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STRONG ASSORTATIVE MATING BY DIET, COLOR, SIZE, AND MORPHOLOGY BUT LIMITED PROGRESS TOWARD SYMPATRIC SPECIATION IN A CLASSIC EXAMPLE: CAMEROON CRATER LAKE CICHLIDS

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Models predict that sympatric speciation depends on restrictive parameter ranges, such as sufficiently strong disruptive selection and assortative mating, but compelling examples in nature have rarely been used to test these predictions. I measured the strength of assortative mating within a species complex of *Tilapia* in Lake Ejagham, Cameroon, a celebrated example of incipient sympatric adaptive radiation. This species complex is in the earliest stages of speciation: morphological and ecological divergence are incomplete, species differ primarily in breeding coloration, and introgression is common. I captured 27 mated pairs in situ and measured the diet, color, size, and morphology of each individual. I found strong assortative mating by color, size, head depth, and dietary source of benthic or pelagic prey along two independent dimensions of assortment. Thus, Ejagham *Tilapia* showed strong assortative mating most conducive to sympatric speciation. Nonetheless, in contrast to a morphologically bimodal *Sarotherodon* cichlid species pair in the lake, Ejagham *Tilapia* show more limited progress toward speciation, likely due to insufficient strength of disruptive selection on morphology estimated in a previous study ($\gamma = 0.16$). This supports the predicted dependence of sympatric speciation on strong assortment *and* strong disruptive selection by examining a potentially stalled example in nature.

KEY WORDS: Adaptive radiation, ecological speciation, female preference, magic trait, mate choice, sexual selection.

Sympatric speciation, the evolution of reproductive isolation within a population without the aid of geographic barriers of any kind, remains one of the most controversial ideas in evolutionary biology after a long polarized history (Coyne and Orr 2004; Gavrilets 2004; Bolnick and Fitzpatrick 2007; Fitzpatrick et al. 2008). Theoreticians finally agree that ecologically driven sympatric speciation is possible (Dieckmann and Doebeli 1999; Kirkpatrick and Ravigné 2002; Gavrilets 2004), but it is predicted to occur only within a very restricted range of parameter space (Matessi et al. 2001; Bolnick and Doebeli 2003; Gavrilets 2005; Burger et al. 2006; Bolnick 2011; Thibert-Plante and Hendry 2011; Norvaišas and Kisdi 2012). Natural selection against intermediate ecologies must be strong enough to drive the evolution of strong assortative mating by ecotype and split the population (Dieckmann and Doebeli 1999; Kirkpatrick and Ravigné 2002; Bolnick and Fitzpatrick 2007). However, if either disruptive selection or assortative mating is not strong enough, speciation either does not occur or becomes stalled in a weakly bimodal phenotype distribution (Matessi et al. 2001; Bolnick and Doebeli 2003; Gavrilets 2005; Burger et al. 2006; Bolnick 2011; Thibert-Plante and Hendry 2011). Sympatric speciation may also be thwarted by substantial costs to mate choice (Otto et al. 2008; de Cara et al. 2008) or loss of bimodality due to Fisherian runaway sexual selection (Norvaišas and Kisdi 2012), among a host of other constraints (Gavrilets 2005; Bolnick and Fitzpatrick 2007; Thibert-Plante and Hendry 2011). However, there is a broad consensus that it is substantially easier if a single "magic" trait has large effects on both ecology and mate choice (Dieckmann and Doebeli 1999; Servedio et al. 2011; Haller et al. 2012; Norvaišas and Kisdi 2012).

There are very few case studies measuring the parameters of sympatric speciation within plausible empirical examples of this process in nature, including Darwin's finches (Huber et al. 2007; Hendry et al. 2009), Lord Howe Island palms (Babik et al. 2009), or Cameroon and Nicaraguan crater lake cichlids (Elmer et al. 2009, 2013; Martin 2012). Thus, it remains entirely unknown whether this process in nature is as sensitive to parameter ranges as predicted. This requires measuring key model parameters in compelling examples of sympatric speciation in the earliest stages of divergence (also see the island-survey approach of Papadopolus et al. 2011). Furthermore, focusing on examples of stalled speciation, in which species clusters remain weakly bimodal in phenotype relative to faster divergence observed in other species of similar age, can provide insight into which parameters may be holding back further progress toward speciation. For this reason, it is also important to measure these parameters in populations that appear not to be speciating in sympatry (Bolnick 2011).

Foremost, estimates of the strength of disruptive selection on ecological traits and the strength of assortative mating are needed. Ideally, these parameters should be measured directly along the ecological axes believed to be driving sympatric divergence (Huber et al. 2007; Pfennig 2007; Snowberg and Bolnick 2008, 2012; Hendry et al. 2009; Bolnick 2011). For example, the strength of assortment by ecology is most relevant to sympatric speciation models (Dieckmann and Doebeli 1999; Snowberg and Bolnick 2008; Bolnick 2011), even if ecological assortment results indirectly from mate choice based on other cues (such as dietary assortment due to size-assortative mating in stickleback: Snowberg and Bolnick 2008). Second, the strength of assortative mating should be measured between diverging species clusters because this reflects the strength of positive assortment driving speciation, the relevant parameter for speciation models (whereas positive assortment within clusters reflects the potential for additional speciation within clusters).

Here, I measured the strength of assortative mating in the flagship example of sympatric speciation: Cameroon crater lake cichlids. Coyne and Orr (2004, p. 152) state, "we know of no more convincing example [of sympatric speciation] in any group." This is due to the convincing monophyly of speciose clades restricted to isolated and uniform lake basins, ruling out any possibility of historical allopatry (Schliewen et al. 1994; Schliewen et al. 2001; Schliewen and Klee 2004; also see Barluenga et al. 2006; Elmer et al. 2010). I focused on the incipient species complex of *Tilapia* (*Coptodon*) from Lake Ejagham, Cameroon, which are in the very earliest stages of divergence: breeding individuals from four nominal species showed some genetic divergence at neutral markers despite frequent introgression (Dunz and Schliewen 2010); morphology was unimodal in a large random sample of at least three species across multiple sites in the lake (Martin 2012); and species were only discernible while displaying temporary breeding coloration (Dunz and Schliewen 2010; Martin 2012). Thus, despite their celebrated status as icons of sympatric speciation, the Ejagham *Tilapia* species complex exists somewhere between panmixia and the earliest stages of speciation (sensu Wu 2001; Mallet et al. 2007; Hendry 2009; Nosil et al. 2009): bimodal sexual coloration, unimodal morphology, and limited genetic differentiation. In contrast, a sister species pair of *Sarotherodon* cichlids has progressed much further along the speciation continuum to complete phenotypic bimodality within the same 0.49 km^2 lake basin (Neumann et al. 2011). Although there are a range of factors that can explain differential progress toward speciation (Berner et al. 2009; Hendry 2009; Bolnick 2011; Martin and Wainwright 2011, 2013; Rosenblum and Harmon 2011), these two young cichlid clades exhibit similar ecology and dispersal capabilities and coexist within the tiny and homogeneous habitat of Lake Ejagham. This suggests that Ejagham *Tilapia* have become stalled in their progress toward speciation for reasons beyond time since colonization, ecology, or effective population size (see also Elmer et al. 2013) despite ongoing disruptive selection on the complex (Martin 2012). Thus, Ejagham *Tilapia* present an ideal system in which to investigate constraints on completion of the sympatric speciation process in nature.

