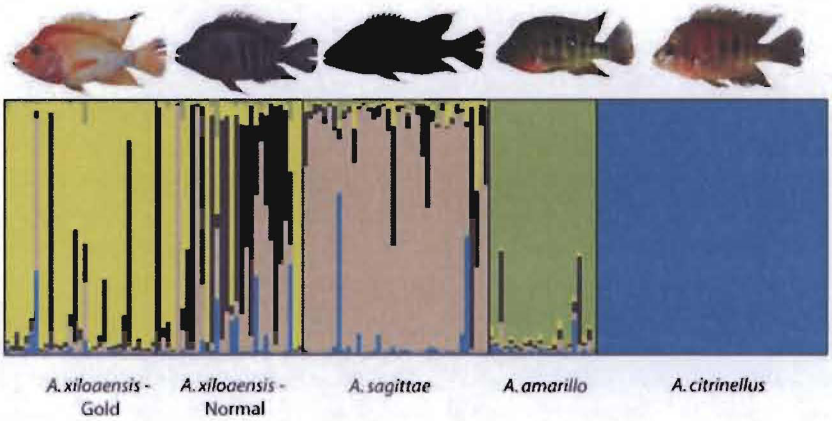

Figure 2. Model-based cluster analyses (with Structure version 2.2) distinguish the genetic differentiation of Lake Managua *A. citrinellus* from the gold and normal *A. xiloaensis* and the sister taxa *A. sagittae*, and *A. amarillo* in Lake Xiloá. *Amphilophus xiloaensis*-gold is shown in yellow, *A. xiloaensis*-normal in gray, *A. sagittae* in beige, *A. amarillo* in green, and the ancestral population *A. citrinellus* from Lake Managua in blue, along with photograph exemplars of each species/morph.

crater lake Xiloá are clearly genetically distinct and sister taxa relative to *A. citrinellus* from the neighboring great lake Managua (Fig. 2, Table 2, Table S2). *Amphilophus amarillo* is the most genetically distinct of the species within Lake Xiloá. Differentiation between gold *A. xiloaensis* and the other species is greater than the differentiation between normal *A. xiloaensis* and the other species (Table 2). Especially, there is more allele sharing between the ecological species *A. xiloaensis*-normal and *A. sagittae* than between any other species (Fig. 2). This may represent occasional hybridization between species, although no such pairs were observed, or be retained ancestral polymorphism because of the young age of the species.

Mating was color assortative in both species, suggesting significant sexual isolation (Table 3). The two species differed in the proportion of gold individuals in the breeding population (*A. sagittae*: 6.7%, *A. xiloaensis*: 19.5%, G^2 test of independence, $G^2 = 26$, df = 1, $P < 0.001$). When the two species are considered together, gold♂–normal♀ matings were more common than normal♂–gold♀ matings (binomial distribution, $n = 43$, $P = 0.032$; *A. sagittae*: $n = 12$, $P = 0.15$; *A. xiloaensis*, $n = 31$, $P = 0.15$) (Table 1). In contrast to the ecologically different species, *A. sagittae* and *A. xiloaensis*, which breed at different depths, gold–gold and normal–normal pairs within both species breed at the same depth (Table 1). Interestingly, mixed pairs breed deeper than pure pairs (Kruskal–Wallis test with Scheirer–Ray–Hare extension, pair type effect, $H = 9.16$, df = 1, $P = 0.003$), independent of the species (species × pair type interaction, $H = 0.31$, df = 1, $P = 0.63$) (Table 1).

We found a pronounced difference in allele frequencies between gold and normal *A. xiloaensis* ($F_{ST} = 0.03$, $P = 0.001$). When only individuals from the same sample locality within the lake are compared (site A, $n = 28$ gold, $n = 30$ normal) there is still significant differentiation between colors ($F_{ST} = 0.03$, $P = 0.001$). Therefore, the differentiation found between colors is not an effect of sample locality. Individuals can be successfully assigned to either gold or normal based on microsatellite allele frequencies (Fig. 3). Cluster analysis as implemented in Structure clearly discerns gold *A. xiloaensis* from Lake Managua *A. citrinellus* (Fig. 2), suggesting that ongoing introgression of a "gold allele" from Managua *A. citrinellus* is not responsible for the gold *A. xiloaensis*. Our estimates of genetic differentiation between color morphs are probably conservative given that some young, phenotypically normal fish might be genetically destined to turn gold later.

