

In Search of the Causes of Evolution:

From Field Observations to Mechanisms



Edited by

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PRINCETON UNIVERSITY PRESS

PRINCETON AND OXFORD

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Published by Princeton University Press, 41 William Street,
Princeton, New Jersey 08540
In the United Kingdom: Princeton University Press, 6 Oxford Street, Woodstock,
Oxfordshire OX20 1TW

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ISBN: 978-0-691-14681-2

ISBN (pbk.): 978-0-691-14695-9

British Library Cataloging-in-Publication Data is available

This book has been composed in [TK]
Printed on acid-free paper ∞
press.princeton.edu
Printed in the United States of America

1 3 5 7 9 10 8 6 4 2
10 9 8 7 6 5 4 3 2 1

Chapter Fourteen

Geographical Mode and Evolutionary Mechanism of Ecological Speciation in Cichlid Fish

Ole Seehausen and Isabel Santos Magalhaes

The spatial context of speciation has been a major issue in evolutionary biology and systematics for nearly seventy years (Abbott et al. 2008; Bolnick and Fitzpatrick 2007; Bush 1969; Coyne and Orr 2004; Gavrillets 2004; Mayr 1942, 1963). Specifically, the theoretical possibility, empirical reality, and relevance of sympatric speciation have been, and continue to be, a significant battleground (Bush 1998; Mayr 1982). Whereas a handful of compelling empirical cases has lately led to fairly broad agreement that sympatric speciation has indeed happened in nature (Bolnick and Fitzpatrick 2007; Coyne and Orr 2004), disagreement prevails over two consequential questions. The first is the question, what in fact constitutes sympatric speciation (Fitzpatrick et al. 2008)? The other one is the question whether sympatric speciation is common, and hence of relevance to understanding the origins and structure of species diversity (Bolnick and Fitzpatrick 2007). Several authors have suggested that instead of discussing the geographical mode of speciation, a more fruitful approach would be to focus on the mechanisms that play major roles in speciation, such as the sources of divergent selection (Grant and Grant 2006; Rundle et al. 2000) and the balance between gene flow, selection, and mate choice (Butlin et al. 2008; Grant and Grant 2008; Nosil and Crespi 2004; Rundle et al. 2000; Thibert-Plante and Hendry 2009). Whereas we strongly advocate the mechanistic approach, we wish to stress that the geography of speciation remains equally important for understanding the structure of biodiversity.

We agree with others that discrete classification of speciation modes into allopatric, sympatric, and parapatric, using criteria of theoretical

population genetics (i.e., $m = 0$, $m = 0.5$, and $0 < m < 0.5$), is not likely to produce major new insights into the importance of modes of speciation. Most cases of speciation are likely to be parapatric by this definition (Bolnick and Fitzpatrick 2007; Fitzpatrick et al. 2008). We further agree with Bolnick and Fitzpatrick (2007) that counting compelling cases of sympatric and allopatric speciation is suffering from ascertainment bias. We believe that the way forward necessitates development of methods for quantitative comparative investigations of the role of spatial structure in as many as possible cases of speciation that cannot be categorized as either allopatric or sympatric. In this chapter we investigate several examples of speciation in an unusually rapid large animal radiation, the cichlid fish of Lake Victoria, East Africa.

We suggest ways to extract quantitative rules about the role of space in speciation. The idea requires investigating many evolutionary replicates of divergence of essentially similar phenotypes that vary in the amount of spatial structure and in the levels of completion of speciation. We apply this idea to twelve divergent phenotype pairs of Lake Victoria cichlid fish, representing 5, 4, and 3 replicates of parallel divergence along water depth gradients in three different trait complexes. We proceed in three steps: first we present a short overview of current thinking on modes of speciation in the large cichlid fish radiations. Then we review the replicates of parallel divergence that our group investigates. Finally, we subject these data to an integrated comparative analysis for testing effects of variation in spatial structure on the progress in speciation.

GEOGRAPHICAL MODES OF SPECIATION IN AFRICAN GREAT LAKE HAPLOCHROMINES

The very high ratio of endemic species to river-dwelling species in and around the large lakes, Lake Victoria and Malawi, has sometimes been taken as support for sympatric speciation at coarse geographical grain (Meyer et al. 1990). On the other hand, in recent years allopatric speciation has been suggested to be the predominant mechanism of speciation in the African Great Lake cichlids (Kocher 2004). In these large lakes, two models of speciation involving geographical isolation may account for a significant portion of the species origination. The first

involves geographical speciation in isolated lake basins or satellite lakes during lake level low stands, followed by secondary sympatry during subsequent lake level high stands (Sturmbauer 1998). Although proposed dozens of times, compelling evidence is rare. A well-supported case comes from Lake Malawi (Genner et al. 2007). Indirect evidence exists for Lake Tanganyika haplochromines (Sturmbauer et al. 2003), whereas for the classical case of the Lake Victoria satellite Lake Nabugabo (Greenwood 1965), evidence remains anecdotal. More generally, the explanatory power of this mechanism appears limited in lakes where many species evolved during periods of relatively insignificant water level fluctuations. The most extreme such case is Lake Victoria after the latest refilling 15,000 years ago (Stager and Johnson 2008).

The second model involves intralacustrine allopatric speciation when populations diverge in isolation on patches of the same type of habitat separated from each other by other, unsuitable habitat. Such speciation has often erroneously been called microallopatric in the cichlid literature, but the latter concept more correctly applies to speciation that appears at coarse grain sympatric (geographical ranges fully overlapping) but allopatric at fine grain (i.e., host-race formation) (Fitzpatrick et al. 2008). Support for intralacustrine allopatric speciation mostly comes from patterns of species distribution and intraspecific genetic differentiation and is mostly confined to cichlid groups that are restricted to patchily distributed habitats like rocky shores (Kocher 2004). However, given that such habitat specialization does not exist in typical riverine haplochromines, and by inference did probably have to evolve from habitat generalist ancestors after these colonized the lakes, it is perhaps unlikely that geographical isolation alone would have been sufficient to initiate the radiations.

On the other hand, there is compelling evidence for sympatric speciation in cichlid fish of other evolutionary lineages that occupy small isolated crater lakes in West Africa and Nicaragua. While these lineages have made small radiations in isolated crater lakes, they have not made larger species flocks despite their occurrence in large lakes too. Mitochondrial and nuclear genomic evidence taken together suggest that all eleven endemic species of crater lake Barombi Mbo in Cameroon have arisen within the lake, which is thought to be too small to allow

within-lake geographical isolation (Schliewen and Klee 2004). While allopatric speciation seems very unlikely indeed in this lake, and also in the nearby lakes Ejagham and Bermin that have smaller radiations too, detailed population-biological analyses required to reveal the fine grain of spatial structure are yet to be conducted. The latter concern also applies to the other compelling case of sympatric speciation in cichlid fish, the heroine cichlids of crater lakes in the vicinity of Lake Nicaragua (Barluenga et al. 2006). The question really is, how much or how little spatial differentiation is required for speciation in cichlid fish and beyond. In this chapter, we attempt to provide an answer to this question for Lake Victoria cichlids.

MODE AND MECHANISM OF SPECIATION IN REPLICATED CASES OF INCIPIENT SPECIATION

General Approach

We identify replicate pairs of phenotypically similar cichlid morphs and species that occur at multiple islands and vary between islands in the extent of their genetic and phenotypic differentiation, that is, in their “progress in speciation” (Nosil and Sandoval 2008). We investigate such replicate pairs for each of the three major classes of phenotypic polymorphisms that have been associated with incipient speciation in Lake Victoria cichlids; male nuptial coloration, trophic (dental) morphology, and X-linked (female) coloration. Using these, we explore the conditions that are associated with variation in the extent of differentiation (i.e., the “progress” in speciation). We ask:

1. Does ecological habitat structure matter to speciation?
2. Which trait complexes are most strongly affected by divergent selection?