Incomplete phenotypic divergence in Ejagham *Tilapia* may be due to weak disruptive selection on trophic morphology (Martin 2012). The largest estimate of disruptive selection in this complex (Martin 2012: Table 1, Fig. 4E: $\gamma = 0.16$) was less than the minimum strength of disruptive selection ($\gamma > 0.25$) necessary for sympatric speciation suggested by a recent theoretical model (Bolnick 2011). However, it is not clear whether disruptive selection is the only factor holding back speciation within this species complex or whether additional factors may be contributing.

I investigated assortative mating parameters within Ejagham *Tilapia* relevant to sympatric speciation theory. I measured (1) the strength of assortative mating by color, size, and morphology, (2) the strength of assortment directly by diet, the primary axis of ecological divergence in this complex, and (3) the dimensionality of assortment along independent canonical correlation axes. My goal was to address whether these factors would constrain or promote sympatric divergence.

Methods

STUDY SYSTEM

Lake Ejagham is exceptional among African cichlid radiations. Among all known lacustrine cichlid radiations, Ejagham is the smallest lake at 0.49 km^2 (also see Nxomani et al. 1999; Seehausen

2006). Second, Ejagham contains not one, but two independent radiations: four endemic species of *Tilapia* (*Coptodon*) (Dunz and Schliewen 2010) and two endemic species of *Sarotherodon* (Neumann et al. 2011). Third, Ejagham defies a recent probabilistic model for the occurrence of cichlid adaptive radiations: Ejagham is a shallow lake (maximum depth: 18 m [Schliewen et al. 2001]) and both cichlid radiations are largely sexually monochromatic, contradicting two major predictors of adaptive radiation across the continent (Wagner et al. 2012).

Ejagham *Tilapia* contains four nominal species corresponding to *Tilapia deckerti*, *Tilapia fusiforme*, *Tilapia ejagham*, and *Tilapia nigrans* (Dunz and Schliewen 2010). All species are biparental substrate spawners, allowing for measurement of assortative pairing while parents defend their offspring. Effective assortative mating may be weaker than the pairings observed due to unknown levels of extra-pair copulation with partners potentially less similar in phenotype than observed in the territorial pair.

I targeted all mated pairs which defended breeding territories from 1 to 0.3 m depth throughout the littoral zone. Breeding pairs of at least two nominal species, *T. fusiforme* and *T. deckerti*, were completely interspersed throughout this depth range. There was also substantial color and morphological variation within *T. fusiforme/deckerti* which may correspond to additional species (*T. nigrans* or *T. fusiforme* "little black"/"large black": Schliewen et al. 2001; Dunz and Schliewen 2010). *T. deckerti* is deeper bodied with a larger epaxial area and breeding pairs often displayed red ventral spotting on an olive body. *T. fusiforme* is more elongate and breeding pairs displayed solid black to gray coloration. Both species displayed red irises and varying degrees of yellow along the ventral surface and caudal fin margins. *T. fusiforme* preferred hollow logs or excavated cavities underneath woody debris, whereas *T. deckerti* guarded open pits. Rare breeding pairs of the large piscivore *T. ejagham* ($n = 2$) were observed guarding territories at 2–3 m depth and this species was probably not included in my sample of mated pairs.

SAMPLING

From 11 to 16 January 2010, *Tilapia* breeding pairs guarding spawning territories, eggs, or fry were collected in situ using a 5 m barrier net and hand net while snorkeling to maintain visual confirmation of pair identity. Pairs were haphazardly targeted for capture by swimming 100 m transects along the littoral zone and attempting to collect any pairs encountered. Although pairs were sampled randomly, they could be assigned to two putative species.

Each fish was euthanized in an overdose of MS-222, labeled, photographed on both sides with a color standard under standardized flash illumination in the field (as in Martin and Johnsen 2007) using a Canon Powershot A1100IS, sampled for muscle tissue for stable isotope analysis, and stored in 95% ethanol. Sex was initially determined from size dimorphism within each pair (males are known to be larger: Schliewen et al. 2001) and verified in the laboratory by dissection of gonads (in all cases the smaller fish in each pair was female).

STABLE ISOTOPE ANALYSES

Assortative mating by diet was estimated from stable isotope ratios of individuals in each pair: δ^{13}C isotope ratio indicates the relative amount of benthic or pelagic carbon from prey consumed and δ^{15}N isotope ratio indicates relative trophic position (Post 2002). Isotope ratios were not calibrated to prey values because all individuals came from a single lake environment (Snowberg and Bolnick 2008). After euthanasia, approximately 5 mg of muscle tissue was removed from the caudal peduncle of each individual and dehydrated in a sealed tube in the field using magnesium perchlorate [$Mg(ClO_4)^2$]. Initial laboratory trials indicated that this dessicant did not bias stable isotopes relative to replicate freezer-preserved matched-pair samples (δ^{13}C: $r^2 = 0.91$; δ^{15}N: $r^2 = 0.95$; $n = 11$ paired samples). Dehydrated samples were dried in the laboratory at 60°C for 24 h before weighing and sent to the UC Davis Stable Isotope Facility for measurement on a PDZ Europa ANCA-GSL elemental analyzer, interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, U.K.).