Table 2. Population differentiation (F_{ST} for microsatellites, ϕ_{ST} equivalent to Weir and Cockerham's θ) between each species and color morph in crater lake Xiloá and neighboring Lake Managua. **represents P<0.001.

	A. xiloaensis-gold	*A. xiloaensis*-normal	*A. sagittae*	*A. amarillo*
A. xiloaensis-gold	—			
A. xiloaensis-normal	0.03**	—		
A. sagittae	0.08**	0.03**	—	
A. amarillo	0.19**	0.16**	0.14**	—
A. citrinellus	0.14**	0.13**	0.12**	0.11**

Table 3. Different indexes of sexual isolation between golds and normals (pair sexual isolation, Levine YA and Yule V) and *G*-test for randomness of mating (Rolán-Alvarez and Caballero 2000; Carvajal-Rodriguez and Rolán-Alvarez 2006). These measures are based on distributions of the different pair types by color, presented in Table 1. SD refers to standard deviation and df to degrees of freedom.

Species	Measure	Value	SD	Test value	df	*P*
A. sagittae	Ipsi	0.86	0.03	t=24.8	222	<0.001
	YA	0.78	0.07	t=10.8	222	<0.001
	V	0.59	0.11	t=5.48	222	<0.001
	G-test			G=37.2	3	<0.001
A. xiloaensis	Ipsi	0.39	0.08	t=5.00	135	<0.001
	YA	0.37	0.11	t=3.57	135	<0.001
	V	0.28	0.10	t=2.89	135	0.004
	G-test			G=9.62	3	0.022

Discussion

The results of this study demonstrate that at least two of the three endemic crater lake Xiloá Midas cichlid species—in parallel across ecologically differentiated species—mate assortatively based on color. In *A. xiloaensis* this is contributing to significant genetic differentiation between the two color morphs in sympatry and we suspect that that is the case also for *A. sagittae*, for which we only assessed behavioral sexual isolation based on color. Incipient speciation by sexual selection and variation in breeding depth was proposed from field behavioral observation of the Midas cichlid complex in Lake Xiloá (then only a single species, *Cichlasoma citrinellum*, was taxonomically recognized for Lake Xiloá) by McKaye (1980). Our study is the first to assess color assortative mate choice behavior using the new multispecies taxonomy and to identify that sexual isolation is contributing to significant sympatric population differentiation based on color. The results suggest the cichlid color morphs should not be considered to be a single polymorphic species, but rather that the color morphs are evolutionarily distinct incipient species, the divergence of which is promoted by sexual selection.

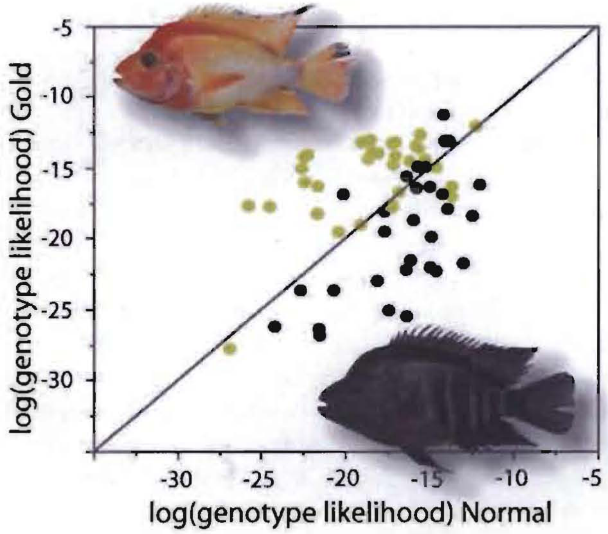

Figure 3. *Amphilophus xiloaensis* genotypes can be highly successfully assigned to either the gold or normal phenotype based on genotype (80.3% correctly assigned). This plot shows the likelihood that a particular individual originates from a given color morph sample. Gold individuals are shown with yellow dots, normal individuals with black dots.

