

INVITED REVIEW

Ancient lakes revisited: from the ecology to the genetics of speciation

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Abstract

Explosive speciation in ancient lakes has fascinated biologists for centuries and has inspired classical work on the tempo and modes of speciation. Considerable attention has been directed towards the extrinsic forces of speciation—the geological, geographical and ecological peculiarities of ancient lakes. Recently, there has been a resurgence of interest in the intrinsic nature of these radiations, the biological characteristics conducive to speciation. While new species are thought to arise mainly by the gradual enhancement of reproductive isolation among geographically isolated populations, ancient lakes provide little evidence for a predominant role of geography in speciation. Recent phylogenetic work provides strong evidence that multiple colonization waves were followed by parallel intralacustrine radiations that proceeded at relatively rapid rates despite long-term gene flow through hybridization and introgression. Several studies suggest that hybridization itself might act as a key evolutionary mechanism by triggering major genomic reorganization/revolution and enabling the colonization of new ecological niches in ancient lakes. These studies propose that hybridization is not only of little impediment to diversification but could act as an important force in facilitating habitat transitions, promoting postcolonization adaptations and accelerating diversification. Emerging ecological genomic approaches are beginning to shed light on the long-standing evolutionary dilemma of speciation in the face of gene flow. We propose an integrative programme for future studies on speciation in ancient lakes.

Keywords: adaptive radiation, ecological innovations, gene flow, hybridization, reproductive isolation, speciation

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Introduction: ancient lakes as evolutionary arenas

The oldest lakes of the world (e.g. the African Rift Lakes, Lake Baikal, Lake Titicaca, Caspian Lake, the Malili Lakes of Sulawesi) shelter endemic faunas that provide the most celebrated examples of ecological diversification and speciation (Fig. 1). The presence of species flocks, endemic monophyletic groups of closely related species, in these ancient lakes has fascinated biologists for centuries (Brooks 1950; Fryer 1991; Martens 1997). It has been noted that members of species

flocks often display super-specialization in resource utilization associated with marked morphological and ecological differences. These observations have sparked much interest in the underlying evolutionary processes that are involved in producing such rapid diversification.

It has long been recognized that ancient lakes provide excellent study systems that enable a detailed analysis of the speciation process and the mechanisms underlying colonization, adaptation and diversification. They act as ‘natural experiments’ from which we can extract principle evolutionary processes and gain a better understanding of how speciation mechanisms may operate at a more general level. The replicated nature of the evolution, the long historical record and the wide

