

Adaptive radiation and hybridization in Wallace's Dreamponds: evidence from sailfin silversides in the Malili Lakes of Sulawesi

Fabian Herder^{1,4,*}, Arne W. Nolte², Jobst Pfaender¹, Julia Schwarzer¹,
Renny K. Hadiaty³ and Ulrich K. Schliewen⁴

¹*Sektion Ichthyologie, Zoologisches Forschungsmuseum Alexander Koenig, Adenauerallee 160, 53113 Bonn, Germany*

²*Institute for Genetics, University of Cologne, Weyertal 121, 50931 Köln, Germany*

³*Ichthyology Laboratory, Division of Zoology, Research Center for Biology, Indonesian Institute of Sciences (LIPI),
Jl. Raya Bogor Km 46, Cibinong 16911, Indonesia*

⁴*Department of Ichthyology, Bavarian State Collection of Zoology (ZSM),
Münchhausenstr 21, 81247 München, Germany*

Adaptive radiations are extremely useful to understand factors driving speciation. A challenge in speciation research is to distinguish forces creating novelties and those relevant to divergence and adaptation. Recently, hybridization has regained major interest as a potential force leading to functional novelty and to the genesis of new species. Here, we show that introgressive hybridization is a prominent phenomenon in the radiation of sailfin silversides (Teleostei: Atheriniformes: Telmatherinidae) inhabiting the ancient Malili Lakes of Sulawesi, correlating conspicuously with patterns of increased diversity. We found the most diverse lacustrine species-group of the radiation to be heavily introgressed by genotypes originating from streams of the lake system, an effect that has masked the primary phylogenetic pattern of the flock. We conclude that hybridization could have acted as a key factor in the generation of the flock's spectacular diversity. To our knowledge, this is the first empirical evidence for massive reticulate evolution within a complex animal radiation.

Keywords: hybridization; introgression; adaptive radiation; Malili Lakes; Telmatherinidae; amplified fragment length polymorphism

1. INTRODUCTION

Introgressive hybridization can play a significant role in evolution by creating novel gene combinations, which in turn may create key innovations leading to expansions of niche, habitat or range (Gilbert 2003; Rieseberg *et al.* 2003; Grant *et al.* 2004). Although it remains debatable whether introgressive hybridization and hybrid speciation contribute significantly to the generation of global species diversity (Arnold 1997; Dowling & Secor 1997; Barton 2001), most authors agree that overall fitness of introgressed populations may reach its highest levels in novel habitats, when selection intensity against introgressed alleles is low and the evolution along new evolutionary trajectories is facilitated (Grant *et al.* 2004; Nolte *et al.* 2005).

Adaptive radiations are prime examples for the 'explosive' genesis of biodiversity in novel habitats such as newly formed lakes. Some of these harbour species flocks containing hundreds of endemic species, e.g. the cichlids of 'Darwin's Dreamponds' (Goldschmidt 1998), i.e. the East African Great Lakes (Turner *et al.* 2001; Kocher 2004). Although these systems have served for decades as natural laboratories for the study of speciation

processes, it is only recently that hybridization has been proposed as a major explanatory factor for the generation of diversity (Seehausen 2004). Indeed, recent molecular phylogenetic evidence shows that introgression and hybrid speciation has occurred within species flocks (Barrier *et al.* 1999; Rüber *et al.* 2001; Salzburger *et al.* 2002; Shaw 2002; Smith *et al.* 2003; Schliewen & Klee 2004; Sullivan *et al.* 2004; Joyce *et al.* 2005). However, unequivocal evidence for large scale hybridization events that are related to the evolution of megadiversity is still lacking.

Reasons for the deficiency in knowledge concerning the evolutionary role of hybridization lie partially in methodological problems (Seehausen 2004). However, the amplified fragment length polymorphism (AFLP) technique (Vos *et al.* 1995) constitutes a powerful tool to investigate phylogenetic relationships within species flocks (Albertson *et al.* 1999; Allender *et al.* 2003; Schliewen & Klee 2004; Sullivan *et al.* 2004) with the advantage to provide numerous unlinked nuclear loci. These can be used to test hypotheses of hybridization by screening for homoplastic effects in phylogenetic trees (Seehausen 2004; see §2d).

A second major problem is the need to study ideally all potential partners of the hybridization scenario in order to quantify the contribution of introgressive hybridization to speciation processes. To overcome the problem of complexity, radiations with lower species richness and less spatial complexity (McKaye *et al.* 2002; Schliewen & Klee 2004) or single pairs of sympatric species (Pigeon *et al.*

* Author for correspondence (f_herder@yahoo.com).

The electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2006.3558> or via <http://www.journals.royalsoc.ac.uk>.