Speciation is a continuum of differentiation and we do not argue that gold and normal have completed this process. However, the "biological species" concept defines species as groups of interbreeding populations that are physically or behaviorally reproductively isolated from other such groups (Mayr 1942). Our inferences from mate choice and population genetics demonstrate that the gold and normal color morphs of *A. sagittae* and *A. xiloaensis* are not freely interbreeding but are significantly, although still incompletely, reproductively isolated from their "conspecifics." That such a differentiation is evolutionarily sympatric can be assumed when there is contemporary sympatry, substantial reproductive isolation, diverging entities that are sister taxa, and allopatric possibilities are unlikely (Coyne and Orr 2004). Gold and normal morphs are found sympatrically in Lake Xiloá with extensively overlapping breeding areas (Fig. 1, Table 1, Table S2) rendering an allopatric origin scenario of the divergence of gold and normal species in parallel within the very small crater lake Xiloá highly unlikely. Midas cichlids in neighboring Lake Managua are both normal and gold, but the recent introgression of gold genotypes from Lake Managua is not responsible for the differentiation between colors of *A. xiloaensis* (Fig. 3), as there is not more allele sharing between *A. citrinellus* and *A. xiloaensis* gold than there is *A. citrinellus* and any other Xiloá species, and the populations are genetically quite distinct. Consequently, all lines of evidence suggest that this diversification occurred under entirely sympatric conditions.

We have shown that the sympatric endemic Midas cichlids in Lake Xiloá are significantly genetically differentiated from each other (Figs. 2, 3; Table 2). *Amphilophus amarillo* is the most differentiated, both in population comparisons and at the level of interspecific allele sharing within individuals. The limnetic morph, *A. sagittae*, is also quite well differentiated from its sister species. The normal color morph of *A. xiloaensis* shares more alleles with its sister taxa than any other species or morph of Midas cichlid in Lake Xiloá, representing either a more recent shared ancestry with *A. sagittae* or ongoing hybridization. Although all species of Midas cichlid will hybridize in the lab (A. Meyer, unpubl. ms.), in the 223 pairs of *A. sagittae* and 136 pairs of

A. xiloaensis observed in the field we found no interspecies hybrid pairs in Xiloá.

Our field data suggest that matings between normal and gold individuals were not overly rare, yet the population has evolved beyond a simple color polymorphism with assortative mating (sensu Gray and McKinnon 2007), as suggested by the significant genetic divergence among color morphs. This significant genetic divergence despite the observed frequency of mixed pairs implies there may be other sources of selection that diminish the reproductive success of the mixed pairs. As suggested by Barlow (1983, 1992), color may affect behavioral interactions between the fish, making mixed pairs less functional and therefore less successful. In line with this hypothesis, we found that in both species mixed pairs breed in deeper water than pure pairs, possibly as a consequence of being forced to use breeding sites that are suboptimal (see also McKaye and Barlow 1976). Hence, we did not find support for previous findings that used old taxonomy (e.g., McKaye 1980), which suggested that pure gold pairs could be breeding in deeper water than pure normals. Midas cichlids are plagued with a very low reproductive success due to intense predation and breeding site competition (McKaye and Barlow 1976; McKaye 1977; Barlow 1992; Vivas and McKaye 2001). Thus, any pair at a slight extra disadvantage (such as poor partner coordination or occupying an inferior breeding site) is even more likely to lose their brood and therefore not contribute successfully to future generations. Second, the rate of matings between genetic normals and golds could be considerably lower than suggested by the field counts of phenotypes if some golds breed with a gold partner before their phenotype has transformed to match their genotype (see Barlow 1998, who proposed this phenomenon alone would be sufficient to prevent gold–normal speciation). That normal♂–gold♀ pairings are significantly rarer than gold♂–normal♀ could be a result of both the above-mentioned mechanisms; females are smaller at their first spawning than males and consequently a greater proportion of young, phenotypically normal females may be genetically gold. Third, there may be natural or sexual selection mechanisms against hybrid progeny even if they have survived past the juvenile stage. We did not find any evidence that ecological factors would play a large role in differentiation of golds and normals but suggest that more research is needed (e.g., possibility gold fish are exposed to a stronger predation pressure due to their greater conspicuousness). Hence, it seems likely that several interacting factors may be involved in the accumulation of significant genetic differentiation between the golds and normals.