MORPHOMETRICS

Preserved specimens were photographed on both sides in the laboratory with a size standard for morphometric measurements. Linear distances of body depth, head depth, orbit diameter, lower jaw length, and ascending process of the premaxilla were measured from laboratory photographs as described in Martin (2012), in which the form of selection on these traits was estimated. Traits were measured from photographs of both sides of each individual and averaged. Standard length (SL) was measured from each specimen using dial calipers. The five morphological traits (not including SL) were then size-corrected by taking residuals from a linear regression of log-transformed trait on the multivariate size axis estimated from the first principal component of the correlation matrix for all six measurements (Bookstein 1991). This multivariate size axis provided a more balanced estimate of residual shape variation between species clusters (i.e., similar slopes [McCoy et al. 2006]) than the univariate size axis of log-transformed SL. Measurements of the 27 mated pairs were pooled with measurements of 523 *Tilapia* individuals (Martin 2012), which showed similar allometric scaling relationships, for more accurate estimation of this linear relationship for size-correction.

COLORIMETRY

Field photographs of breeding individuals immediately following euthanasia were used for quantification of lightness and red/yellow coloration. Individuals were photographed once on each side and measurements from each image were averaged. Each photograph was converted to the CIELAB color space (International Commission on Illumination 1971) using ImageJ (Supplementary Fig. S1). The CIELAB color space is a device-independent space which is conveniently divided into the three color dimensions targeted in this study: lightness (L), green (negative) to red/magenta (positive) chroma (A), and blue (negative) to yellow (positive) chroma (B). This provides the most relevant color dimensions for measurements of overall lightness, red chroma, and yellow chroma, the main components of Ejagham *Tilapia* breeding coloration. CIELAB aims for perceptual uniformity within the human vision system; thus, photographic measurements of color may fail to capture additional elements of cichlid vision, such as UV reflectance (Timelthaler 2010).

Mean lightness values were sampled from four rectangular regions on each individual (Supplementary Fig. S1) and divided by the mean lightness of a standard white background in each image. The first and second principal components describing variation across these four regions were retained, which described overall lightness (value PC1) and the difference in dorsal/ventral lightness (value PC2).

Mean red chroma was sampled from the iris and ventral lateral surface (Supplementary Fig. S1A). Mean yellow chroma was sampled from the ventral lateral surface and the caudal fin (Supplementary Fig. S1B). In addition, for all four regions the proportional coverage by red or yellow was estimated from the number of pixels greater than zero (i.e., more red/yellow than green/blue, respectively) divided by the total number of pixels sampled in each region. The four color proportions were arcsine (square-root) transformed to meet Gaussian assumptions. The first three principal components from these eight color measurements were retained as estimates of overall red and yellow chroma (color PC1), relative amount of red or yellow (color PC2), and red ventral coloration (color PC3). To assess whether additional assortment by morphology remained between pairs independent of assortment due to sexual coloration, residuals from multiple regression analyses were used to remove the variance explained by breeding coloration (including all 12 measurements of color and value) from the morphological traits of head depth and SL. The strength of assortment by head depth and SL was then remeasured.

STATISTICAL ANALYSES

Assortative mating between the sexes was compared for diet (δ^{13}C and δ^{15}N stable isotope ratios), SL, size-corrected morphological traits (body depth, head depth, orbit diameter, jaw length, ascending process), lightness (lightness PC1–2), and red–yellow coloration (color PC1–3). Traits exhibiting significant linear correlations between the mated pairs were retained for canonical correlation analysis and multiple regression (Table 1, Supplementary Table S1). Red ventral coloration loaded heavily on the second canonical axis and was also included for these analyses. Canonical correlation analysis identifies linear combinations of these traits which show the strongest correlation between the mated pairs. Each canonical axis represents an independent dimension of assortment. Canonical correlations between the mated pairs were calculated using the CCA function in the vegan package (Oksanen et al. 2005) in R (R Development Core Team 2012). Significance of canonical axes was assessed from Wilks' lambda statistic using permutation tests. For some statistical analyses and graphical displays, individuals were assigned to nominal species categories (*T. deckerti* and *T. fusiforme*) based on the two clusters identified on the first canonical assortment axis. Isotope ratios were normally distributed (Shapiro–Wilk test, $P > 0.05$) and compared using *t*-tests.

Results

INCOMPLETE MORPHOLOGICAL AND DIETARY DIVERGENCE

Species clusters partially overlapped along major axes of morphological and dietary variation (Fig. 1A,C). Morphological divergence between species clusters was incomplete along any single trait axis measured (Fig. 2). Species clusters did not differ in relative trophic position (δ^{15}N: $t = 0.14$, $P = 0.893$), but were significantly different in relative proportions of benthic or pelagic prey in their diets (δ^{13}C: $t = 5.63$, $P = 1e^{-6}$; Fig. 1). However, there was overlap in the range of δ^{13}C isotope ratios among breeding individuals in the two species clusters; discriminant analyses of species identity based on δ^{13}C and δ^{15}N isotope ratios misclassified 23.2% (12/52) of individuals.

ASSORTATIVE MATING BY DIET, COLOR, SIZE, AND MORPHOLOGY

Despite incomplete morphological and dietary divergence, I found strong assortative mating by overall color intensity, yellow–red coloration, overall lightness, SL, head depth, and dietary source of benthic or pelagic carbon (Fig. 2; Table 1). Assortative mating was strongest along the principal color and lightness axes (Fig. 2; Table 1). Color and lightness values also loaded more heavily than morphological traits on the first two canonical correlation axes (Table 1).

Assortment by diet probably resulted indirectly from direct assortment by other mating cues. Three assortment traits were each significantly correlated with diet in a multiple regression