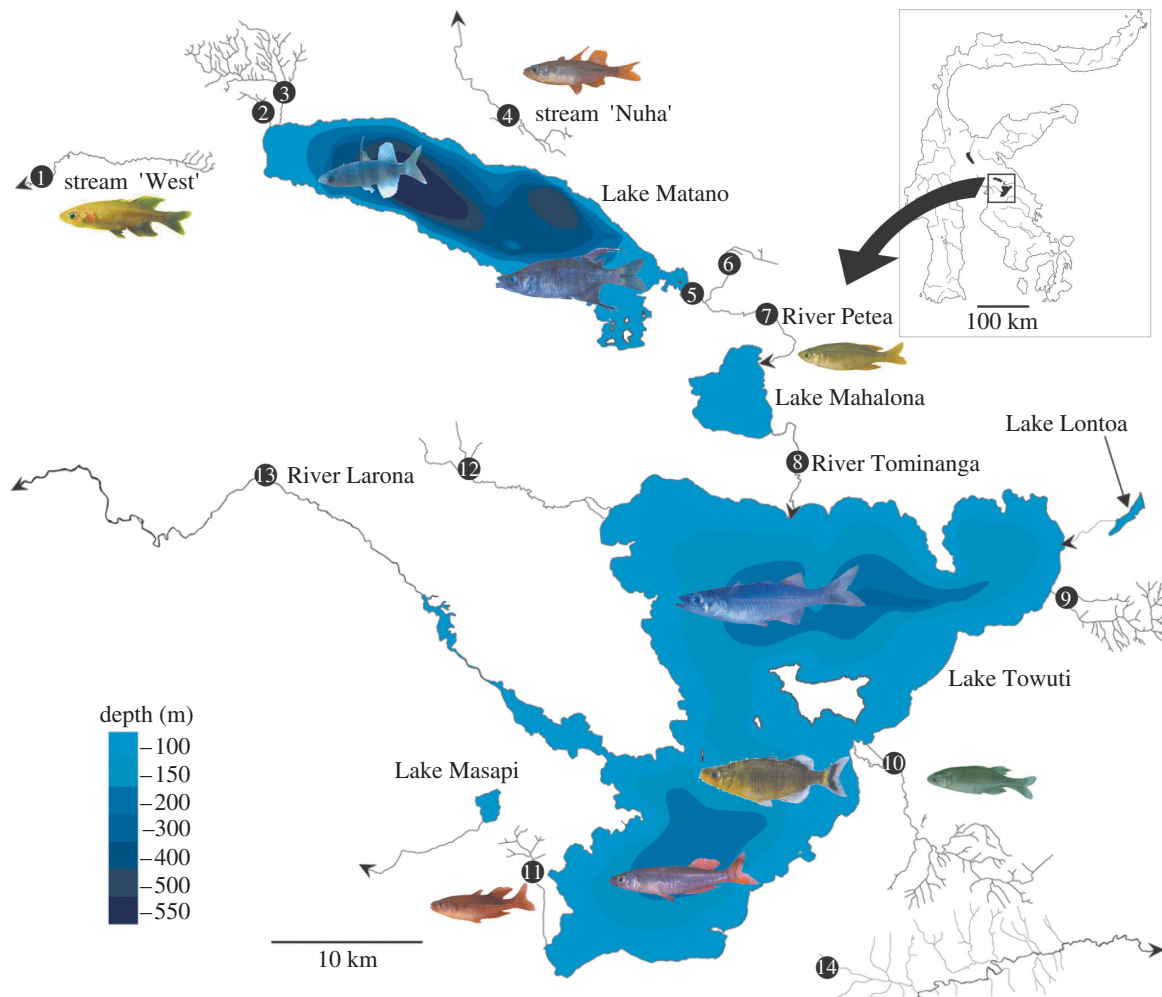


Figure 1. The Malili Lakes system and its endemic sailfin silversides. L. Matano is a very deep (590 m) graben-lake, drained by the steep R. Petea to L. Mahalona, from where the flow continues to L. Towuti. L. Towuti is drained to the sea. Lake Lontoa is connected by a stream to L. Towuti; no telmatherinids were found in L. Masapi. Fish inside the lakes represent the major groups of lake phenotypes. Sampling locations of stream telmatherinids are indicated as dots, with numbers referring to location names specified in electronic supplementary material Table 2. Map by von Rintelen & Zitzler, modified with permission.

1997; Lu & Bernatchez 1999; Turgeon *et al.* 1999; McKinnon & Rundle 2002) have been developed as models. However, to understand the mechanisms shaping whole radiations, model systems should be larger than a single species pair but small enough to be covered almost completely. Recent molecular phylogenetic evidence based on freshwater gastropods (von Rintelen *et al.* 2004) and fishes (Roy *et al.* 2004) suggest strongly that the organismic diversity of the Malili Lakes of Central Sulawesi (Indonesia) may represent such a system. This ancient lake system (Brooks 1950) consists of the three main lakes, Matano, Towuti and Mahalona, and two smaller lakes, Lontoa and Masapi (figure 1). They harbour an endemic species flock of sailfin silversides (Teleostei: Atheriniformes: Telmatherinidae; see figures 1 and 2), which exhibit a conspicuous chromatic polymorphism in male coloration in several species consisting of 'yellow' and 'blue' colour components; they are endemic either to Lakes Towuti and Mahalona or to isolated L. Matano alone (Kottelat 1990, 1991; Herder *et al.* in press). Their diversity comprises the genera *Paratherina*, *Tominanga* and *Telmatherina*. Lake Matano's *Telmatherina* can be assigned according to shape of their second dorsal and anal fins to 'roundfins' and 'sharpfins' (Kottelat 1991), with considerably higher diversity in the predominantly benthic

sharpfins than in the predominantly pelagic roundfins (Herder *et al.* in press; supplementary table 1). In rivers and streams of the Malili Lakes drainage system, as well as in several isolated neighbouring watersheds, morphologically more or less distinct stream populations (*Telmatherina* cf. *bonti*) were recently discovered (figure 1; supplementary table 2).

Here, we use multilocus AFLP marker and mitochondrial haplotypes to test for phylogenetic signals of ancient or ongoing introgressive hybridization during species flock formation. Tests based on the multilocus dataset are applied to demonstrate that hybridization is a common phenomenon in the radiation of sailfin silversides. There is a conspicuous correlation of the high morphological diversity and pronounced patterns of hybridization in the adaptive radiation of L. Matano's sharpfins. We discuss findings in the context of hybrid speciation in species flocks.

2. MATERIAL AND METHODS

(a) Study system

The Malili Lakes system is located in the central highland of Sulawesi. Its main lakes have a size of 24–561 km², are characterized by crystal clear water and are interconnected by rivers or streams, allowing for contact among sailfin silverside