Throughout, our definition of “species” is one close to the genotypic cluster definition (Mallet 1995). We consider two groups species if they differ significantly in neutral multilocus genotypes and maintain these differences in sustained sympatry. We measure such differentiation using at least nine microsatellite loci.

Replicated Polymorphisms

MALE NUPTIAL COLORATION

The most frequent intra- and interspecific polymorphism in male nuptial coloration involves blue versus yellow-red flank coloration of males (Seehausen and van Alphen 1999). We investigated replicate populations of one taxon pair that epitomizes this type of polymorphism. *Pundamilia pundamilia* and *P. nyererei* are a geographically completely sympatric pair of sibling species endemic to the lake proper (plate XXa). The distribution of *P. nyererei* is nested within that of *P. pundamilia*. Independent evidence for speciation in this phenotype pair comes from neutral marker differentiation among sympatric populations (plate XXc). Where the phenotypes are significantly differentiated incipient species, many differences between them have been documented (reviewed in Seehausen 2009). These include differences in depth distribution (Seehausen 1997; Seehausen et al. 2008), stomach contents (Bouton et al. 1997), stable isotopes (Mrosso et al. unpublished), parasites (Maan, Van Rooijen, et al. 2008), color vision (Carleton et al. 2005; Maan et al. 2006; Seehausen et al. 2008), female mate choice (Haesler and Seehausen 2005; Seehausen and van Alphen 1998; Stelkens et al. 2008; van der Sluijs, van Dooren, et al. 2008), male aggression bias (Dijkstra, Hemelrijk, et al. 2008; Dijkstra et al. 2006; Dijkstra et al. 2007; Verzijden et al. 2009), and male behavioral dominance (red > blue) (Dijkstra et al. 2005). To test for evidence of selection, we calculated and compared F_{ST} in opsin genes with F_{ST} in unlinked microsatellites.

Trophic Morphology

The genus *Neochromis* is composed of epilithic algae scrapers with various degrees of specialization. At sites in turbid waters, *Neochromis greenwoodi* is a rather unspecialized omnivorous scraper. At islands with clearer water and hence better algae growth, the species assumes a more specialized scraper morphology with a strongly decurved dorsal head profile, subequally bicuspid oral teeth in the outer tooth rows in both oral jaws, and many bands of oral teeth (Bouton et al. 1999;

Seehausen 1996). At clear water offshore islands it is generally replaced by its allopatric sister species *N. omnicaeruleus* with similar but even more specialized algae scraper morphology. At Bihiru Island the species *N.* “Bihiru scraper” takes their position. At some of the islands with a relatively specialized scraper population of either species, a second sympatric phenotype occurs. This morph is distinguished by unicuspid teeth in the outer tooth rows, teeth arranged in fewer bands, and usually a less strongly decurved dorsal head profile (plate XXb). The distribution of the ratio unicuspid/bicuspid teeth is at all three islands consistent with disruptive selection on the dental morphology (fig. 14.1c). We found that the replicate pairs of these sympatric morphs show within-island phenotype-environment correlations between dentition type and water depth: the unicuspid morphs tend to occupy deeper waters (Magalhaes et al. unpubl. data). At one island (Makobe) we collected stable isotope data, and these suggest long-term differences in diet: the unicuspid morph tends to be more “limnetic” (H.D.J. Mrosso et al. unpublished). Subsequently, we refer to these sympatric phenotypes as ecomorphs. We studied the sympatric polymorphism at three different islands, using phenotypic information and neutral genetic markers.

To test for evidence of selection, we estimated P_{ST} values for morphology and for dentition. P_{ST} values are equivalent to Q_{ST} values (Spitze 1993) but may be influenced by environmental and non-additive genetic effects. P_{ST} values for morphology were estimated from Principal Components. Between- and within-ecomorph components of phenotypic variances were estimated by performing an analysis of variance using SPSS version 14.0 (SPSS Inc.). Bootstrapping over individuals, using R (<http://www.r-project.org/>), was performed to calculate confidence intervals. P_{ST} values were quantified as the proportion of variance in quantitative traits attributable to differences among ecomorphs ($P_{ST} = \sigma^2_{gb}/\sigma^2_{gb} + 2(h^2\sigma^2_{g\tau w})$, where σ^2_{gb} and $\sigma^2_{g\tau w}$ are the among-ecomorph and within-ecomorph variance components respectively (Spitze 1993) and h^2 is the heritability. We have assumed a heritability of 0.5 (Merilä 1997; Bernatchez 2004; Ostbye et al. 2005; Leinonen et al. 2006). P_{ST} values were considered significantly different from multilocus F_{ST} values when their 95 percent confidence intervals did not overlap.

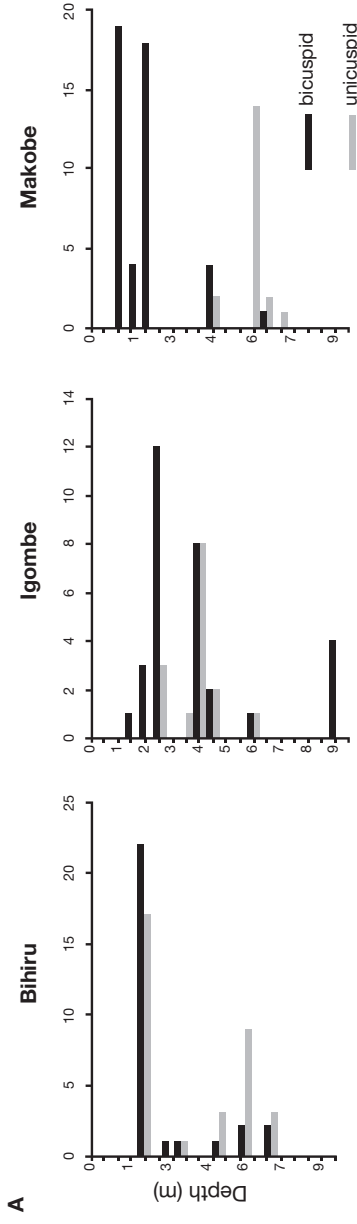


Figure 14.1. Ecological, phenotypic, and genetic divergence between *Neochromis* ecomorphs at three islands (data for the same island are presented in the same column). (a) Depth distributions of ecomorphs. (b) The shore types (from left to right): steep with large boulders, intermediate slope and boulder size, gentle with small boulders. (c) Frequency distributions of tooth phenotypes, quantified as the ratio unicuspid/bicuspid teeth. (d) Measures of neutral versus adaptive differentiation between sympatric ecomorphs. P_{ST} values (with their 95% confidence intervals) versus multilocus F_{ST} values (with their 95% confidence intervals; 9 μ sat loci). Data from left to right refer to morphometric PC1, PC2, and PC3, number of tooth rows and percentage of unicuspid teeth in the outer tooth row. The solid lines indicate the multilocus F_{ST} value. The dotted lines indicate the 95% confidence intervals of the F_{ST} value.