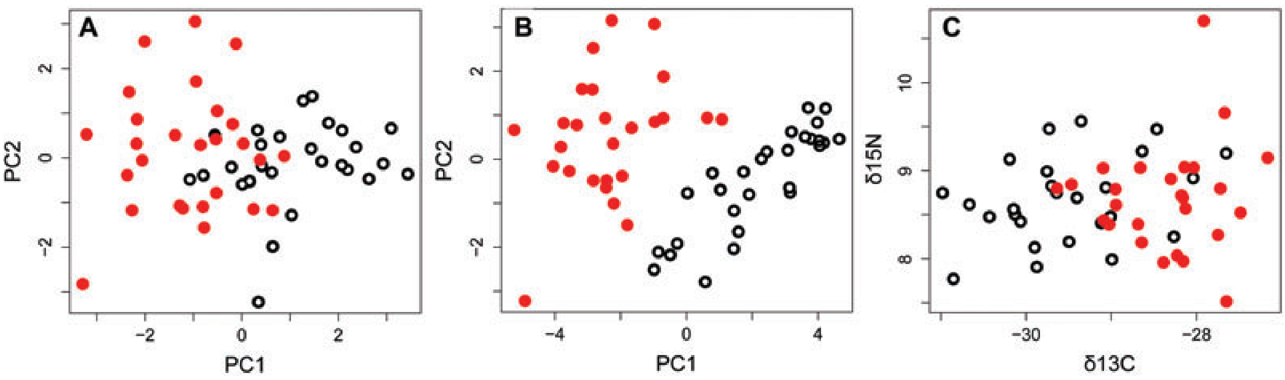

Figure 1. Major axes of variation in (A) morphology, (B) breeding coloration, and (C) diet between mated pairs in two incipient species within the Ejagham *Tilapia* species complex. (A) First two principal components of morphological variation (explaining 43% and 23% of the total morphological variance, respectively) for size-corrected measures of jaw length, ascending process, orbit diameter, head depth, and body depth in mated pairs. (B) First two principal components of variation in breeding coloration (explaining 64% and 15% of the total color variance, respectively) for the 12 measurements of coloration and lightness from photographs of freshly euthanized mated pairs. (C) Stable isotope ratios of mated pairs indicating relative trophic position ($\delta^{15}N$) and benthic–pelagic source of prey ($\delta^{13}C$).

Table 1. Strength of assortative mating along canonical correlation axes (CCA1–3) and by diet ($\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios), color, standard length (SL), and size-corrected morphology within breeding pairs of Ejagham *Tilapia*. Pearson correlation coefficients (*r*) and *P* values are indicated for each linear correlation. Proportion of variance explained (λ) is included for each principal component axis. Loadings for traits retained for canonical correlation analysis are shown on the right side of the table, with the highest loadings on each axis highlighted in bold.

	Trait	λ	r	P	Canonical loadings			
	CCA1		0.97	$1e^{-6}$***[1]	CCA1		CCA2	
	CCA2		0.92	$3e^{-5}$***[1]	♂ on ♀	♀ on ♂	♂ on ♀	♀ on ♂
	CCA3		0.56	0.170[1]				
Diet	$\delta^{13}C$: benthic-pelagic prey		0.51	0.008**				
	$\delta^{15}N$: trophic position		0.07	0.717				
Color	Color PC1: intensity	0.59	0.86	$1e^{-8}$***	**−0.93**	**−0.89**	0.09	−0.14
	Color PC2: yellow–red	0.22	0.46	0.016*	0.46	0.36	−0.08	0.02
	Color PC3: red ventral	0.10	0.10	0.630	0.50	−0.45	**0.50**	**0.51**
	Lightness PC1: light–dark	0.85	0.79	$1e^{-6}$***	**0.88**	**0.79**	0.18	0.44
	Lightness PC2: ventral–dorsal	0.09	0.23	0.239				
Morphology	SL		0.77	$2e^{-6}$***	**0.83**	**0.80**	−0.26	−0.11
	Head depth		0.64	0.0003***	0.69	0.58	**0.44**	**0.41**
	Body depth		0.18	0.356				
	Orbit diameter		0.04	0.969				
	Ascending process		0.35	0.070				
	Jaw length		0.18	0.375				

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

[1] Permutation test using Wilks' lambda.

analysis: head depth, SL, and red ventral coloration (Supplementary Table S1). Dietary assortment between mated pairs was not observed ($r = 0.105$, $P = 0.611$) after removing the significant effect of SL on diet ($r = 0.684$, $P = 2e^{-8}$).

Assortment by morphology also may have resulted indirectly from assortment by sexual coloration. Pairs weakly assorted by SL ($r = 0.441$, $P = 0.021$), but not head depth ($r = 0.088$, $P = 0.661$), after removing the variance explained by sexual coloration in multiple regression analyses.

DIMENSIONALITY OF ASSORTMENT

Canonical correlation analysis identified two significant, independent axes of assortment across the traits measured (Table 1, Fig. 3). CCA1 described the major axis of differentiation between

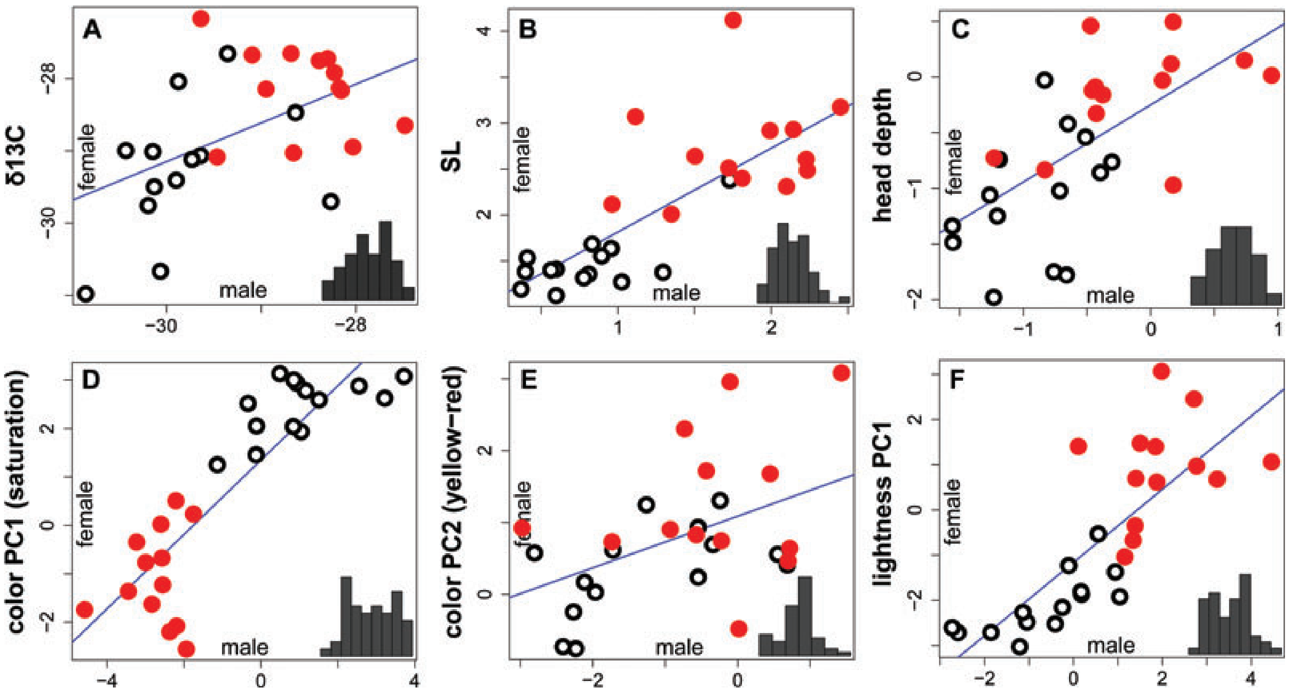

Figure 2. Significant univariate axes of assortative mating by (A) benthic–pelagic source of prey (δ^{13}C), (B) standard length, (C) head depth, (D) overall color saturation (color PC1), (E) yellow–red color axis (color PC2), and (F) overall lightness (lightness PC1). Males in each pair are plotted relative to females on the abscissa with color indicating categorical assignment of each pair to species clusters (*Tilapia deckerti* ● or *Tilapia fusiforme* ●). Trait histograms for all breeding individuals are shown in the lower right corner of each plot.