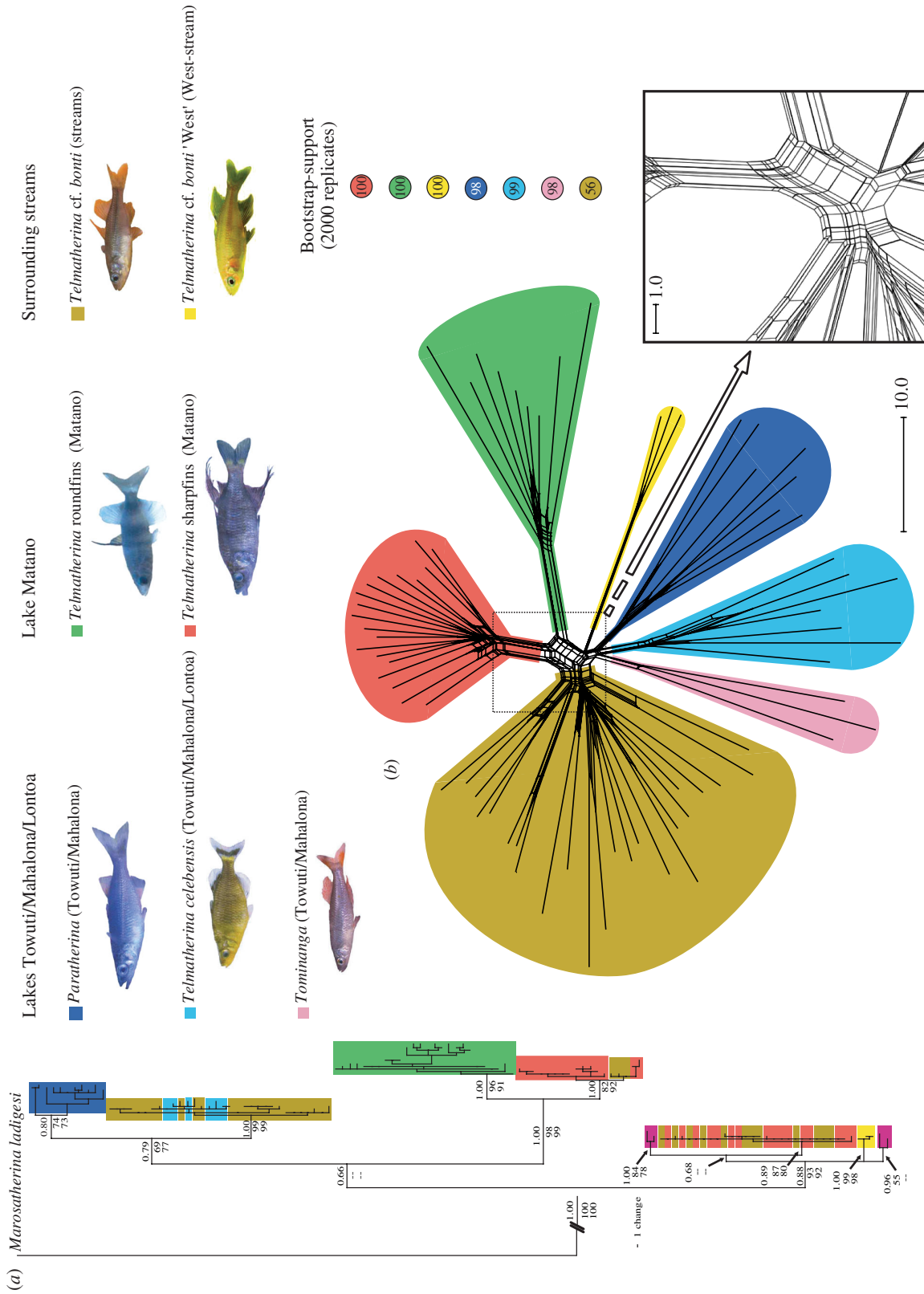


Figure 2. Phylogenies of Malili Lakes telmatherinids. (a) Bayesian tree of mitochondrial haplotypes, rooted with *Marosatherina* (SW-Sulawesi). The numbers above branches refer to Bayesian posterior probabilities of the major nodes. Bootstrap and jackknife values (more than 50%; upper and lower values, respectively) are shown below branches. (b) Distance-based phylogenetic AFLP network. Compatible collections of splits are displayed tree-like, conflicting signal as boxes. Strongest conflict is indicated for the 'stream-clade' and for *L. Matano's* 'sharpfins'. Substantial conflict is also present in the centre of the network, suggesting strong reticulate patterns within the whole species-flock. Enlarged figures and specimens are provided in electronic supplementary material figures 1 and 2.

populations. The lakes are estimated to be approximately 1–2 Myr old (unpublished information cited by von Rintelen *et al.* 2004); however, a thorough estimation of their age is unavailable to date.

(b) *Material*

Material was collected from several sites both in the lakes and in the rivers (figure 1) by F.H., J.P., J.S. and R.K.H. in 2002 and 2004. Specimens were marked individually, a fin-clip was stored in 99% ethanol and the specimen was subsequently preserved in 4% formalin and later transferred to 70% ethanol for storage. Voucher specimens are stored in fish collections of the Bavarian State Collection of Zoology, Munich (ZSM).

(c) *Molecular methods*

A mtDNA fragment of 665–677 bp spanning part of the cytochrome b gene, threonine and proline tRNAs and part of the D-Loop was sequenced for 124 individuals. Bayesian methods and maximum parsimony (MP) analyses were performed, including calculation of Bayesian posterior probability, bootstrap and jackknife branch support values. AFLPs were analysed for 74 individuals, resulting in 1327 polymorphic fragments. Based on the fragment matrix, distances were calculated and used for creating a phylogenetic network with the Neighbour-Net algorithm (Bryant & Moulton 2004) and to calculate bootstrap support for single clades. For details on species identification, material and molecular methods see electronic supplementary material methods. The DNA sequences have been deposited in the Gene Bank (see electronic supplementary material).

(d) *Detection of hybridization*

Seehausen (2004) suggested and Schlieven & Klee (2004) successfully applied a tree-based method to identify species with a hybrid genome by using large numbers of AFLP-loci. As hybrids should be intermediate to the parental species, because they carry a mosaic of parental characters, the inclusion of a hybrid taxon introduces an excess of homoplasies and therefore conflict in the subset of clades that contributed to hybridization. Consequently, the removal of the putative hybrid taxon should decrease the amount of homoplasies and hence increase statistical (e.g. bootstrap) support for those nodes that unite descendants from taxa which gave rise to a hybrid taxon. In contrast, removal of a non-hybridizing taxon should not affect support for the respective nodes.

As analysis of nuclear and mitochondrial markers revealed cytonuclear discordance indicating hybridization between *L. Matano's* sharpfins and stream telmatherinids, the AFLP matrix was used to test for the presence of introgressed alleles, present as homoplastic information shared by sharpfins and stream telmatherinids. Bootstrap support of the 'stream-clade' was examined in comparison with removal of the 'sharpfin-clade' or respectively 19 samples randomly chosen from the dataset (100 control experiments). To control for the potential effect of removing complete clades from the dataset, five additional removal experiments were conducted, excluding all samples of each of the remaining clades which were significantly supported in previous analyses (*Telmatherina celebensis*, *Tominanga*, *Paratherina*, *L. Matano's* roundfins, *T. cf. bonti* 'West'). Number of excluded samples was standardized by adding randomly chosen samples to a total number of 19. Tests were conducted one-way, as

maximum support (100%) of the sharpfin-clade did not allow unrestricted observation of changing support *vice versa*.

The bootstrap-method does not allow straightforward testing for signal of alternative phylogenetic hypotheses (unvisualized nodes) hidden in the tree by dominant conflicting signal. Therefore, Canonical Phylogenetic Ordination (Giannini 2003) was applied to test for potential signal of *L. Matano's* monophyly in the AFLP data matrix. To achieve this, 5000 full model Monte Carlo permutations were performed to the complete data set, the data set exclusive the stream-clade and 100 random exclusions of 24 samples, respectively, using CANOCO 4.0 (ter Braak & Smilauer 1998; Schlieven & Klee 2004).

(e) *Assessment of phenotypic diversity*

Adaptive radiation is detectable by the criteria of common ancestry, phenotype–environment correlation, trait utility and rapid speciation (Schluter 2000). Body shape of fishes has been demonstrated to reflect ecological variation in species flocks and can therefore be used as a test trait in the ecological opportunity hypothesis (Rüber & Adams 2001). To evaluate phenotypic diversity in *L. Matano's* sharpfins and roundfins with respect to the potential hybrid-partner 'stream telmatherinids', morphological variation in both lake clades is compared with that of nine riverine populations from all over the Malili Lakes system.