The gold–normal color polymorphism is also known to exist, although much more rarely, in some other large neotropical cichlid species (e.g., *Parachromis dovii*, *P. managuensis*, *Hypsophrys nicaraguensis*, and *Petenia splendida*), and to be sex-linked in some African species (e.g., McKaye and Stauffer 1986). In no other species, however, is a gold morph found so pervasively as in the Midas cichlid complex (Webber et al. 1973). This pattern has led to the assumption that the gold–normal polymorphism is frequency-dependent, favoring golds when they are rare (Barlow 1983). However, a frequency-dependent selection regime is not mutually exclusive with speciation, but might actually contribute to it indirectly by favoring persistence of the morphs during the diversification progress (Gray and McKinnon 2007). Furthermore, although currently no pure gold species is described in the Midas cichlid species complex, this does not exclude the possibility that one is, or will be, evolving. The level of genetic differentiation we have reported between normal and gold *A. xiloaensis* is as strong or stronger than what we have found with the same genetic markers between some ecologically and morphologically described species of this extremely young assemblage of species (e.g., great lakes *A. labiatus* vs. *A. citrinellus* [Klingenberg et al. 2003; Barluenga and Meyer 2004]) and on par with the differentiation between *A. xiloaensis*-normal and *A. sagittae*-normal within Lake Xiloá. It is possible, if not likely, that differentiation between gold and normal individuals is at varying stages in different members of this species complex: previous results have already shown that in *A. citrinellus* and *A. labiatus* from the Nicaraguan great lakes, gold–normal genetic differentiation, based on the same microsatellite loci as used in this study, is low (e.g., $F_{ST} \leq 0.01$) or absent (Barluenga and Meyer 2004). Future research will show whether Lake Xiloá will continue to represent the most significant degree of genetic divergence between color morphs. Indeed, the case of normal and gold Midas cichlids provides evidence for how disruptive sexual selection can be an important contributor to the speciation process in different ecological and geographic settings, including sympatry.

Whether complete reproductive isolation will develop between color morphs of cichlids in general, or the Midas cichlid species complex in particular, can only be speculated. However, when mate choice is influenced by a single locus and is under differential selection recombination cannot break down this unit and rapid divergence may occur even in sympatry (Maynard Smith 1966; Gavrilets 2004). We suggest that the simple trait of gold or normal may drive such rapid divergence. Intriguingly, despite incomplete assortative mating by color, normal and gold Midas cichlids of Lake Xiloá are genetically diverging and clearly represent sympatric incipient species. Therefore, sympatric divergence in the Midas cichlid species complex can be driven by both ecological speciation (Barluenga et al. 2006) as well as sexual selection. Future research will focus on the relative strength and speed by which these two forces might lead to the formation of species in this species complex in the crater lakes of Nicaragua.

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LITERATURE CITED

Allender, C. J., O. Seehausen, M. E. Knight, G. F. Turner, and N. Maclean. 2003. Divergent selection during speciation of Lake Malawi cichlid fishes inferred from parallel radiations in nuptial coloration. Proc. Natl. Acad. Sci. U.S.A. 100:14074–14079.

Barlow, G. W. 1983. The benefits of being gold: behavioral consequences of polychromatism in the midas cichlid, *Cichlasoma citrinellum*. Environ. Biol. Fish. 8:235–247.

———. 1992. Is mating different in monogamous species? The Midas cichlid fish as a case study. Am. Zool. 32:91–99.

———. 1998. Sexual-selection models for exaggerated traits are useful but constraining. Am. Zool. 38:59–69.

Barlow, G. W., W. Rogers, and R. V. Cappeto. 1977. Incompatibility and assortative mating in the Midas cichlid. Behav. Ecol. Sociobiol. 2:49–59.

Barluenga, M., and A. Meyer. 2004. The Midas cichlid species complex: incipient sympatric speciation in Nicaraguan cichlid fishes? Mol. Ecol. 13:2061–2076.

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

Bolnick, D. I., and B. M. Fitzpatrick. 2007. Sympatric speciation: models and empirical evidence. Ann. Rev. Ecol. Evol. Syst. 38:459–487.