the two species clusters, defined primarily by color intensity, lightness, and SL (Fig. 3). CCA1 was significantly correlated with benthic or pelagic source of prey carbon (δ^{13}C: $r = 0.610$, $P = 2e^{-6}$). CCA2 described a significant axis of differentiation within both species clusters, defined primarily by red ventral coloration and head depth (Fig. 3B–C, Table 1). CCA2 was not correlated with benthic or pelagic source of prey carbon (δ^{13}C: $r = 0.054$, $P = 0.704$).

Discussion

Sympatric speciation models predict that phenotypic divergence within a population cannot occur without strong disruptive selection and strong assortative mating, ideally resulting from the same magic trait (Dieckmann and Doebeli 1999; Kirkpatrick and Ravigné 2002; Gavrilets 2004; Bolnick and Fitzpatrick 2007; Otto et al. 2008; Bolnick 2011). However, the dependence of this process on the magnitude of these parameters has never been directly tested in nature (but see other measurements of sympatric speciation parameters, e.g., Seehausen et al. 2008; Babik et al. 2009; Elmer et al. 2009, 2013; Martin 2012). Here, I measured the strength of assortment observed in mated pairs within a species complex which may be stalled in a phase of incomplete morphological separation. My goal was to address whether incomplete speciation in Ejagham *Tilapia* could be due merely to the observed moderate strength of disruptive selection (Martin 2012).

(1) STRONG ASSORTATIVE MATING BY COLOR, SIZE, AND MORPHOLOGY

The observed strength of assortative mating ($r = 0.46$–0.86; Table 1, Fig. 2) was often well above the threshold required for morphological separation within an empirically motivated sympatric speciation model (Bolnick 2011: $r > 0.6$). Moreover, significant assortment along the two independent canonical axes identified (Fig. 2, Table 1) was very strong ($r = 0.92$–0.97). These canonical correlation coefficients were optimized from multiple traits and thus may be artificially high; however, canonical axes more accurately describe mate choice which is nearly always based on a complex range of traits across multiple sensory modalities and courtship stages (Hebets and Papaj 2005), particularly in cichlids (Martin 2010). Thus, canonical axes might be the most appropriate measure of the strength of assortative mating (Bolnick 2011).

(2) SIGNIFICANT ASSORTMENT BY DIET

Most directly relevant to models of sympatric speciation by natural selection, assortative mating by diet was quite strong ($r = 0.51$; Table 1). A similar direct field estimate of assortative mating by diet is known only from unimodal populations of stickleback

($r = 0.353$–0.507; Snowberg and Bolnick 2008, 2012). As in stickleback (Snowberg and Bolnick 2008), assortment by diet in Ejagham *Tilapia* probably results indirectly from strong assortative mating by size. Size may, in fact, be one of the most ubiquitous examples of a magic trait affecting both ecology and mate choice across taxa (Servedio et al. 2011).

(3) SIGNIFICANT ASSORTMENT ALONG TWO INDEPENDENT CANONICAL AXES

I unexpectedly found evidence of positive assortment within the pooled species clusters (Fig. 3B–C, Table 1). This assortment axis (CCA2) was largely independent of the interspecific divergence axis (CCA1: Fig. 3; Table 1), which suggests that individuals are pairing based on two different sets of cues: interspecific species recognition cues (CCA1) and an independent set of intraspecific cues (CCA2; note that these are both axes of positive assortment, not sexual selection [Kirkpatrick and Ravigne 2002], because only mated pairs were used for this analysis). If each subsequent speciation event within Ejagham *Tilapia* is driven by assortative mating based on a new set of mating cues (as suggested by the multivariate differences in breeding coloration among nominal species in this complex [Dunz and Schliewen 2010], rather than univariate variation along a single dimension [see also Harmon et al. 2005; Hohenlohe and Arnold 2011]), this observation suggests that some positive assortment within species clusters may be available for future divergence events. If multiple axes of positive assortment frequently coexist within diverging populations, this suggests that positive assortment may not need to evolve from random mating after each speciation event as is widely assumed within theoretical models (Dieckmann and Doebeli 1999; Otto et al. 2008). Indeed, surveys of the dimensionality of sexual isolation suggest that reproductive isolation between populations frequently occurs along multiple independent axes, but there may be an upper limit to this dimensionality (Hohenlohe and Arnold 2011; Nosil and Hohenlohe 2012).

(4) PLAUSIBLE MAGIC TRAITS

SL exhibited one of the strongest correlations between mated pairs and was also strongly correlated with diet. Moreover, SL contributed to assortative mating ($r = 0.44$, $P = 0.02$) after statistically removing the variance explained by sexual coloration. No estimate of the strength and form of selection on SL is available due to the correlation between SL and the fitness proxy used in Martin (2012). Nonetheless, SL is highly divergent among three Ejagham *Tilapia* species, suggesting divergent selection. If SL is under strong disruptive selection, contributes to assortment independently from other traits measured, and these are pleiotropic effects of the same genetic loci (Servedio et al. 2011), this trait may be a strong candidate for a large-effect magic trait (sensu Haller et al. 2012) substantially facilitating speciation in this system.

Head depth experienced the strongest nonlinear selection gradient observed in this complex (correlational selection on head depth and ascending process: $\gamma = 0.16$; Fig. 4E in Martin 2012), consistent with predictions from fluid-dynamic modeling of suction-feeding performance on benthic versus pelagic prey (Holzman et al. 2012: see epaxial area/jaw protrusion speed). However, head depth did not contribute to assortment independently of sexual coloration and was only weakly associated with CCA2 (Table 1), indicating a low effect size (Haller et al. 2011). Thus, the trait experiencing the strongest disruptive selection observed does not appear to be a magic trait which could facilitate sympatric speciation.