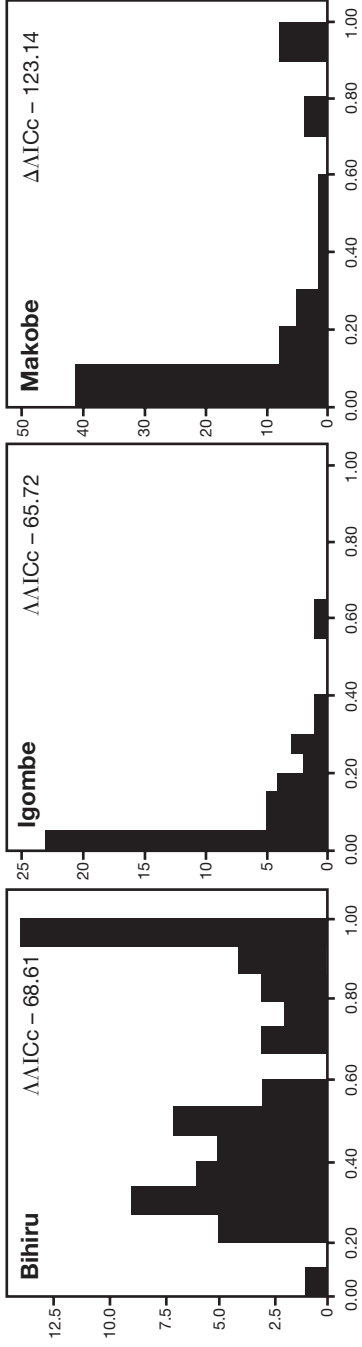
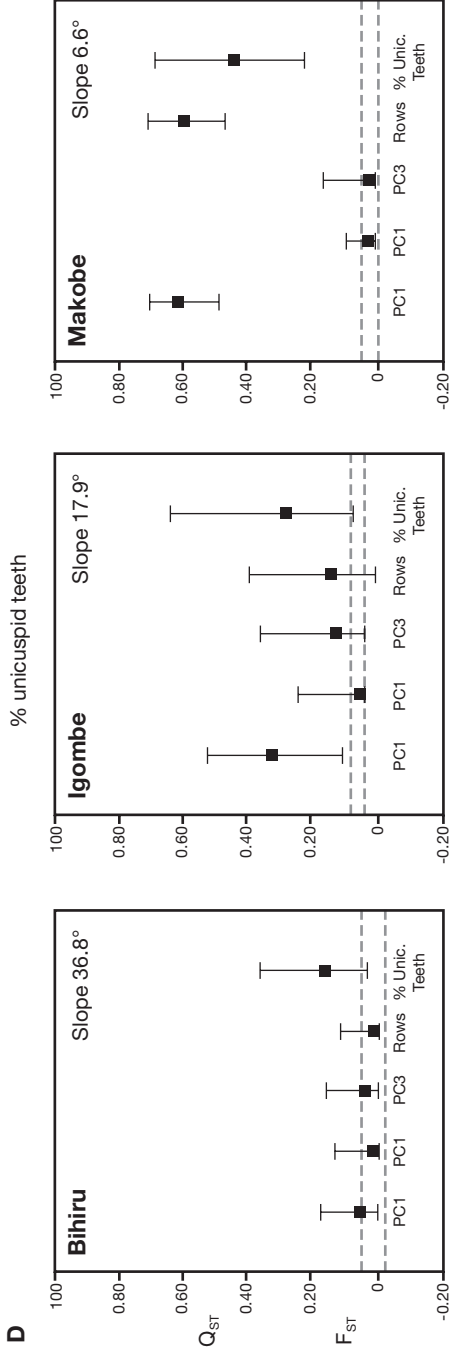
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Figure 14.1. (Continued)

X-linked (Female) Color

Very similar X-linked female color polymorphisms are found in several genera of Lake Victoria cichlids, and in multiple species within some of the genera. This type of polymorphism involves an orange blotched (OB; black blotches on orange), a white blotched (WB; black blotches on white), and a normal “plain” phenotype (plate XXc). OB and WB color is sex linked in all populations for which data are available (Lande et al. 2001). Sex linkage of both OB and WB has been attributed to weak physical linkage between two different major effect genes for color and (dominant) female determination, which together are linked to the original (recessive) female determining X chromosome (Seehausen et al. 1999). Blotched individuals are female, unless additional autosomal male determining genes are present that more than compensate for the dominant female effect of the OB and WB genes. OB and WB males are extremely rare (<1%) in nature in most studied populations (Lande et al. 2001; Maan, Eshuis, et al. 2008; Seehausen et al. 1999). Species possessing OB or WB morphs usually, but not always, also possess a plain (P) morph in both sexes, which is considered the ancestral condition. Theoretical studies modeling this polymorphism suggested that sympatric speciation may be possible through the interaction of selection on sex reversal and sexual selection (Lande et al. 2001).

Most of the knowledge on this type of polymorphism derives from studies on *N. omnicaeruleus*. Some populations of this species possess all three female color morphs: WB, OB, and P, others have just two (generally OB and P), and again others have just P morphs. Geographically the three morphs are fully sympatric with WB nested in OB and P, and OB nested in P (plate XXc). There is no evidence of microhabitat differentiation (fig. 14.4a) (Maan, Eshuis, et al. 2008; Seehausen and Bouton 1997) and only weak evidence for morphological differentiation between color morphs (Magalhaes et al. unpubl. data; Seehausen et al. 1999). Yet, behavioral investigations had found partially assortative mating preferences based on this color variation (Seehausen et al. 1999), and assortative aggression biases among the females (Dijkstra, Seehausen, et al. 2008). We have reported frequencies of the three *N. omnicaeruleus* color morphs in a natural population for several years and found a large numerical deficiency of intermediate phenotypes

suggesting nonrandom mating and/or strong disruptive selection on the phenotype (fig. 14.2b) (Maan, Eshuis, et al. 2008; Magalhaes et al. unpubl. data; Seehausen et al. 1999). We investigated the differentiation between the color morphs at this island and in *N. greenwoodi* at Igombe Island using phenotypic and neutral genetic markers (Magalhaes et al. unpubl. data).

A METHOD FOR ASSESSING AND COMPARING EFFECTS OF FINE GRAIN SPATIAL STRUCTURE

In order to compare the relative importance to speciation of spatial ecological structure between the three common types of polymorphisms, we calculated for each replicate phenotype pair the correlation between numerical prevalence of either phenotype and water depth. A correlation coefficient (R) of 1 is the equivalent of saying that phenotypes have zero spatial overlap. A correlation coefficient of 0 is the equivalent of saying that phenotypes have complete spatial overlap. We then asked if variation in the progress of speciation (measured by the global μ sat F_{ST}), was explained by the variation in spatial overlap, by the kind of phenotypic polymorphism, or the interaction between these. We first calculated Pearson's Correlation Coefficients between global F_{ST} and the phenotype*depth correlation. We then calculated a general linear model with global F_{ST} as dependent variable, type of polymorphism (3 types) as factor, and the correlation coefficient (R) between phenotype and water depth as a covariate.

To express spatial overlap in a general currency that is comparable across studies, we propose to express the correlation coefficient between phenotype and water depth in terms of an estimate of spatial opportunity for cross-mating between morphs. We can calculate potential m as

$$m = \frac{(1-R)}{2}$$

where m ranges from 0 (two spatially isolated populations) to 0.5 (a spatially panmictic population). Importantly, our estimate of m is not an estimate of actual gene flow, but one of the potential for gene flow between morphs if there was no behavioral assortative mating (note

that seasonality does not contribute to reproductive isolation between these incipient species).

RESULTS

We will first review our results for each of the three polymorphisms, followed by a discussion on the quantitative comparative analysis of all phenotype pairs.

1. Male Nuptial Coloration

Evidence for incipient speciation among geographically sympatric male nuptial color morphs is seen in significant neutral marker differentiation (we used 11 microsatellites) at three of our five islands (plate XXc). Evidence for $m > 0$ is seen in replicate pairs studied at five islands: sympatric populations of different phenotype are often more similar to each other in their allele frequencies at neutral loci (11 unlinked microsatellites) than allopatric populations of same phenotype (fig. 14.3a). The extent of sympatric differentiation at neutral loci exhibits continuous variation between replicate phenotype pairs (plate XXc). The extent of sympatric differentiation in male nuptial coloration too exhibits continuous variation (plate XXb). In contrast, the extent of sympatric differentiation in a visual pigment gene exhibits rather discontinuous variation (plate XXd). Where phenotypes are differentiated incipient species, “Blue” lives in more shallow waters than “Red.” in more blue shifted ambient light (plate XXa). Haplochromine cichlids of Lake Victoria express four or five different opsin genes, each coding a visual pigment with peak sensitivities at different wavelength. The most variable of these genes among the cichlids of Lake Victoria is the long wavelength sensitive opsin gene (*LWS*). *LWS* alleles of individuals of the blue *Pundamilia* species are blue shifted by 15 nm relative to those of fish of the red sister species. We determined this through in-vitro mutagenesis of the red shifted allele, expression of both proteins and absorbance measurements (Seehausen et al. 2008; Terai et al. 2006).