Landmark-based geometric morphometric methods were applied to 1–21 mature males from each population/phenotype. Morphometric analyses are based on formalin-preserved adult male specimens that were not deformed or twisted. Standardized digital pictures of 260 preserved specimens were used to collect 20 *x* and *y* coordinates of homologous landmarks as shown in supplementary figure 3, using the TPS software (Rohlf 2003). Subsequent analyses were conducted applying the IMP software package (Sheets 2002). Differences among the specimens in the sets of coordinates due to scaling, rotation and translation were removed using the geometric morphometric approach of placing the specimen in Partial Procrustes Superimposition on the iteratively estimated mean reference form, using the Generalized Procrustes Analysis procedure (Zelditch *et al.* 2004). After superimposition, data were converted into components of the Partial Warp axes of a thin-plate spline model of deformations and along uniform axes of deformation due to shear and dilation as described in Nolte & Sheets (2005).

Principal components were calculated to extract major axes of variation in sharpfins, roundfins and stream phenotypes, based on the covariance matrix derived from the partial warp scores. To compare differences in 'morphospace' between clades as identified by AFLP markers, individual scores of principal components 1 and 2 (explaining together 42.83% of the total variance) were plotted. To visualize the morphospace (Joyce *et al.* 2005), positions of individuals of every single phenotypic group were connected by lines, marking thereby group specific morphospaces. Morphospace of clades was identified by surrounding outlines of all included group specific morphospaces.

3. RESULTS AND DISCUSSION

(a) *Molecular phylogenies*

By using multiple markers, we detected large scale hybridization events, particularly in the *L. Matano* subsystem. Both maternally inherited mitochondrial markers and multilocus nuclear AFLP markers were

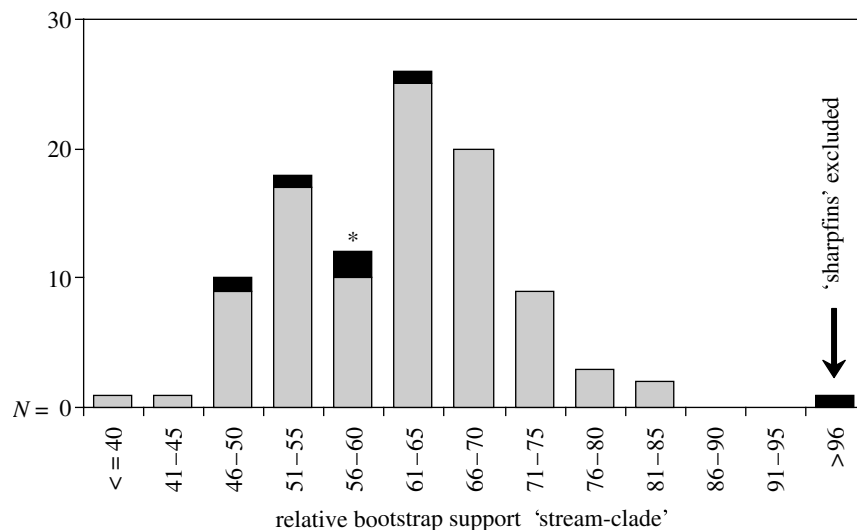


Figure 3. Test for signal of hybridization. Relative bootstrap support (2000 replicates) for the clade of 'stream phenotypes' when removing experimentally selected samples. Bars display 97 out of 100 random removals (three experimental topologies did not support stream phenotypes as monophyletic). Black sections illustrate complete removal of each of the six remaining clades, corrected to standardized number of exclusions (19) by adding randomly selected samples. Asterisk marks the stream phenotypes support value given the complete dataset. The arrow denotes the far outside value (99%) resulting from the exclusion of all sharpfins.

applied to (i) test for phylogenetic signals indicating ancient or ongoing introgressive hybridization during species flock formation and (ii) to provide a robust phylogenetic hypothesis for a novel, highly diverse model-system for the study of speciation.

Comparing discordant phylogenetic relationships derived from mtDNA haplotypes and nuclear AFLP markers (figure 2), we detected four possible hybridization events within the sailfin silversides species-flock. (i) Lake Towuti's/Mahalona's *T. celebensis* and stream inhabiting *T. cf. bonti* populations surrounding these lakes share one haplotype-clade, despite nuclear data indicating that *T. celebensis* represents a distinct lineage, closely related to *Paratherina* and *Tominanga* from the same lakes. (ii) *Tominanga* are monophyletic according to AFLPs, but carry haplotypes of two different lineages, which are closely related to stream phenotypes from the Matano-surrounding and to parts of L. Matano's sharpfins. (iii) Telmatherinids from isolated 'Nuha' stream north of the L. Matano drainage carry haplotypes of the 'sharpfin' clade, but belong to stream phenotypes according to AFLPs. (iv) Finally, the highly diverse L. Matano's sharpfins are supported strongly as a monophylum by AFLP data, whereas they carry two fundamentally different mtDNA haplotype-clades. One of these is the sister-clade to the 'roundfin'-clade, while the other is closely related to stream haplotypes. There is no apparent lineage sorting among haplotypes of sharpfins and stream phenotypes from the neighbourhood of L. Matano. Moreover, roughly half of sharpfin phenotypes are found to be represented in both haplotype-clades, suggesting recent or ongoing geneflow from streams into the lacustrine sharpfin-assemblage as well as within it. Telmatherinids from River Petea, connecting L. Matano with L. Mahalona, carry the 'stream-haplotypes' and are the only stream-dwelling specimens assigned to L. Matano's sharpfins by AFLPs.

Interestingly, stream phenotypes (except populations from stream West and River Petea) are assigned by AFLPs

to a single clade that, in sharp contrast to the other lineages, exhibits substantial phylogenetic conflict. This is visualized in the phylogenetic network as boxes in its centre (figure 2). Likewise, tree-based bootstrap tests support the AFLP-clades poor in conflicts with comparatively high bootstrap values, but the conflict-rich stream-clade (*T. cf. bonti* without *T. cf. bonti* 'West' and R. Petea) is only weakly supported (figure 2).