(5) TWO SCENARIOS FOR INCOMPLETE SYMPATRIC SPECIATION

In contrast to minimal ecological and morphological divergence, sexual coloration was the most divergent feature within the Ejagham *Tilapia* species complex (Fig. 1) and showed the strongest correlation between mated pairs (Fig. 2, Table 1). This suggests two plausible scenarios. (1) Initially strong disruptive ecological selection on trophic morphology and diet may have indirectly driven the evolution of strong assortative mating by sexual coloration through a reinforcement-like process (Dieckmann and Doebeli 1999). As the phenotype distribution flattened and species clusters slightly diverged in ecology, disruptive selection may have then weakened to moderate levels, insufficient to complete the evolution of morphological bimodality; however, strong assortative mating remained, which maintained bimodality in sexual coloration. (2) Alternatively, strong disruptive sexual selection on sexual coloration and magic traits, such as SL, may have been the primary driver of morphological divergence within Ejagham *Tilapia*, indirectly causing ecological divergence between species clusters. However, without additional strong disruptive selection on ecology, the evolution of phenotypic bimodality could not be completed (see also strong assortative mating by color in a Nicaraguan crater lake cichlid pair [Elmer et al. 2009] and hamlets [Puebla et al. 2007]).

Although Cameroon crater lake cichlids have traditionally been considered examples of ecologically driven speciation (Coyne and Orr 2004), pronounced differences in sexual coloration within incipient species complexes in these lakes (Martin 2012) parallel the many cichlid species complexes in African Great Lakes in which ecologically equivalent species differ only in breeding coloration (Streelman and Danley 2003; Martin and Genner 2009). Models rely on ecological divergence to stabilize disruptive selection and species persistence (Coyne and Orr 2004; van Doorn et al. 2009; Norvaišas and Kisdi 2012) and, yet, ecological divergence often appears to lag far behind sexual

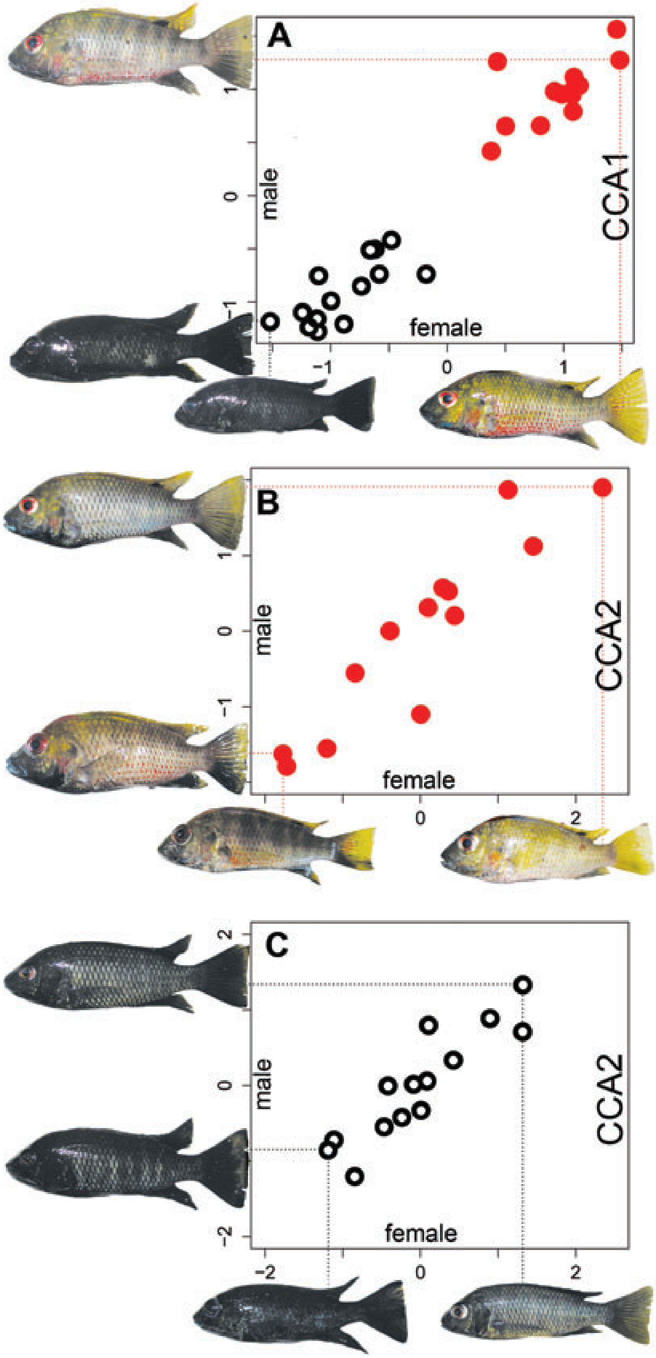

Figure 3. Significant canonical correlation axes of assortative mating based on coloration, morphology, and SL of the mated pairs (Table 1). (A) The primary axis of assortative mating corresponds to the two species clusters (*Tilapia deckerti* ● and *Tilapia fusiforme* ●). (B,C) A second independent axis of assortative mating describes significant assortment by red ventral coloration and head depth within species clusters. Representative photographs of male–female pairs are shown at opposite extremes of each axis. CCA2 is divided into (B) *Tilapia deckerti* and (C) *Tilapia fusiforme* plots for clarity.

divergence in many rapidly speciating systems (McPeek and Brown 2000; Elmer et al. 2009; Martin and Genner 2009; Arnegard et al. 2010). Thus, sexual selection may play a leading role in driving sympatric speciation of Cameroon crater lake cichlids, in contrast to most theoretical models (Coyne and Orr 2004; van Doorn et al. 2009; Norvaišas and Kisdi 2012; but see Higashi et al. 1999; Turner and Burrows 1995).

CONCLUSION

Here, I demonstrated that a celebrated example of incipient sympatric speciation in a tiny African lake is well within the parameter ranges for mate choice most conducive to sympatric speciation due to (1) strong assortative mating by color, size, and morphology along two independent dimensions, (2) strong assortment by diet resulting indirectly from size-assortative mating, and (3) plausible magic traits such as SL. Nonetheless, Ejagham *Tilapia* was morphologically unimodal with minimal ecological divergence (Fig. 1). This is consistent with incomplete morphological divergence predicted by many theoretical models without strong assortative mating *and* strong disruptive selection (Matessi et al. 2001; Bolnick and Doebeli 2003; Gavrilets 2005; Burger et al. 2006; Otto et al. 2008; Bolnick 2011). Although previous estimates of disruptive selection on head depth/ascending process were moderately strong (Martin 2012: $\gamma = 0.16$), insufficient strength of selection is the most likely constraint identified so far slowing or preventing morphological divergence. I cannot rule out that speciation in this complex may be slow, rather than stalled, due to the young age of Ejagham, small effective population size, or complex genetic architectures of trophic morphology, mate preferences, or sexual coloration. Alternatively, I cannot rule out species collapse due to the recent human-caused invasion of *Parauchenoglanis* catfish in 2000–2001 (Martin 2012). However, a second endemic sister species pair of *Sarotherodon* cichlids in Ejagham appears to show complete morphological separation on the same timescale at lower census population sizes (Neumann et al. 2011).