Ambient subsurface light composition changes markedly with increasing water depth, not just in its intensity but in its wavelength composition. The slope of this light gradient varies with water transparency and physical shore slope (steep when water is turbid and when

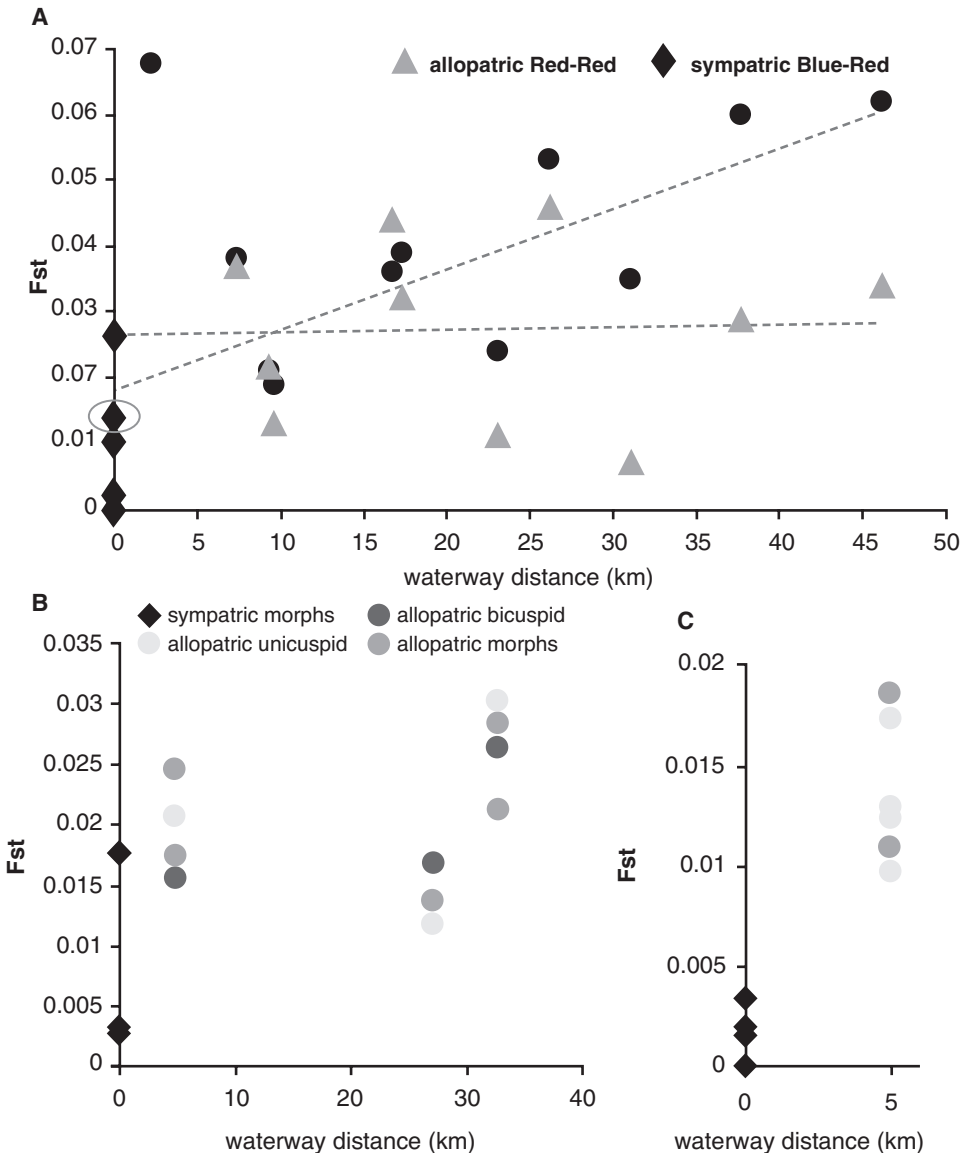


Figure 14.3. Evidence for divergence with gene flow. Microsatellite-based estimates of F_{ST} between sympatric phenotypes (black diamonds) are generally smaller than those between allopatric populations of the same phenotype, here plotted against the geographical distance between sampling sites. (a) Blue and red male nuptial color morphs of *Pundamilia*. (b) Tooth shape morphs of *Neochromis*. (c) X-linked color morphs of *Neochromis* (orange = allopatric different color, gray = allopatric same color).

the shore slopes steeply), whereas its magnitude (i.e., the difference in spectral composition between the ends of the gradient) was very similar at all studied sites. Given constant gradient magnitude among the five islands studied, the length of the gradient correlates negatively with its slope. It follows that the physical distance in meters between the extreme environments becomes shorter as the slope gets steeper. This variation in the gradient slope explains 87 percent of the variance in the extent of phenotypic differentiation along water depth, 65 percent of that in genotypic differentiation at the *LWS* locus, and 90 percent of that in genotypic differentiation at eleven microsatellites. If the gradient is very short and steep, progress in speciation is not observed, despite the presence of considerable variation in color and mating preferences. Speciation is hence not constrained by lack of suitable genetic variation (Seehausen et al. 2008).

Earlier we had studied similar variation in *LWS* genes and male nuptial coloration in *Neochromis greenwoodi*, but in this case, divergently adapted populations occur in geographical parapatry, namely on different islands. In this case we again saw strong adaptation in the *LWS* gene, but not much progress toward speciation. Only a very incomplete association of *LWS* alleles with color was observed (Terai et al. 2006). Taking the two investigations together, we observed adaptation whenever migration between contrasting environments was reduced ($0.5 > m = 0$), but we observed speciation only when $0.5 > m > 0$, whereas neither when migration between contrasting environments was unreduced (i.e., $m \sim 0.5$), nor when it was close to zero.

2. Trophic Morphology

Evidence for incipient speciation among geographically sympatric trophic ecomorphs is seen in significant neutral marker differentiation at one of our three islands (Igombe Island, $F_{ST} = 0.018$, $p < 0.001$). Ecomorphs occupying the same island (sympatric morphs) are often more similar to each other in their allele frequencies at neutral loci (9 unlinked microsatellites) than allopatric populations belonging to the same ecomorph (fig. 14.3b), implying $m > 0$ between the ecomorphs. Islands vary in the extent of phenotypic differentiation between ecomorphs, from single traits to more than ten different traits. Just like among the five replicate pairs in male nuptial coloration, the extent of

differentiation between sympatric ecomorphs exhibits continuous variation, both in the number of traits that are divergent, and in the extent of their divergence (fig. 14.31d).

Phenotypic differentiation (P_{ST}) between ecomorphs was generally stronger than the neutral genetic differentiation (F_{ST}) (fig. 14.1d). At Makobe Island, divergence in quantitative functional traits, as measured by P_{ST} , was high for several morphological traits. The P_{ST} values and their confidence intervals for PC1 ($P_{ST} = 0.433$), number of tooth rows ($P_{ST} = 0.471$), and percentage of unicuspid teeth ($P_{ST} = 0.3$) did not overlap with the confidence interval of the F_{ST} value, indicating that differentiation along these axes was higher than expected by drift alone, and that several traits might be under divergent selection. When compared to the results from Makobe, the divergence in phenotypic traits between ecomorphs at Igombe Island was low. Only for PC1 and the percentage of unicuspid teeth did the C.I.s not overlap with the C.I. of the F_{ST} estimate, indicating divergent selection acted on fewer morphological traits than at Makobe. At Bihiru Island, P_{ST} values were even lower. With the exception of the percentage of unicuspid teeth, all confidence intervals overlapped fully with the confidence interval of the F_{ST} value.