(b) Detection of hybridization

Based on discordant mtDNA and AFLP phylogenetic hypotheses as well as on basal conflict in stream phenotypes and sharpfins as displayed in the AFLP network, we hypothesized that massive hybridization between sharpfins and stream telmatherinids has occurred. To test if there is significant conflict signal in the multilocus nuclear dataset only, bootstrap homoplasy excess tests (Seehausen 2004) were performed by evaluating support values for the stream-clade with and without the 19 sharpfin-individuals, i.e. the putative hybrid clade. We found strong evidence for the hybridization scenario as the support drastically increased from 56% to 99% when all 19 samples of the sharpfin-clade were removed. In contrast, only a minimal rise or even a decline of bootstrap support values was detected when 19 randomly chosen samples were removed (100 removal experiments; mean stream-clade support = 61.6%; figure 3). Accordingly, the one-by-one exclusion of complete other clades, including the roundfins, did not result in a significantly raised bootstrap support of the stream-clade. To evaluate the potential effect of R. Petea stream fish, the only stream population assigned by AFLPs to the sharpfins, we repeated the experiment without R. Petea samples, with an almost identical result (data not shown). We conclude that the discordance between mtDNA based phylogenetic hypothesis and the AFLP-based phylogenetic network, as well as the detected conflict within the AFLP dataset is caused most likely by a substantial partition of stream-clade *T. cf. bonti* alleles in

the sharpfin genotypes. Signal of introgression is apparent not only according to the cytonuclear phylogenetic conflict, potentially reflecting rare invasions of the lake population, but also according to intranuclear conflict alone.

Based on the finding of massive introgression into sharpfins and the fact that one of the sharpfin haplotype-clades was identified as sister group to the roundfin haplotypes, we hypothesized that Lake Matano telmatherinids are originally of monophyletic origin. To test for remains of a hypothetical ancient signal of *L. Matano*'s monophyly (all sharpfins + roundfins) in the AFLP data, we used Canonical Phylogenetic Ordination (Giannini 2003; CPO). For the complete data set ($N=74$), CPO resulted in no significant statistical support for this signal. However, the monophyly hypothesis was supported strongly ($P=0.002$) when the potential hybrid partner stream-clade (as identified above by AFLP phylogenetic analysis; 24 samples) was removed. No significant support was observed in 100 control tests, each excluding 24 samples randomly chosen from the whole dataset. We conclude that roundfins and sharpfins descend from a common ancestor ('proto-Matano'), but the respective phylogenetic signal was masked in the full dataset by introgressed alleles derived from hybridization with stream telmatherinids.

Our data suggest that only sharpfins, rather than both sharpfins and roundfins or proto-Matano telmatherinids, have been affected by introgressive hybridization. There are three supporting arguments: (i) according to the logic of tree-based homoplasy-detection (Seehausen 2004), homoplastic signals among one of two sister clades and its hybridization partner would decrease support of the node connecting both sister clades. Removal of the hybridization partner would increase support of this sister group relationship. In contrast, introgressed alleles present in both sister clades due to speciation after ancestral hybridization are unlikely to affect this node. The CPO experiment uncovered a significant monophyly signal for a roundfin + sharpfin-clade after removal of putative homoplastic signals, supporting a scenario of hybridization after separation of roundfins and sharpfins. (ii) Likewise, 'bootstrap-tests' revealed homoplastic signal only among stream-clade and sharpfins, not among stream-clade and roundfins. (iii) Observed differential response of bootstrap-support corresponds with the split pattern of haplotype distribution, suggesting recent or even ongoing geneflow between sharpfins and stream phenotypes. In contrast, these arguments do not support the alternative hypothesis of a major hybridization event predating the split of roundfins and sharpfins and affecting the proto-Matano telmatherinids, as suggested by the 'hybrid swarm hypothesis' (Seehausen 2004). Following the latter scenario, signal due to introgression should be detectable in roundfins and sharpfins; therefore, homoplastic stream-clade signal should affect the position of 'Matano' relative to the stream-clade in the phylogenetic network, but would not mask monophyly of roundfins + sharpfins. Likewise, we would expect to find a response when excluding roundfins in the bootstrap-removal experiment. Additionally, the pattern of haplotype distribution can hardly be explained by ancient hybridization, as the 'introgressed' haplotypes are absent in roundfins and

incomplete lineage-sorting is evident in sharpfins and stream fish. Therefore, it is suggested that the ancient phylogenetic signal of *L. Matano*'s monophyly is masked in the complete dataset by noise due to alleles introgressed from the stream-clade. Hence, telmatherinids of Lake Matano are a primarily monophyletic group with two ancient sub-clades. Interestingly, the only connection of *L. Matano* and the other lakes, R. Petea, is inhabited by the only known river-dwelling sharpfins (*T. sp.* 'Petea'), which carry 'stream-like' haplotypes.

(c) *Phenotypic diversity*

Landmark-based geometric morphometry demonstrates that diversity of body shapes is conspicuously higher in *L. Matano*'s introgressed sharpfins than in the non-introgressed sympatric roundfins or in the geographically scattered stream phenotypes (figure 4). Moreover, both lake clades differ from each other and from the stream fish in morphospace area occupied, most likely reflecting adaptation to different ecological opportunities (Rüber & Adams 2001). In combination with our finding of masked ancient monophyly of *L. Matano*'s telmatherinids and obviously porous species boundaries suggesting young or even ongoing processes of divergence, we suggest that *L. Matano*'s sailfin silversides represent an adaptive radiation (Schluter 2000). However, direct evidence for environment-phenotype correlation or trait utility of *L. Matano*'s telmatherinids is pending, a topic presently under investigation.

(d) *Concluding remarks*

Molecular and morphological findings demonstrate that sailfin silversides of the Malili Lakes exhibit patterns typical for an adaptive radiation: emergence of ecological and phenotypic diversity in a rapidly multiplying lineage, characterized by low levels of neutral genetic divergence (Schluter 2000; Joyce *et al.* 2005). Our findings of widespread hybridization within an adaptive radiation and between its constituent species and external taxa, and conspicuous correlation of introgression and diversity in telmatherinids of *L. Matano*, support the hypothesis (Seehausen 2004) that hybridization could increase the likelihood and speed of the evolution of animal radiations. While the role of hybridization in evolution has been analysed for decades by botanists (Stebbins 1959; Grant 1981; Arnold 1997; Rieseberg 1997; Hegarty & Hiscock 2004; Wissemann 2005), the notion that hybridization could enhance rather than retard (Mayr 1963) the genesis of biodiversity has rarely been approached in animal systems (Seehausen 2004). In contrast to polyploidy-triggered speciation common in plants, homoploid hybrid speciation is more difficult to detect (Hegarty & Hiscock 2004), and most animals lack selfing which may help stabilizing the genome after hybridization (Coyne & Orr 2004; Hegarty & Hiscock 2004). However, homoploid hybrid speciation depending on ecological selection is plausibilized in theoretical models (Buerkle *et al.* 2000), takes place in nature (Rieseberg *et al.* 2003; Gross & Rieseberg 2005) and has been shown in species flocks (Schliewen & Klee 2004; Howarth & Baum 2005). In line with several reported single cases of homoploid hybrid speciation in animals (Dowling & DeMarais 1993; Salzburger *et al.* 2002; Smith *et al.* 2003; Schliewen & Klee 2004; Mallet 2005; Nolte *et al.* 2005), our results