Bond, A. B. 2007. The Evolution of color polymorphism: crypticity, searching images, and apostatic selection. Annu. Rev. Ecol. Evol. Syst. 38:489–514.

Carvajal-Rodriguez, A., and E. Rolan-Alvarez. 2006. JMATING: a software for the analysis of sexual selection and sexual isolation effects from mating frequency data. BMC Evol. Biol. 6:40.

Coyne, J. A. 2007. Sympatric speciation. Curr. Biol. 17:R787–R788.

Coyne, J. A., and H. A. Orr. 2004. Speciation. Sinauer Associates, Sunderland, MA.

Dickman, M. C., M. Schliwa, and G. W. Barlow. 1988. Melanophore death and disappearance produces color metamorphosis in the polychromatic Midas cichlid (*Cichlasoma citrinellum*). Cell Tissue Res. 253:9–14.

Dieckmann, U., and M. Doebeli. 1999. On the origin of species by sympatric speciation. Nature 400:354–357.

Falush, D., M. Stephens, and J. K. Pritchard. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164:1567–1587.

Gavrilets, S. 2004. Fitness landscapes and the origin of species. Princeton Univ. Press, Princeton, NJ.

Gavrilets, S., A. Vose, M. Barluenga, W. Salzburger, and A. Meyer. 2007. Case studies and mathematical models of ecological speciation. 1. Cichlids in a crater lake. Mol. Ecol. 16:2893–2909.

Gray, S. M., and J. S. McKinnon. 2007. Linking color polymorphism maintenance and speciation. Trends Ecol. Evol. 22:71–79.

Higashi, M., G. Takimoto, and N. Yamamura. 1999. Sympatric speciation by sexual selection. Nature 402:523–526.

INETER. 2008. Instituto Nicaragüense de Estudios Territoriales: Laguna de Xiloa [online]. Available at http://www.ineter.gob.ni/Direcciones/Recursos Hidricos/HIDROGRAFIA WEB/Lagunas. Accessed November 10, 2008.

Kellogg, K. A., J. A. Markert, J. R. Stauffer, Jr., and T. D. Kocher. 1995. Microsatellite variation demonstrates multiple paternity in lekking cichlid fishes from Lake Malawi, Africa. Proc. R. Soc. Lond. B 260:79–84.

Klingenberg, C. P., M. Barluenga, and A. Meyer. 2003. Body shape variation in cichlid fishes of the *Amphilophus citrinellus* species complex. Biol. J. Linn. Soc. 80:397–408.

Kutterolf, K., A. Freundt, W. Pérez, H. Wehrmann, and H.-U. Schmincke. 2007. Late Pleistocene to Holocene temporal succession and magnitudes of highly explosive volcanic eruptions in west-central Nicaragua. J. Volcanol. Geotherm. Res. 163:55–82.

Maynard Smith, J. 1966. Sympatric speciation. Am. Nat. 100:637–650.

Mayr, E. 1942. Systematics and the origin of species. Columbia Univ. Press, New York, NY.

McKaye, K. R. 1977. Competition for breeding sites between the cichlid fishes of Lake Jiloa, Nicaragua. Ecology 58:291–302.

———. 1980. Seasonality in habitat selection by the gold color morph of *Cichlasoma citrinellum* and its relevance to sympatric speciation in the family Cichlidae. Environ. Biol. Fish. 5:75–78.

McKaye, K. R., and G. W. Barlow. 1976. Competition between color morphs of the Midas cichlid, *Cichlasoma citrinellum*, in Lake Jiloá, Nicaragua. Pp. 467–475 *in* T. B. Thorson, ed. Investigations of the ichthyofauna of Nicaraguan Lakes. University of Nebraska School of Life Sciences, Lincoln, NE.

McKaye, K. R., and J. R. Stauffer Jr. 1986. Description of a gold cichlid (Teleostei: Cichlidae) from Lake Malawi, Africa. Copeia 1986:870–875.

McKaye, K. R., J. R. Stauffer Jr., E. P. Van Den Berghe, R. Vivas, L. J. L. Perez, J. K. McCrary, R. Waid, A. Konings, W.-J. Lee, and T. D. Kocher. 2002. Behavioral, morphological and genetic evidence of divergence of the Midas cichlid species complex in two Nicaraguan crater lakes. Cuadernos de Investigación de la UCA 12, Dirección de Investigación de la Universidad Centroamericana, Managua, Nicaragua, pp. 19–47.