The three islands had different combinations of water clarity, shore slope steepness, and boulder size, generating environmental gradients that differed in steepness and in spatial linearity. Clear water and gentle lake floor slopes make the transition from algae growth dominated shallow water to poorly illuminated deeper water long and gradual. Small boulders generate a locally homogeneous light environment, allowing for a spatially linear gradient of light-dependent resources that only depends on water depth. Large boulders, to the contrary, create a spatial mosaic of light habitats and associated resource abundances. The extent of phenotypic differentiation between trophic ecomorphs was predicted by the slope and length of the environmental gradient (in this case simply water depth; fig. 14.1d), similar to the case of male nuptial color polymorphism and visual adaptation. However, neutral genetic differentiation was generally weaker, and variation in the extent of neutral genetic differentiation was not well predicted by the gradient slopes, nor did it correlate with the extent of phenotypic differentiation (Magalhaes et al. unpubl. data).

3. *X-linked (Female) Color*

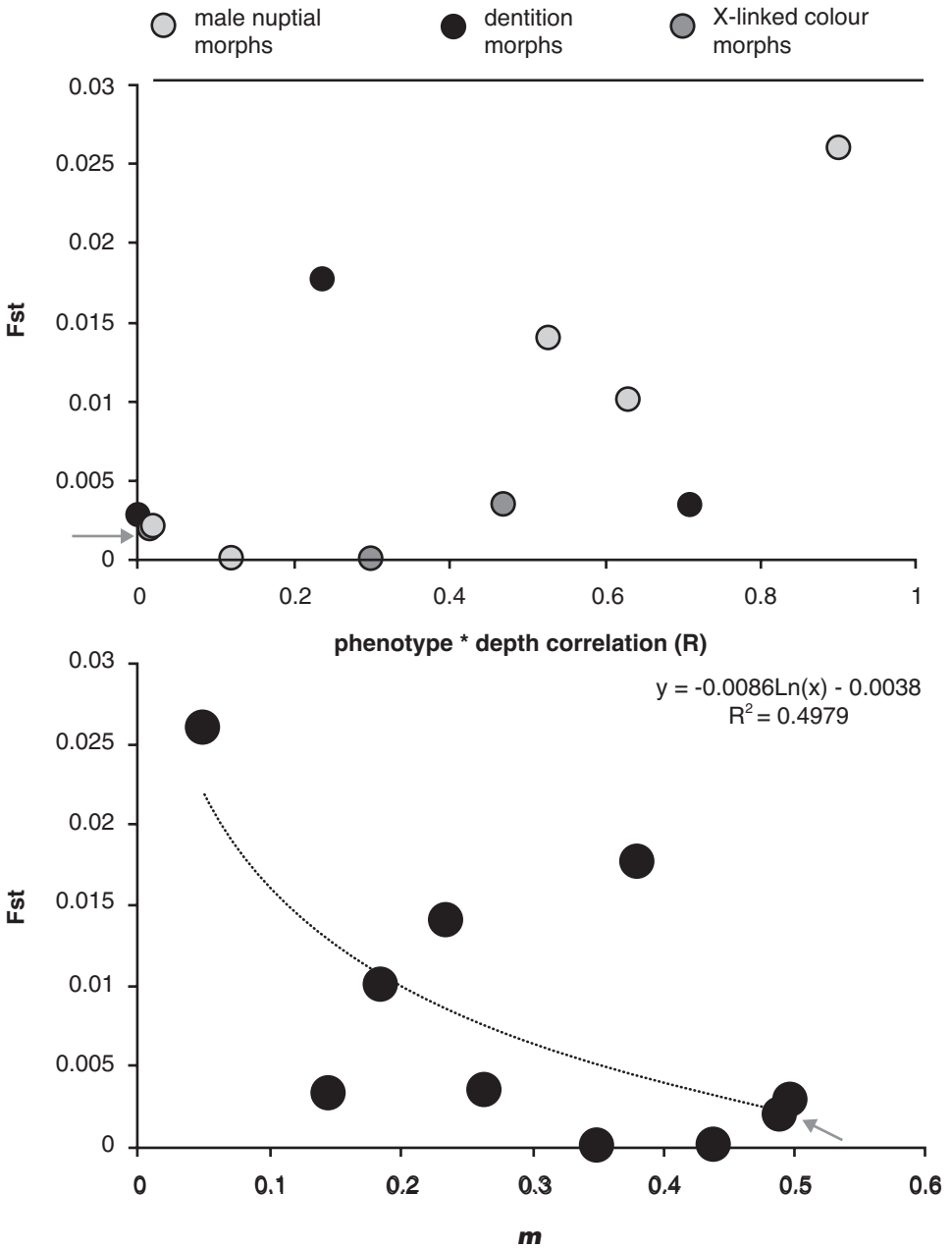
We found evidence for non-random mating among the OB and P color morphs of *Neochromis omnicaeruleus* at Makobe Island, as indicated by significant assignment tests based on neutral markers (9 microsatellites), linkage disequilibrium at many loci, and significant F_{ST} at two of the 9 loci. However, it appears as if speciation had not progressed beyond the stage of incompletely assortative mating, since multilocus F_{ST} did not differ significantly between any of the color morph pairs (Magalhaes et al. unpubl. data). This was so despite the presence of slight morphological and perhaps dietary differences (HJD Mrosso et al. unpublished data), and despite the presence of matching and quite discrete female color polymorphisms (Seehausen et al. 1999) and male mating preference polymorphisms (Pierotti et al. 2009 and fig. 14.2), which most likely are maintained by some form of disruptive selection. Hence, here too, neither lack of suitable genetic variation nor the form of selection constrain the progress in speciation.

Different color morphs from the same island were generally more similar to each other in their allele frequencies than the same (or different) color morphs from different islands (fig. 14.3c), implying that incipient differentiation between sympatric morphs is maintained despite gene flow. We could not detect any significant differences in depth distribution between these morphs at Makobe Island (fig. 14.2a), nor any other microdistribution differences.

In another investigation of a WB/P color polymorphism in the distantly related *Paralabidochromis chilotes* (plate XXc), we did observe population genetic and behavioral evidence for complete speciation into sister species fixed for different X-linked female color. However, in this case the incipient species occupy different islands, though fully nested geographically with evidence for gene flow, suggesting parapatric speciation with a large component of geographical isolation (M. Pierotti et al. unpublished data).

4. *Effects of Spatially Fine Grained Environmental Structure*

The correlation between prevalence of either phenotype and water depth was significant for the male nuptial color morphs in *Pundamilia* at three of five islands, and for the dentition morphs of *Neochromis* at



one of three islands (Makobe Island), but was not significant in the other cases, indicating no or only weak associations between phenotype and water depth. Over all eleven morph pairs, there was a significant positive correlation between the multilocus F_{ST} and the magnitude of the phenotype*water depth correlation ($R = 0.62$, p (1-tailed) = 0.02; fig. 14.4). In a general linear model with multilocus F_{ST} as dependent variable, the type of polymorphism did not explain variation in F_{ST} (2df, $F = 0.63$, $p = 0.56$), whereas there was a trend for the magnitude of the phenotype*water depth correlation to explain variation in F_{ST} (1df, $F = 3.8$, $p = 0.09$).

Calculating the spatial opportunity for gene flow between phenotypes, we find that significant progress in the speciation process was observed in the range $0 \leq m \leq 0.4$ and that three of four cases were in the range $0 \leq m \leq 0.25$. The spatial opportunity for gene flow (m) predicts F_{ST} as

$$F_{ST} = -0.0086Ln(m) - 0.0038$$

DISCUSSION

Ecologically Parapatric Speciation along Depth-mediated Habitat Gradients

The population genetics and ecology of morph differentiation in eleven different pairs of geographically sympatric morphs of Lake Victoria cichlid fish suggest that the extent of spatial segregation of spawning sites along the sloping lake floor predicts the progress toward speciation. Such speciation, even though completely sympatric at coarse geographical scale (plate XX), is ecologically parapatric at fine grain. We estimate that speciation is possible with m , here defined as the spatial opportunity for cross-mating between morphs, between 0 and 0.4, and

Figure 14.4. Progress in speciation as a function of the phenotype-depth correlation and m . Progress in speciation as the multilocus F_{ST} value, measured using 9–11 microsatellites, in 11 pairs of sympatric morphs and incipient species of Lake Victoria cichlids. (a) Multilocus F_{ST} as a function of the phenotype-depth correlation. See legend for symbol explanation. Orange arrowhead points to one orange data point hidden behind a green data point. (b) Multilocus F_{ST} as a function of the potential for gene flow, m .

when m is below 0.25 perhaps even likely. If the spatial opportunity for cross-mating between morphs is as similar now as in the past, speciation is close to sympatric even in its population genetic definition. We observed several morph pairs that were not spatially segregated at all, hence $m \sim 0.5$. None of these pairs revealed significantly differentiated allele frequencies at nine to eleven microsatellite loci. This applied to all three major classes of phenotypic polymorphisms that have been associated with incipient speciation in Lake Victoria cichlids; male nuptial coloration, trophic (dental) morphology, and X-linked (female) coloration.