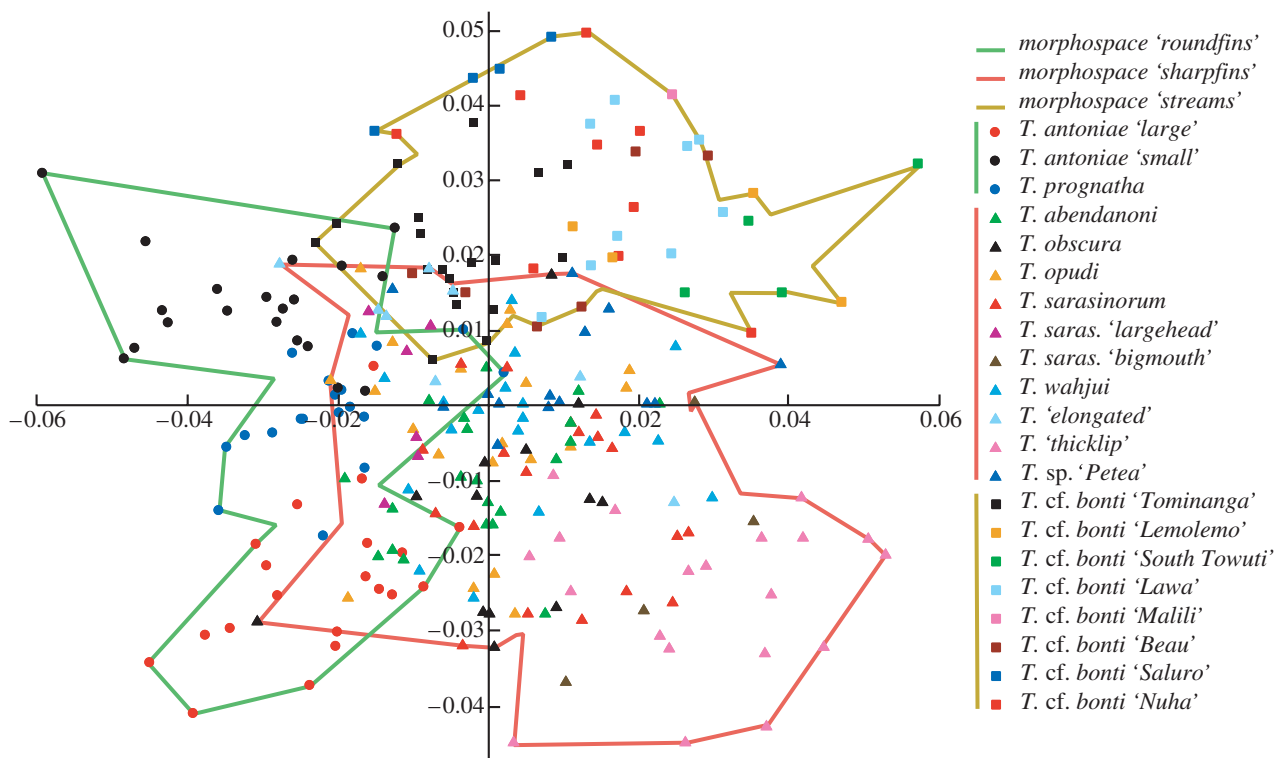


Figure 4. Principal component scatterplot (axes 1 and 2) based on geometric morphometry. Lines surround areas in morphospace occupied by the endemic radiations of Lake Matano's sharpfins and roundfins and stream populations inhabiting surrounding water bodies. Both sympatric lake clades largely differ from each other and from the allopatric stream populations. Morphospace occupied by sharpfins is conspicuously higher than by roundfins or stream phenotypes.

highlight the likely underestimated importance of reticulate processes playing an important and widespread role in speciation and adaptive evolution (Coyne & Orr 2004; Seehausen 2004).

Hybridization may enhance divergence within flocks by hybrid speciation or by introgressive hybridization enriching a population's gene pool without affecting its species' cohesion immediately (Wu 2001; Seehausen 2004; Grant *et al.* 2005). One process which could give rise to evolutionary novelty is transgressive segregation (Rieseberg *et al.* 1999), which is suggested to explain a unique component of body shape in hybrids of freshwater fish (Nolte & Sheets 2005). Though there are indications for constraints of phenotypic diversity shaped by transgressive segregation (Albertson & Kocher 2005), its potential for enhancing genetic variability and thus for increasing divergence in the initial stages of radiations has become obvious (Schliewen & Klee 2004; Bell & Travis 2005).

Potential hybridization events, e.g. in cichlid species flocks of the East African Lakes, may have remained hidden rather than being nonexistent due to necessarily incomplete taxon sampling in these highly diverse model systems with hundreds of species. Here, the small size of the Malili radiations, their intermediate complexity, and their apparently porous species boundaries open a novel and unique analytical window to the early phases of the origin of complex species flocks under the influence of reticulate genomic exchange. The simultaneous study of ecologically and sexually selected characters, e.g. male coloration and trophic adaptations, should allow testing of the role of transgressive segregation in early stages of processes generating megadiversity. With the phylogenetic framework elaborated in this study, the foundation for those studies is available.

When visiting Sulawesi in the late mid-nineteenth century, Alfred Wallace did not anticipate the existence of this ancient lake system. If he had chosen a slightly different route over Sulawesi, maybe not the Galapagos finches, but the sailfin silversides of the Malili Lakes would have become the most popular reference system for speciation research. As an appreciation of Wallace's contribution to evolutionary biology this new model system is here suggested to be called 'Wallace's Dreamponds'.

We thank LIPI for the permit to conduct research in Indonesia. We are deeply grateful to INCO for their outstanding logistic support at the lakes. For invaluable assistance in the field we thank J. Herder and J. Frommen. Fieldwork greatly benefited from logistic support in Indonesia by T. von Rintelen. W. Wickler kindly supported a pilot study, which enabled successful grant application. D. Tautz is acknowledged for ongoing support in many aspects to U.K.S. and A.N. We thank B. Klee for methodical help with AFLPs and J.W. Wägele for inspiring discussion on phylogenetic networks. The manuscript benefited from critical comments and constructive suggestions by J. Frommen, M. Glaubrecht, S. Gray, B. Misof, T. von Rintelen, L. Rüber, J. McKinnon and O. Seehausen. Our view of hybridization benefited from the workshop on the topic conducted by T. Hauser. This study was funded by research grants from the Deutsche Forschungsgemeinschaft to U.K.S. and by a graduate fellowship donated by the Rheinische Friedrich-Wilhelms-Universität Bonn to F.H.