Overall, this study suggests that sympatric speciation depends on large magnitudes of both disruptive selection and assortative mating as predicted by theory in a widely celebrated, but apparently stalled example of this process in nature (also see Bolnick 2011). This study also complements a growing body of work investigating differential progress toward ecological speciation and adaptive radiation across similar environments (Berner et al. 2009; Hendry 2009; Nosil et al. 2009; Martin and Wainwright 2011, 2013; Rosenblum and Harmon 2011; Martin 2012; Wagner et al. 2012).

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LITERATURE CITED

Arnegard, M. E., P. B. McIntyre, L. J. Harmon, M. L. Zelditch, W. G. R. Crampton, J. K. Davis, J. P. Sullivan, S. Lavoué, and C. D. Hopkins. 2010. Sexual signal evolution outpaces ecological divergence during electric fish species radiation. Am. Nat. 176:335–356.

Babik, W., R. K. Butlin, W. J. Baker, A. S. Papadopulos, M. Boulesteix, M. C. Anstett, C. Lexer, I. Hutton, and V. Savolainen. 2009. How sympatric is speciation in the *Howea* palms of Lord Howe Island? Mol. Ecol. 18:3629–3638.

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

Berner, D., A.-C. Grandchamp, and A. P. Hendry. 2009. Variable progress toward ecological speciation in parapatry: stickleback across eight lake-stream transitions. Evolution 63:1740–1753.

Bolnick, D. I. 2011. Sympatric speciation in threespine stickleback: why not? Int. J. Ecol. 2011:1–15.

Bolnick, D. I., and M. Doebeli. 2003. Sexual dimorphism and adaptive speciation: two sides of the same ecological coin. Evolution 57:2433–2449.

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

Bookstein F. L. 1991. Morphometric tools for landmark data: geometry and biology. Cambridge Univ. Press, Cambridge, MA.

Burger, R., K. A. Schneider, and M. Willensdorfer. 2006. The conditions for speciation through intraspecific competition. Evolution 60:2185–2206.

Coyne, J. A., and H. A. Orr. 2004. Speciation. Sinauer Associates, Inc., Sunderland, U.K.

de Cara, M. A. R., N. H. Barton, and M. Kirkpatrick. 2008. A model for the evolution of assortative mating. Am. Nat. 171:580–596.

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

Dunz, A. R., and U. K. Schliewen. 2010. Description of a *Tilapia* (*Coptodon*) species flock of Lake Ejagham (Cameroon), including a redescription of *Tilapia deckerti* Thys van den Audenaerde, 1967. Spix 33:251–280.

Elmer, K. R., T. K. Lehtonen, and A. Meyer. 2009. Color assortative mating contributes to sympatric divergence of Neotropical cichlid fish. Evolution 63:2750–2757.

Elmer, K. R., H. Kusche, T. K. Lehtonen, and A. Meyer. 2010. Local variation and parallel evolution: morphological and genetic diversity across a species complex of Neotropical crater lake cichlid fishes. Phil. Trans. Roy. Soc. B 365:1763–1782.

Elmer, K. R., T. K. Lehtonen, S. Fan, and A. Meyer. 2013. Crater lake colonization by Neotropical cichlid fishes. Evolution 67:281–288.

Fitzpatrick, B. M., J. A. Fordyce, and S. Gavrilets. 2008. What, if anything, is sympatric speciation? J. Evol. Biol. 21:1452–1459.

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

———. 2005. “Adaptive speciation”—it is not that easy: reply to Doebeli et al. Evolution 59:696–699.

Haller, B. C., L. F. De Leon, G. Rolshausen, K. M. Gotanda, and A. P. Hendry. 2012. Magic traits: distinguishing the important from the trivial. TREE 27:4–6.

Harmon, L. J., J. J. Kolbe, J. M. Cheverud, and J. B. Losos. 2005. Convergence and the multidimensional niche. Evolution 59:409–421.

Hebets, E. A., and Papaj, D. R. 2005. Complex signal function: developing a framework of testable hypotheses. Behav. Ecol. Soc. 57:197–214.

Hendry, A. P. 2009. Ecological speciation! Or the lack thereof? Can. J. Fish. Aqua. Sci. 66:1383–1398.

Hendry, A. P., S. K. Huber, L. F. De Leon, A. Herrel, and J. Podos. 2009. Disruptive selection in a bimodal population of Darwin’s finches. Proc. Roy. Soc. B. 276:753–759.

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

Hohenlohe, P. A., and S. J. Arnold. 2011. Dimensionality of mate choice, sexual isolation, and speciation. Proc. Natl. Acad. Sci. USA 107:16583–16588.

Holzman, R., D. C. Collar, R. S. Mehta, and P. C. Wainwright. 2012. An integrative modeling approach to elucidate suction-feeding performance. J. Exp. Biol. 215:1–13.

Huber, S. K., L. F. De Leon, A. P. Hendry, E. Bermingham, and J. Podos. 2007. Reproductive isolation of sympatric morphs in a population of Darwin’s finches. Proc. Roy. Soc. B 274:1709–1714.

Kirkpatrick, M., and V. Ravigné. 2002. Speciation by natural and sexual selection: models and experiments. Am. Nat. 159:S22–S35.

Mallet, J., M. Beltran, W. Neukirchen, and M. Linares. 2007. Natural hybridization in heliconiine butterflies: the species boundary as a continuum. BMC Evol. Biol. 7:28.

Martin, C. H. 2010. Unexploited females and unreliable signals of male quality in a Malawi cichlid bower polymorphism. Behav. Ecol. 21:1195–1202.

———. 2012. Weak disruptive selection and incomplete pheonytpic divergence in two classic examples of sympatric speciation: Cameroon crater lake cichlids. Am. Nat. 180:E90–E109.