Despite absence of differentiation at neutral DNA loci, several of these fully sympatric morph pairs were phenotypically nevertheless strongly differentiated. Failure to proceed in speciation can hence not be explained by lack of suitable genetic variation. Neither can it be explained by lack of selection. The frequency distribution of tooth shape morphs revealed evidence for response to disruptive selection in a case of $m \sim 0.5$. Similarly, the frequency distribution of the X-linked female coloration at Makobe Island was consistent with disruptive selection. Finally, female preference polymorphism is expected to exert disruptive selection on male nuptial coloration in *Pundamilia* even at the sites where $m \sim 0.5$ (Stelkens et al. 2008).

Where gene flow between the morphs is restricted by even small amounts of fine grain spatial segregation, these polymorphisms appear to often but not always achieve the stage of incipient species with isolation-by-adaptation (Nosil et al. 2008) permitting the onset of divergence at neutral unlinked DNA loci. The polymorphisms that we studied are widespread and common in Lake Victoria. Hence, geographically sympatric ecologically parapatric speciation may be widespread too in haplochromine cichlid fish of Lake Victoria, but ecologically fully sympatric speciation may be rare or absent. The very weak population differentiation that we observe between allopatric populations of several Lake Victoria cichlid species, occupying habitat patches that are separated by many kilometers of unsuitable habitat (fig. 14.3), suggests that the scope for completely allopatric speciation may be limited. Interestingly, we observed complete speciation between populations with divergent visual adaptations only in geographical sympatry / ecological parapatry (Seehausen et al. 2008). In geographical allopatry,

in contrast, divergent adaptation was observed, but no speciation (measured as the completeness of association between opsin alleles and male nuptial coloration (Terai et al. 2006)). It is tempting to speculate that speciation at the rates seen in the Lake Victoria cichlid fish radiation may sometimes require migration and gene-flow to generate selection for assortative mating in a reinforcement-like process.

Support from Evolutionary Species Area Relationships

We are not suggesting that geographically sympatric speciation is the most frequent mode of speciation in haplochromine cichlids. The shape of the evolutionary species area relationship for African cichlid fish (Seehausen 2006) suggests a combination of more sympatric-like and more allopatric-like speciation in the evolution of cichlid species flocks. The number of endemic species has a flat relationship with lake size across several orders of magnitude of lake size variation, but suddenly increases steeply when lakes become larger than 1000 km² surface area. It seems that speciation did indeed occur in many lakes without opportunity for geographical isolation, but that many more species are produced in large lakes where there is greater opportunity for geographical isolation. The two arms of the curve are reminiscent of the relatively flat and steep slopes (\approx) respectively of intra- and interprovincial species area curves in island biogeography. Scope for allopatric speciation is significant between provinces (or islands), but not within (Rosenzweig 1997; Rosenzweig 2001).

*Is Divergent Adaptation along Aquatic Depth Gradients
a “Magic trait”?*

Sympatric speciation has been reported in lake-dwelling fishes probably more often than in other vertebrates. Lakes (and the sea) differ from many terrestrial vertebrate environments (and many river environments) by the additional spatial dimension of water depth and the associated strong environmental gradients that exist within even small habitat patches. Water depth mediates gradients in light intensity and composition, oxygen concentration, and temperature. These in turn affect resources, predators, parasites, sensory and signaling requirements, and hence call for divergent adaptation along the gradients within aquatic populations. If variable depth adaptation causes deviation from

random mating, adaptation to depth may act like a “magic trait” in speciation. This scenario has a lot in common with host-plant-based mating of phytophagous insects, but with the difference that the discreteness of habitat patches may make speciation in phytophagous insects microallopatric, whereas the gradient-like nature of the habitat makes speciation in lacustrine fish ecologically parapatric. Our data suggest that such speciation may be typical in the rapid adaptive radiation of Lake Victoria cichlid fish.

A non-exhaustive literature survey on speciation in other fish suggests that divergent adaptation along water-depth-mediated habitat gradients may explain the apparent propensity for geographically sympatric speciation in lacustrine fish more generally. Divergent adaptation along depth gradients (to feeding and spawning requirements) appears to be critical to ecological speciation of whitefish (Vonlanthen et al. 2008), and ciscoes (Ohlberger et al. 2008; Ohlberger et al. 2009), and is involved in speciation of sticklebacks (Boughman 2001), and silversides (Herder et al. 2008). The distribution of spawning sites suggests some depth segregation of sister species also in classical cases of sympatric speciation in lakes (Barluenga et al. 2006; Schliewen et al. 2001). Finally, similar divergent adaptation along depth gradients is likely to drive ecologically parapatric speciation in the sea too, both in fish (Hyde et al. 2008) and in invertebrates (Grahame et al. 2006).

While we here conclude that geographically sympatric speciation may be more common than previously thought, a corollary of our observations is that spatial environmental structure does indeed have strong impact on the likelihood of ecological speciation with gene flow through permitting fine grain parapatric conditions nested in coarser grain sympatric conditions. Three predictions follow: (1) geographically sympatric speciation is expected to be less common in terrestrial systems that lack the strong third dimension at least for vertebrates. This prediction finds circumstantial support in existing reviews of speciation in terrestrial vertebrates (Coyne and Price 2000). (2) Classical cases of sympatric speciation in lacustrine fish may reveal themselves as ecologically parapatric at fine grain upon closer examination. The test of this prediction awaits quantitative phenotype distribution mapping in the Cameroonian and Nicaraguan crater lakes. (3) When disruptive selection on traits not associated with water depth nevertheless is associated with

significant progress in speciation, other yet hidden phenotype-space associations may often be discovered on closer inspection.

REFERENCES

- Abbott, R. J., M. G. Ritchie, and P. M. Hollingsworth. 2008. Speciation in plants and animals: pattern and process—Introduction. *Philos. Trans. R. Soc. B* 363: 2965–2969.
- Adams, C. E., A. J. Wilson, and M. M. Ferguson. 2008. Parallel divergence of sympatric genetic and body size forms of Arctic charr, *Salvelinus alpinus*, from two Scottish lakes. *Biol. J. Linn. Soc.* 95: 748–757.
- Barluenga, M., K. N. Stolting, W. Salzburger, M. Muschick, and A. Meyer. 2006. Sympatric speciation in N
- Berlocher, S. H., and J. L. Feder. 2002. Sympatric speciation in phytophagous insects: Moving beyond controversy? *Annu. Rev. Entomol.* 47: 773–815.
- Blais, J., C. Rico, C. van Oosterhout, J. Cable, G. F. Turner, G. F., and L. Bernatchez. 2007. MHC adaptive divergence between closely related and sympatric African cichlids. *PLoS ONE* 2, e734. doi: 10.1371/journal.pone.0000734.
- Bolnick, D. I., and B. M. Fitzpatrick. 2007. Sympatric speciation: Models and empirical evidence. *Annu. Rev. Ecol. Evol. Syst.* 38: 459–487.
- Boughman, J. W. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature* 411: 944–948.
- Bouton, N., O. Seehausen, and J.J.M. van Alphen. 1997. Resource partitioning among rock-dwelling haplochromines (Pisces : Cichlidae) from Lake Victoria. *Ecology of Freshwater Fish* 6: 225–240.
- Bouton, N., F. Witte, J.J.M. van Alphen, A. Schenk, and O. Seehausen. 1999. Local adaptations in populations of rock-dwelling haplochromines (Pisces : Cichlidae) from southern Lake Victoria. *Proc. R. Soc. London B* 266: 355–360.
- Bush, G. ed. 1998. *The Conceptual Radicalization of an Evolutionary Biologist. Endless Forms: Species and Speciation*. New York: Oxford University Press.
- Bush, G. L. 1969. Sympatric host race formation and speciation in frugivorous flies of genus *Rhagoletis* (Diptera, Tephritidae). *Evolution* 23: 237–.
- Butlin, R. K., J. Galindo, and J. W. Grahame. 2008. Sympatric, parapatric or allopatric: the most important way to classify speciation? *Philosophical Transactions of the Royal Society B-Biological Sciences* 363: 2997–3007.
- Carleton, K. L., J.W.L. Parry, J. K. Bowmaker, D. M. Hunt, and O. Seehausen. 2005. Colour vision and speciation in Lake Victoria cichlids of the genus *Pundamilia*. *Mol. Ecol.* 14: 4341–4353.