REFERENCES

- Albertson, R. C. & Kocher, T. D. 2005 Genetic architecture sets limits on transgressive segregation in hybrid cichlid fishes. *Evolution* **59**, 686–690.
- Albertson, R. C., Markert, J. A., Danley, P. D. & Kocher, T. D. 1999 Phylogeny of a rapidly evolving clade: the

- cichlid fishes of Lake Malawi, East Africa. *Proc. Natl Acad. Sci. USA* **96**, 5107–5110. (doi:10.1073/pnas.96.9.5107)
- Allender, C. J., Seehausen, O., Knight, M. E., Turner, G. F. & Maclean, N. 2003 Divergent selection during speciation of Lake Malawi cichlid fishes inferred from parallel radiations in nuptial coloration. *Proc. Natl Acad. Sci. USA* **25**, 14 074–14 079. (doi:10.1073/pnas.2332665100)
- Arnold, M. L. 1997 *Natural hybridisation and evolution*. Oxford, UK: Oxford University Press.
- Barrier, M., Baldwin, B. G., Robichaux, R. H. & Purugganan, M. D. 1999 Interspecific hybrid ancestry of a plant adaptive radiation: allopolyploidy of the Hawaiian silversword alliance (Asteraceae) inferred from floral homeotic gene duplications. *Mol. Biol. Evol.* **16**, 1105–1113.
- Barton, N. H. 2001 The role of hybridization in evolution. *Mol. Ecol.* **10**, 551–568. (doi:10.1046/j.1365-294x.2001.01216.x)
- Bell, M. A. & Travis, P. 2005 Hybridization, transgressive segregation, genetic covariation, and adaptive speciation. *Trends Ecol. Evol.* **20**, 358–361. (doi:10.1016/j.tree.2005.04.021)
- Brooks, J. L. 1950 Speciation in ancient lakes. *Q. Rev. Biol.* **25**, 30–60. (doi:10.1086/397375)
- Bryant, D. & Moulton, V. 2004 Neighbor-net: an agglomerative method for the construction of phylogenetic networks. *Mol. Biol. Evol.* **21**, 255–265. (doi:10.1093/molbev/msh018)
- Buerkle, C. A., Morris, R. J., Asmussen, M. A. & Rieseberg, L. H. 2000 The likelihood of homoploid hybrid speciation. *Heredity* **84**, 441–451. (doi:10.1046/j.1365-2540.2000.00680.x)
- Coyne, J. A. & Orr, H. A. 2004 *Speciation*. Sunderland, MA: Sinauer Associates.
- Dowling, T. E. & DeMarais, B. D. 1993 Evolutionary significance of introgressive hybridization in cyprinid fishes. *Nature* **362**, 444–446. (doi:10.1038/362444a0)
- Dowling, T. E. & Secor, C. L. 1997 The role of hybridization in the evolutionary diversification of animals. *Ann. Rev. Ecol. Syst.* **28**, 593–619. (doi:10.1146/annurev.ecolsys.28.1.593)
- Giannini, N. P. 2003 Canonical phylogenetic ordination. *Syst. Biol.* **52**, 684–695. (doi:10.1080/10635150390238888)
- Gilbert, L. E. 2003 Adaptive novelty through introgression in *Heliconius* wing patterns: evidence for shared genetic “tool box” from synthetic hybrid zones and a theory of diversification. In *Ecology and evolution of taking flight: butterflies as a model system* (ed. C. L. Boggs, B. W. Ward & P. R. Ehrlich), pp. 281–318. Chicago, IL: University of Chicago Press.
- Goldschmidt, T. 1998 *Darwin's dreampond*. Cambridge, MA: MIT Press.
- Grant, V. 1981 *Plant speciation*. New York, NY: Columbia University Press.
- Grant, P. R., Grant, B. R., Markert, J. A., Keller, L. F. & Petren, K. 2004 Convergent evolution of Darwin's Finches caused by introgressive hybridization and selection. *Evolution* **58**, 1588–1599.
- Grant, P. R., Grant, B. R. & Petren, K. 2005 Hybridization in the recent past. *Am. Nat.* **166**, 56–67. (doi:10.1086/430331)
- Gross, B. L. & Rieseberg, L. H. 2005 The ecological genetics of homoploid hybrid speciation. *J. Hered.* **96**, 241–252. (doi:10.1093/jhered/esi026)
- Hegarty, M. J. & Hiscock, S. J. 2004 Hybrid speciation in plants: new insights from molecular studies. *New Phytol.* **165**, 411–423. (doi:10.1111/j.1469-8137.2004.01253.x)
- Herder, F., Schwarzer, J., Pfaender, J., Hadiaty, R. K. & Schliwen, U. K. In press. Preliminary checklist of sailfin silversides (Teleostei: Atheriniformes: Telmatherinidae) in the Malili Lakes of Sulawesi (Indonesia), with a synopsis of systematics and threats. *Verhandlungen der Gesellschaft für Ichthyologie*.
- Howarth, D. G. & Baum, D. A. 2005 Genealogical evidence for homoploid hybrid speciation in an adaptive radiation of *Scaevola* (Goodeniaceae) in the Hawaiian Islands. *Evolution* **59**, 948–961.
- Joyce, D. A., Lunt, D. H., Bills, R., Turner, G. F., Katongo, C., Duftner, N., Sturmhuber, C. & Seehausen, O. 2005 An extant cichlid fish radiation emerged in an extinct Pleistocene lake. *Nature* **435**, 90–95. (doi:10.1038/nature03489)
- Kocher, T. D. 2004 Adaptive evolution and explosive speciation: the cichlid fish model. *Nat. Rev. Genet.* **5**, 288–298. (doi:10.1038/nrg1316)
- Kottelat, M. 1990 Sailfin silversides (Pisces: Telmatherinidae) of Lakes Towuti, Mahalona and Wawontoa (Sulawesi, Indonesia) with descriptions of two new genera and two new species. *Ichthyol. Explor. Freshwaters* **1**, 35–54.
- Kottelat, M. 1991 Sailfin silversides (Pisces: Telmatherinidae) of Lake Matano, Sulawesi, Indonesia, with descriptions of six new species. *Ichthyol. Explor. Freshwaters* **1**, 321–344.
- Lu, G. Q. & Bernatchez, L. 1999 Correlated trophic specialization and genetic divergence in sympatric lake whitefish ecotypes (*Coregonus clupeaformis*): support for the ecological speciation hypothesis. *Evolution* **53**, 1491–1505.
- Mallet, J. 2005 Hybridization as an invasion of the genome. *Trends Ecol. Evol.* **20**, 229–237. (doi:10.1016/j.tree.2005.02.010)
- Mayr, E. 1963 *Animal species and evolution*. Cambridge, MA: The Belknap press.
- McKaye, K. R. *et al.* 2002 Behavioral, morphological and genetic evidence of divergence of the Midas cichlid species complex in two Nicaraguan crater lakes. *Cuadernos de la Investigacion de la UCA* **12**, 19–47.
- McKinnon, J. S. & Rundle, H. D. 2002 Speciation in nature: the threespine stickleback model systems. *Trends Ecol. Evol.* **17**, 480–488. (doi:10.1016/S0169-5347(02)02579-X)
- Nolte, A. W. & Sheets, H. D. 2005 Shape based assignment tests suggest transgressive phenotypes in natural sculpin hybrids (Teleostei, Scorpaeniformes, Cottidae). *Front. Zool.* **2**, 1–12. (doi:10.1186/1742-9994-2-11)
- Nolte, A., Freyhof, J., Stemshorn, K. & Tautz, D. 2005 An invasive lineage of sculpins, *Cottus* sp. (Pisces, Teleostei) in the Rhine with new habitat adaptations has originated from hybridization between old phylogenetic groups. *Proc. R. Soc. B* **272**, 2379–2387. (doi:10.1098/rspb.2005.3231)
- Pigeon, D., Chouinard, A. & Bernatchez, L. 1997 Multiple modes of speciation involved in the parallel evolution of sympatric morphotypes of lake whitefish (*Coregonus clupeaformis*, Salmonidae). *Evolution* **51**, 196–205.
- Rieseberg, L. H. 1997 Hybrid origins of plant species. *Ann. Rev. Ecol. Syst.* **28**, 359–389. (doi:10.1146/annurev.ecolsys.28.1.359)
- Rieseberg, L. H., Archer, M. A. & Wayne, R. K. 1999 Transgressive segregation, adaptation, and speciation. *Heredity* **83**, 363–372. (doi:10.1038/sj.hdy.6886170)
- Rieseberg, L. H. *et al.* 2003 Major ecological transitions in wild sunflowers facilitated by hybridisation. *Science* **301**, 1211–1216. (doi:10.1126/science.1086949)
- Rohlf, F. J. 2003 *TpsDig, digitize landmarks and outlines, version 1.39*. New York, NY: Department of Ecology and Evolution, State University of New York at Stony Brook.
- Roy, D., Docker, M. F., Hehanussa, P. E., Heath, D. D. & Haffner, G. D. 2004 Genetic and morphological data supporting the hypothesis of adaptive radiation in the endemic fish of Lake Matano. *J. Evol. Biol.* **17**, 1268–1276. (doi:10.1111/j.1420-9101.2004.00783.x)