Martin, C. H., and M. J. Genner. 2009. High niche overlap between two successfully coexisting pairs of Lake Malawi cichlids. Can. J. Fish. Aquat. Sci. 66:579–588.

Martin, C. H., and S. Johnsen. 2007. A field test of the Hamilton-Zuk hypothesis in the guppy *Poecilia reticulata*. Beh. Ecol. Soc. 61:1897–1909.

Martin, C. H., and P. C. Wainwright. 2011. Trophic novelty is linked to exceptional rates of morphological diversification in two adaptive radiations of *Cyprinodon* pupfishes. Evolution 65:2197–2212.

———. 2013. Multiple fitness peaks on the adaptive landscape drive adaptive radiation in the wild. Science 339:208–211.

Matessi, C., A. Gimelfarb, and S. Gavrilets. 2001. Long-term buildup of reproductive isolation promoted by disruptive selection: how far does it go? Selection 2:41–64.

McCoy, M. W., B. M. Bolker, C. W. Osenberg, B. G. Miner, and J. R. Vonesh. 2006. Size correction: comparing morphological traits among populations and environments. Oecologia 148:547–554.

McPeek, M. A., and J. M. Brown. 2000. Building a regional species pool: diversification of the *Enallagma* damselflies in Eastern North America. Ecology 81:904–920.

Neumann, D., M. L. J. Stiassny, and U. K. Schliewen. 2011. Two new sympatric *Sarotherodon* species (pisces: Cichlidae) endemic to Lake Ejagham, Cameroon, west-central Africa, with comments on the *Sarotherodon galilaeus* species complex. Zootaxa 20:5326.

Norvaišas, P., and E. Kisdi. 2012. Revisiting Santa Rosalia to unfold a degeneracy of classic models of speciation. Am. Nat. 180:388–393.

Nosil, P., and P. A. Hohenlohe. 2012. Dimensionality of sexual isolation during reinforcement and ecological speciation in *Timema cristinae* stick insects. Evol. Ecol. Res. 14:467–485.

Nosil, P., L. J. Harmon, and O. Seehausen. 2009. Ecological explanations for (incomplete) speciation. TREE 24:145–156.

Nxomani, C., A. J. Ribbink, and R. Kirby. 1999. DNA profiling of *Tilapia guinasana*, a species endemic to a single sinkhole, to determine the genetic divergence between color forms. Nucleic Acids 20:1781–1785.

Oksanen, J. J., Kindt, R., Legendre, P., and O'Hara, R. B. 2005. Vegan: community ecological package. Version 2.0–3. Available at http://cran.r-project.org/. Accessed March 21, 2013.

Otto, S. P., M. R. Servedio, and S. L. Nuismer. 2008. Frequency-dependent selection and the evolution of assortative mating. Genetics 179:2091–112.

Papadopolus, A. S. T., Baker, W. J., Crayn, D., Butlin, R. K., Kynast, R. G., Hutton, I., and Savolainen, V. 2011. Speciation with gene flow on Lord Howe Island. Proc. Nat. Acad. Sci. USA 108:13188–13193.

Pfennig, K. 2007. Facultative mate choice drives adaptive hybridization. Science 318:965–967.

Post, D. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703–718.

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

R Development Core Team. 2012. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austra. Available via http://www.R-project.org/.

Rosenblum, E. B., and L. J. Harmon. 2011. "Same same but different": replicated ecological speciation at White Sands. Evolution 65:946–960.

Schliewen, U. K., and B. Klee. 2004. Reticulate sympatric speciation in Cameroonian crater lake cichlids. Front. Zoo. 1:5.

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

Schliewen, U., K. Rassmann, M. Markmann, J. Markert, T. Kocher, and D. Tautz. 2001. Genetic and ecological divergence of a monophyletic cichlid species pair under fully sympatric conditions in Lake Ejagham, Cameroon. Mol. Ecol. 10:1471–1488.

Seehausen, O. 2006. African cichlid fish: a model system in adaptive radiation research. Proc. Roy. Soc. B 273:1987–1998.

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 455:620–626.

Servedio, M. R., G. S. Van Doorn, M. Kopp, A. M. Frame, and P. Nosil. 2011. Magic traits in speciation: "magic" but not rare? TREE 26:389–397.

Snowberg, L. K., and D. I. Bolnick. 2008. Assortative mating by diet in a phenotypically unimodal but ecologically variable population of stickleback. Am. Nat. 172:733–739.

———. 2012. Partitioning the effects of spatial isolation, nest habitat, and individual diet in causing assortative mating within a population of threespine stickleback. Evolution 66:3582–3594.

Streelman, T. J., and P. D. Danley. 2003. The stages of vertebrate evolutionary radiation. TREE 18:126–131.

Thibert-Plante, X., and A. P. Hendry. 2011. Factors influencing progress toward sympatric speciation. J. Evol. Biol. 24:2186–2196.

Timelthaler, G. 2010. The crater lake cichlids of Cameroon: visual ecology, opsin molecular evolution and implications for sympatric speciation. Soc. Study Evol. Ann. Conf. Poster.

Turner, G. F., and M. T. Burrows. 1995. A model of sympatric speciation by sexual selection. Proc. Roy. Soc. B 260:287–292.

Wagner, C. E., L. J. Harmon, and O. Seehausen. 2012. Ecological opportunity and sexual selection together predict adaptive radiation. Nature 487:366–369.

Wu, C-I. 2001. The genic view of the process of speciation. J. Evol. Biol. 14:851–865.

Van Doorn, G. S., P. Edelaar, and F. J. Weissing. 2009. On the origin of species by natural and sexual selection. Science 326:1704–1707.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Generalized linear model describing dietary source of benthic or pelagic prey (δ^{13}C isotope ratios) in mated pairs as a function of significant assortment axes: color, lightness, size, and head depth. Traits significantly affecting diet are highlighted in bold.

Figure S1. Illustration of color and value sampling locations on photograph of freshly euthanized breeding individual in CIELAB color space. (1) Original photograph indicating five color or value sampling locations: ventral pectoral girdle, lateral surface ventral to the lateral line between the insertions of the pectoral and anal fins, dorsal lateral surface of the epaxial region, caudal fin, and iris. (2) Ventral sampling location converted into three-dimensional CIELAB color space with axes of L, lightness or darkness; a, red or green, and b, yellow or blue. (3) Intensity of color or value in the three dimensions (L, a, b) of the CIELAB color space. Sampling locations for value (L, $n = 4$), red or green coloration (a, $n = 2$), and yellow or blue coloration (b, $n = 2$) are indicated.