- Coyne, J., and H. A. Orr. 2004. *Speciation*. Sunderland, MA: Sinauer Associates.
- Coyne, J. A., and T. Price. 2000. Little evidence for sympatric speciation in island birds. *Evolution* 54: 2166–2171.
- Dijkstra, P. D., C. Hemelrijk, O. Seehausen, and T.G.G. Groothuis. 2008. Colour polymorphism and intrasexual competition in assemblages of cichlid fish. *Behav. Ecol.*: 138–144.
- Dijkstra, P. D., O. Seehausen, B.L.A. Gricar, M. E. Maan, and T.G.G. Groothuis. 2006. Can male-male competition stabilize speciation? A test in Lake Victoria haplochromine cichlid fish. *Behav. Ecol. Sociobiol.* 59: 704–713.
- Dijkstra, P. D., O. Seehausen, and T.G.G. Groothuis. 2005. Direct male-male competition can facilitate invasion of new colour types in Lake Victoria cichlids. *Behav. Ecol. Sociobiol.* 58: 136–143.
- . 2008. Intrasexual competition among females and the stabilization of a conspicuous colour polymorphism in a Lake Victoria cichlid fish. *Proceedings of the Royal Society B-Biological Sciences* 275: 519–526.
- Dijkstra, P. D., O. Seehausen, M.E.R. Pierotti, and T.G.G. Groothuis. 2007. Male-male competition and speciation: aggression bias towards differently coloured rivals varies between stages of speciation in a Lake Victoria cichlid species complex. *J. Evol. Biol.* 20: 496–502.
- 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, NJ: Princeton University Press.
- Genner, M. J., P. Nichols, G. Carvalho, R. L. Robinson, P. W. Shaw, A. Smith, and G. F. Turner. 2007. Evolution of a cichlid fish in a Lake Malawi satellite lake. *Proc. R. Soc. London B* 274: 2249–2257.
- Grahame, J. W., C. S. Wilding, and R. K. Butlin. 2006. Adaptation to a steep environmental gradient and an associated barrier to gene exchange in *Littorina saxatilis*. *Evolution* 60: 268–278.
- Grant, B. R., and P. R. Grant. 2008. Fission and fusion of Darwin's finches populations. *Philos. Trans. R. Soc. B*, doi: 10.1098/rstb.2008.0051.
- Grant, P. R., and B. R. Grant. 2006. Evolution of character displacement in Darwin's finches. *Science* 313: 224–226.
- Greenwood, P. 1965. The cichlid fishes of Lake Nabugabo, Uganda. *Bull. Br. Mus. nat. Hist. (Zool.)* 12: 315–357.
- Haesler, M. P., and O. Seehausen. 2005. Inheritance of female mating preference in a sympatric sibling species pair of Lake Victoria cichlids: implications for speciation. *Proc. R. Soc. London B* 272: 237–245.

- Herder, F., J. Pfaender, and U. K. Schliewen. 2008. Adaptive sympatric speciation of polychromatic “roundfin” sailfin silverside fish in Lake Matano (Sulawesi). *Evolution* 62: 2178–2195.
- Hyde, J. R., C. A. Kimbrell, J. E. Budrick, E. A. Lynn, and R. D. Vetter. 2008. Cryptic speciation in the vermilion rockfish (*Sebastes miniatus*) and the role of bathymetry in the speciation process. *Mol. Ecol.* 17: 1122–1136.
- Kocher, T. D. 2004. Adaptive evolution and explosive speciation: The cichlid fish model. *Nature Reviews Genetics* 5: 288–298.
- Lande, R., O. Seehausen, and J.J.M. van Alphen. 2001. Mechanisms of rapid sympatric speciation by sex reversal and sexual selection in cichlid fish. *Genetica* 112: 435–443.
- Leinonen, T., J. M. Cano, H. Makinen, and J. Merilä. 2006. Contrasting patterns of body shape and neutral genetic divergence in marine and lake populations of threespine sticklebacks. *Journal of Evolutionary Biology* 19: 1803–1812.
- Maan, M. E., B. Eshuis, M. P. Haesler, M. V. Schneider, J.J.M. van Alphen, and O. Seehausen. 2008. Color polymorphism and predation in a Lake Victoria cichlid fish. *Copeia*: 621–629.
- Maan, M. E., K. D. Hofker, J.J.M. van Alphen, and O. Seehausen. 2006. Sensory drive in cichlid speciation. *Am. Nat.* 167: 947–954.
- Maan, M. E., A.M.C. Van Rooijen, J.J.M. van Alphen, and O. Seehausen. 2008. Parasite-mediated sexual selection and species divergence in Lake Victoria cichlid fish. *Biol. J. Linn. Soc.* 94: 53–60.
- Magalhaes, I., B. Lundsgaard-Hansen, S. Mwaiko, and O. Seehausen. Unpubl. data. Eco-morphological but not genetic differentiation within cichlid fish populations correlates with the slope of resource gradients.
- Magalhaes, I., S. Mwaiko, M. Schneider, and O. Seehausen. 2009. Divergent selection and phenotypic plasticity during incipient speciation in Lake Victoria cichlid fish. *J. Evol. Biol.* in press.
- Magalhaes, I., S. Mwaiko, and O. Seehausen. Unpubl. data. Sympatric colour polymorphisms associated with non-random gene flow in cichlid fish of Lake Victoria.
- Magalhaes, I. S., and O. Seehausen. Genetics of male colour in a Lake Victoria cichlid species pair and its role in sympatric speciation. Unpublished manuscript.
- Mallet, J. 1995. A species definition for the modern synthesis. *Trends Ecol. Evol.* 10: 294–299.
- Mayr, E. 1942. *Systematics and the Origin of Species*, New York: Columbia University Press.
- . 1963. *Animal Species and Evolution*. Cambridge, MA: Belknap Press.