- Rüber, L. & Adams, D. C. 2001 Evolutionary convergence of body shape and trophic morphology in cichlids from Lake Tanganyika. *J. Evol. Biol.* **14**, 325–332. (doi:10.1046/j.1420-9101.2001.00269.x)
- Rüber, L., Meyer, A., Sturmbauer, C. & Verheyen, E. 2001 Population structure in two sympatric species of the Lake Tanganyika cichlid tribe Eretmodini: evidence for introgression. *Mol. Ecol.* **10**, 1207–1225. (doi:10.1046/j.1365-294X.2001.01259.x)
- Salzburger, W., Baric, S. & Sturmbauer, C. 2002 Speciation via introgressive hybridization in East African cichlids? *Mol. Ecol.* **11**, 619–625. (doi:10.1046/j.0962-1083.2001.01438.x)
- Schliewen, U. K. & Klee, B. 2004 Reticulate sympatric speciation in Cameroonian crater lake cichlids. *Front. Zool.* **1**, 1–12. (doi:10.1186/1742-9994-1-5)
- Schluter, D. 2000 *The ecology of adaptive radiation*. Oxford series in ecology and evolution. Oxford, UK: Oxford University Press.
- Seehausen, O. 2004 Hybridization and adaptive radiation. *Trends Ecol. Evol.* **19**, 198–207. (doi:10.1016/j.tree.2004.01.003)
- Shaw, K. L. 2002 Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: What mtDNA reveals and conceals about modes of speciation in Hawaiian crickets. *Proc. Natl Acad. Sci. USA* **99**, 16 122–16 127. (doi:10.1073/pnas.242585899)
- Sheets, H. D. 2002 *IMP-integrated morphometrics package*. New York, NY: Canisius College, Buffalo, Department of Physics.
- Smith, P. F., Konings, A. & Kornfield, I. 2003 Hybrid origin of a cichlid population in lake Malawi: implications for genetic variation and species diversity. *Mol. Ecol.* **12**, 2497–2504. (doi:10.1046/j.1365-294X.2003.01905.x)
- Stebbins, G. L. 1959 The role of hybridization in evolution. *Proc. Am. Philos. Soc.* **103**, 231–251.
- Sullivan, J. P., Lavoue, S., Arnegard, M. E. & Hopkins, C. D. 2004 AFLPs resolve phylogeny and reveal mitochondrial introgression within a species flock of African electric fish (Mormyroidea: Teleostei). *Evolution* **58**, 825–841.
- ter Braak, C. J. F. & Smilauer, P. 1998 CANOCO reference manual and user's guide to canoco for windows. 351. Wageningen, The Netherlands: Centre for Biometry.
- Turgeon, J., Estoup, A. & Bernatchez, L. 1999 Species flock in the North American great lakes: molecular ecology of Lake Nipigon Ciscoes (Teleostei: Coregonidae: Coregonus). *Evolution* **53**, 1857–1871.
- Turner, G. F., Seehausen, O., Knight, M. E., Allender, C. J. & Robinson, R. L. 2001 How many species of cichlid fishes are there in African Lakes? *Mol. Ecol.* **10**, 793–806. (doi:10.1046/j.1365-294x.2001.01200.x)
- von Rintelen, T., Wilson, A. B., Meyer, A. & Glaubrecht, M. 2004 Escalation and trophic specialization drive adaptive radiation of freshwater gastropods in ancient lakes on Sulawesi, Indonesia. *Proc. R. Soc. B* **271**, 2841–2849. (doi:10.1098/rspb.2004.2842)
- Vos, P. *et al.* 1995 AFLP: a new technique for DNA fingerprinting. *Nucl. Acids Res.* **23**, 4407–4414.
- Wissemann, V. 2005 Evolution by hybridization. The influence of reticulate evolution on the biosymmetrical patterns and processes in plants. *Theory Biosci.* **123**, 223–233. (doi:10.1016/j.thbio.2004.09.003)
- Wu, C.-I. 2001 The genic view of the process of speciation. *J. Evol. Biol.* **14**, 851–865. (doi:10.1046/j.1420-9101.2001.00335.x)
- Zelditch, M. L., Swiderski, D. L., Sheets, H. D. & Fink, W. L. 2004 *Geometric morphometrics for biologists*. London, UK: Academic Press.