Meirmans, P. G., and P. H. Van Tienderen. 2004. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. Mol. Ecol. Notes 4:792–794.

Michalakis, Y., and L. Excoffier. 1996. A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. Genetics 142:1061–1064.

Noack, K., A. B. Wilson, and A. Meyer. 2000. Broad taxonomic applicability of microsatellites developed for the highly polymorphic neotropical cichlid, *Amphilophus citrinellum*. Anim. Genet. 31:151–152.

Paetkau, D., R. Slade, M. Burden, and A. Estoup. 2004. Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. Mol. Ecol. 13:55–65.

Piry, S., A. Alapetite, J.-M. Cornuet, D. Paetkau, L. Baudouin, and A. Estoup. 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. J. Hered. 95:536–539.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Puebla, O., E. Bermingham, F. Guichard, and E. Whiteman. 2007. Colour pattern as a single trait driving speciation in *Hypoplectrus* coral reef fishes? Proc. R. Soc. Lond. B 274:1265–1271.

Rannala, B., and J. L. Mountain. 1997. Detecting immigration by using multilocus genotypes. Proc. Natl. Acad. Sci. U.S.A. 94:9197–9201.

Rolán-Alvarez, E., and A. Caballero. 2000. Estimating sexual selection and sexual isolation effects from mating frequencies. Evolution 54:30–36.

Rosenberg, N. A. 2004. DISTRUCT: a program for the graphical display of population structure. Mol. Ecol. Notes 4:137–138.

Roulin, A. 2004. The evolution, maintenance and adaptive function of genetic colour polymorphism in birds. Biol. Rev. 79:815–848.

Salzburger, W., I. Braasch, and A. Meyer. 2007. Adaptive sequence evolution in a color gene involved in the formation of the characteristic egg-dummies of male haplochromine cichlid fishes. BMC Biol. 5:51.

Sanetra, M., F. Henning, S. Fukamachi, A. Meyer. 2009. A microsatellite-based genetic linkage map of the cichlid fish, *Astatotilapia burtoni* (Teleostei): A comparison of genetic architectures among rapidly speciating cichlids. Genetics 182:387–397.

Schliewen, U. K., D. Tautz, and S. Paabo. 1994. Sympatric speciation suggested by monophyly of crater lake cichlids. Nature 368:629–632.

Seehausen, O., Y. Terai, I. S. Magalhaes, K. L. Carleton, H. D. J. Mrosso, R. Miyagi, I. Van Der Sluijs, M. V. Schneider, M. E. Maan, H. Tachida, *et al.* 2008. Speciation through sensory drive in cichlid fish. Nature 455:620–627.

Stauffer, J. R., Jr., and K. R. McKaye. 2002. Descriptions of three new species of cichlid fishes (Teleostei: Cichlidae) from Lake Xiloá, Nicaragua. Cuadernos de Investigación de la U.C.A 12, Dirección de Investigación de la Universidad Centroamericana, Managua, Nicaragua, pp. 1–18.

Stauffer, J. R., Jr., J. K. McCrary, and K. E. Black. 2008. Three new species of cichlid fish (Teleostei: Cichlidae) in Lake Apoyo, Nicaragua. Proc. Biol. Soc. Wash. 121:117–129.

Vivas, R., and K. R. McKaye. 2001. Habitat selection, feeding ecology, and fry survivorship in the *Amphilophus citrinellus* species complex in Lake Xiloá. J. Aquacult. Aq. Sci. IX:32–48.

Webber, R., G. W. Barlow, and A. H. Brush. 1973. Pigments of a color polymorphism in a cichlid fish. Comp. Biochem. Physiol. 44B:1127–1135.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of populations structure. Evolution 38:1358–1370.

Wilson, A. B., K. Noack-Kunnmann, and A. Meyer. 2000. Incipient speciation in sympatric Nicaraguan crater lake cichlid fishes: sexual selection versus ecological diversification. Proc. R. Soc. Lond. B 267:2133–2141.