- Mayr, E. 1982. *The Growth of Biological Thought: Diversity, Evolution and Inheritance*. Cambridge, MA: Belknap Press.
- Merilä, J. 1997. Quantitative trait and allozyme divergence in the greenfinch (*Carduelis chloris*, Aves: Fringillidae). *Biological Journal of the Linnean Society* 61: 243–266.
- Meyer, A., T. D. Kocher, P. Basasibwaki, and A. C. Wilson. 1990. Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature* 347: 550–553.
- Nosil, P., and B. J. Crespi. 2004. Does gene flow constrain adaptive divergence or vice versa? A test using ecomorphology and sexual isolation in *Timema cristinae* walking-sticks. *Evolution* 58: 102–112.
- Nosil, P., S. R. Egan, and D. J. Funk. 2008. Heterogeneous genomic differentiation between walking-stick ecotypes: “Isolation by adaptation” and multiple roles for divergent selection. *Evolution* 62: 316–336.
- Nosil, P., and C. P. Sandoval. 2008. Ecological niche dimensionality and the evolutionary diversification of stick insects. *PlosOne* 3: e1907.
- Ohlberger, J., T. Mehner, G. Staaks, and F. Hölker, F. 2008. Temperature-related physiological adaptations promote ecological divergence in a sympatric species pair of temperate freshwater fish, *Coregonus* spp. *Funct. Ecol.* 22: 501–508.
- Ohlberger, J., G. Staaks, T. Petzoldt, T. Mehner, and F. Hölker, F. 2008. Physiological specialization by thermal adaptation drives ecological divergence in a sympatric fish species pair. *Evol. Ecol. Res.* 10: 1173–1185.
- Ostbye, K., P. A. Amundsen, L. Bernatchez, A. Klemetsen, R. Knudsen, R. Kristoffersen, T. F. Naesje, and K. Hindar. 2006. Parallel evolution of ecomorphological traits in the European whitefish *Coregonus lavaretus* (L.) species complex during postglacial times. *Mol. Ecol.* 15: 3983–4001.
- Ostbye, K., T. F. Naesje, L. Bernatchez, O. T. Sandlund, and K. Hindar. 2005. Morphological divergence and origin of sympatric populations of European whitefish (*Coregonus lavaretus* L.) in Lake Femund, Norway. *Journal of Evolutionary Biology* 18: 683–702.
- Pierotti, M.E.R., J. A. Martín-Fernández, and O. Seehausen. 2009. Mapping individual variation in male mating preference space: multiple choice in a colour polymorphic cichlid fish. *Evolution*. doi:10.1111/j.1558-5646.2009.00716.x
- Rosenzweig, M. L. 1997. Tempo and mode of speciation. *Science* 277: 1622–1623.
- . 2001. Loss of speciation rate will impoverish future diversity. *Proc. Natl Acad. Sci. USA* 98: 5404–5410.
- Rundle, H. D., L. Nagel, J. W. Boughman, and D. Schluter. 2000. Natural selection and parallel speciation in sympatric sticklebacks. *Science* 287: 306–308.

- Saint-Laurent, R., M. Legault, and L. Bernatchez. 2003. Divergent selection maintains adaptive differentiation despite high gene flow between sympatric rainbow smelt ecotypes (*Osmerus mordax* Mitchell). *Mol. Ecol.* 12: 315–330.
- Schliewen, U., and B. Klee. 2004. Reticulate sympatric speciation in Cameroonian crater lake cichlids. *Frontiers in Zoology* 1, doi: 10.1186/1742-9994-1-5.
- Schliewen, U., K. Rassmann, M. Markmann, J. Markert, T. D. 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.
- Schliewen, U., D. Tautz, and S. Pääbo. 1994. Sympatric speciation suggested by monophyly of crater lake cichlids. *Nature* 368: 306–308.
- Seehausen, O. 1996. *Lake Victoria Rock Cichlids. Taxonomy, Ecology and Distribution.*: Zevenhuizen, NL: Cichlid Press.
- . 1997. Distribution of and reproductive isolation among color morphs of a rock-dwelling Lake Victoria cichlid (*Haplochromis nyererei*). *Ecology of Freshwater Fish* 6: 57–.
- . 2002. Patterns in fish radiation are compatible with Pleistocene desiccation of Lake Victoria and 14 600 year history for its cichlid species flock. *Proc. R. Soc. London B* 269: 491–497.
- . 2006. African cichlid fish: a model system in adaptive radiation research. *Proc. R. Soc. London B* 273: 1987–1998.
- . 2009. *Progressive levels of trait divergence along a “speciation transect” in the Lake Victoria cichlid fish Pundamilia.* Ecology and Speciation. Cambridge: Cambridge University Press.
- Seehausen, O., and N. Bouton. 1997. Microdistribution and fluctuations in niche overlap in a rocky shore cichlid community in Lake Victoria. *Ecology of Freshwater Fish* 6: 161–173.
- Seehausen, O., E. Koetsier, M. V. Schneider, L. J. Chapman, C. A. Chapman, M. E. Knight, G. F. Turner, J.J.M. van Alphen, and R. Bills. 2003. Nuclear markers reveal unexpected genetic variation and a Congolese-Nilotic origin of the Lake Victoria cichlid species flock). *Proc. R. Soc. London B* 270: 2637–2638.
- Seehausen, O., Y. Terai, I. S. Magalhaes, K. L. Carleton, H.D.J. Mrosso, R. Miyagi, I. van der Sluijs, et al. 2008. Speciation through sensory drive in cichlid fish. *Nature* 455: 620–623.
- Seehausen, O., and J.J.M. van Alphen. 1998. The effect of male coloration on female mate choice in closely related Lake Victoria cichlids (*Haplochromis nyererei* complex). *Behav. Ecol. Sociobiol.* 42: 1–8.
- . 1999. Can sympatric speciation by disruptive sexual selection explain rapid evolution of cichlid diversity in Lake Victoria? *Ecology Letters* 2: 262–271.

- Seehausen, O., J.J.M. van Alphen, and R. Lande. 1999. Color polymorphism and sex ratio distortion in a cichlid fish as an incipient stage in sympatric speciation by sexual selection. *Ecology Letters* 2: 367–378.
- Spitze, K. 1993. Population-structure in *Daphnia-Obtusa*—Quantitative genetic and allozymic variation. *Genetics* 135: 367–374.
- Stäger, J. C., and T. C. Johnson. 2008. The late Pleistocene desiccation of Lake Victoria and the origin of its endemic biota. *Hydrobiologia* 596: 5–16.
- Stelkens, R. B., M.E.R. Pierotti, D. A. Joyce, A. M. Smith, I. Van der Sluijs, and O. Seehausen. 2008. Female mating preferences facilitate disruptive sexual selection on male nuptial colouration in hybrid cichlid fish. *Phil. Trans. Roy. Soc. Series B* 363: 2861–2870.
- Sturmbauer, C. 1998. Explosive speciation in cichlid fishes of the African Great Lakes: a dynamic model of adaptive radiation. *J. Fish Biol.* 53: 18–36.
- Sturmbauer, C., U. Hainz, S. Baric, E. Verheyen, and W. Salzburger. 2003. Evolution of the tribe Tropheini from Lake Tanganyika: synchronized explosive speciation producing multiple evolutionary parallelism. *Hydrobiologia* 500: 51–64.
- Terai, Y., O. Seehausen, T. Sasaki, K. Takahashi, S. Mizoiri, T. Sugawara, T. Sato, et al. 2006. Divergent selection on opsins drives incipient speciation in Lake Victoria cichlids. *Plos Biology* 4: 2244–2251.
- Thibert-Plante, X., and A. P. Hendry. 2009. Five questions on ecological speciation addressed with individual-based simulations. *Journal of Evolutionary Biology* 22: 109–123.
- van der Sluijs, I., J.J.M. van Alphen, and O. Seehausen. 2008. Preference polymorphism for coloration but no speciation in a population of Lake Victoria cichlids. *Behav. Ecol.* 19: 177–183.
- van der Sluijs, I., T.J.M. Van Dooren, K. D. Hofker, J.J.M. van Alphen, R. B. Stelkens, and O. Seehausen, O. 2008. Female mating preference functions predict sexual selection against hybrids between sibling species of cichlid fish. *Philos. Trans. R. Soc. B* 363: 2871–2877.
- Verzijden, M. N., J. Zwinkels, and C. ten Cate. 2009. Cross-fostering does not influence the mate preferences and territorial behaviour of males in Lake Victoria cichlids. *Ethology* 115: 39–48.
- Vonlanthen, P., D. Roy, A. G. Hudson, C. R. Largiader, D. Bittner, and O. Seehausen. 2008. Divergence along a steep ecological gradient in Lake whitefish (*Coregonus sp.*). *J. Evol. Biol.*, doi: 10.1111/j.1420-9101.2008.01670.