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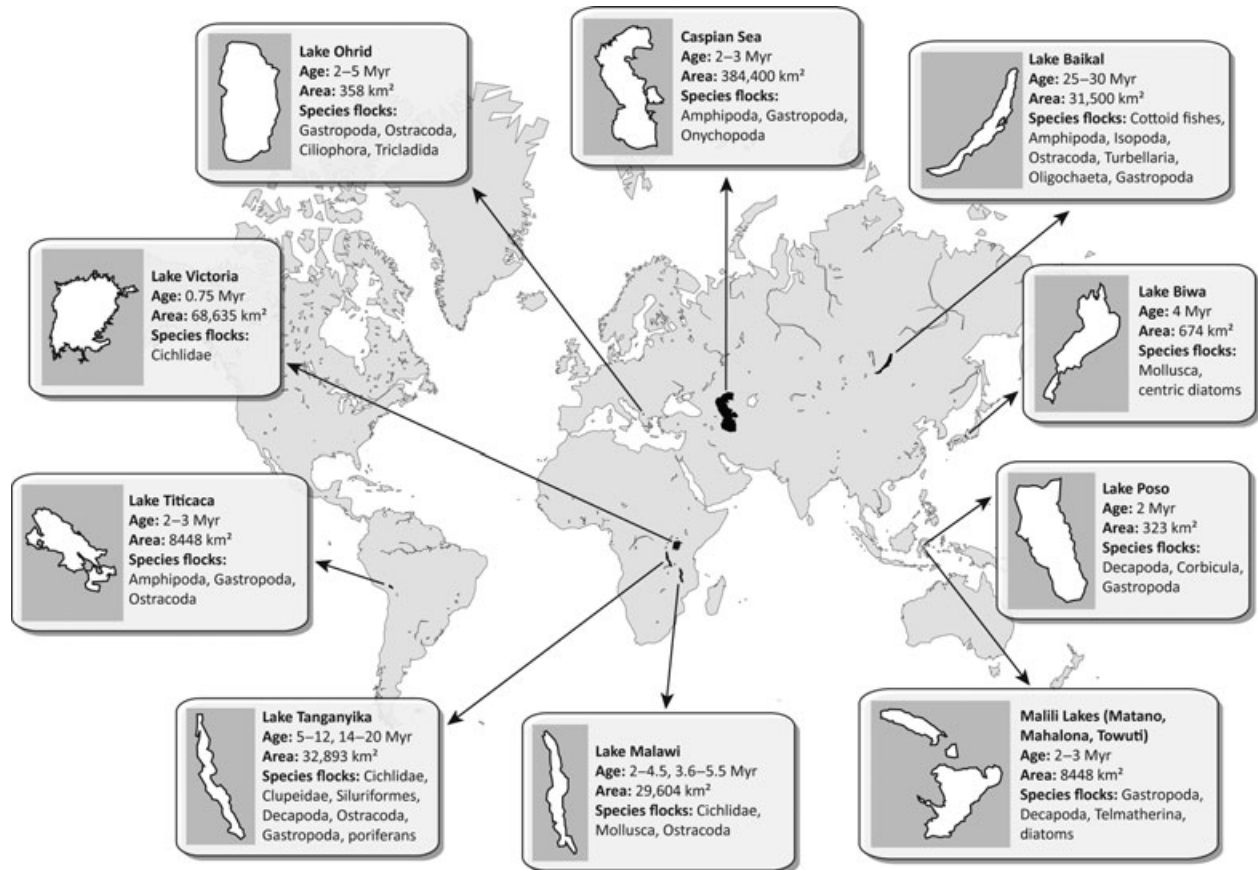


Fig. 1 Endemic diversity and physical features of major ancient lakes of the world. Data from Martens (1997) and Schön & Martens (2004).

assortment of adaptive and nonadaptive radiations make ancient lakes ideal settings for the study of speciation. Indeed, such observations inspired a large body of research. The accelerated progress that has been made on speciation in ancient lakes since Brooks's (1950) and Martens's (1997) classical reviews make a re-evaluation of the processes regulating speciation in these systems timely.

Here, we contrast classical methods and new trends in speciation research in ancient lakes. We first examine the influence of geological history and unique attributes of ancient lakes on diversification and review research on the timing and pace of ancient lakes radiations. Secondly, we synthesize the evidence supporting the main categories of speciation mechanisms in ancient lakes and show that the traditional debate on allopatric and sympatric speciation failed to capture the complexity of most radiation scenarios. Moreover, we show that hybridization is currently emerging as an important yet understudied evolutionary force that can promote genomic reorganization and radiation. Finally, we propose that future research should focus on (i) advancing a few nonvertebrate model systems of adaptive radiation,

(ii) investigating the pattern and tempo of diversification by using a snap-shot research approach at the sister-clade, sister-species and population levels, (iii) employing multilocus phylogenetic studies for revealing not only the basic evolutionary history of the group but also potential reticulate patterns of speciation, (iv) advancing population genomics studies for detecting genomic regions under selection and speciation genes, (v) integrating research across multiple lakes and multiple taxa and (vi) promoting multidisciplinary investigations on the same study systems (e.g. geological, paleolimnological, ecological, morphometric, phylogenetics, genomics).

Patterns of ancient lakes radiations: geography and biology

The stability paradox

Ancient lakes are commonly portrayed as long-lived, isolated and relatively stable environments with low extinction rates and a steady increase in the number of species. However, upon close scrutiny, most ancient

lakes reveal both steady limnological ageing processes (Mackay 1998) and complex physical/chemical histories owing to drastic geological and climatic changes. Although Lake Victoria appears to date back 750 000 years, it experienced a major regression or complete drought about 18 000–15 000 years ago. Similarly, the older Lake Malawi (4–5 Myr) and Tanganyika (9–12 Myr) experienced a coincidental severe water-level drop as recently as 135 000–75 000 years ago (Scholz *et al.* 2007). Lake Titicaca has also experienced major fluctuations that separated the basin into three to five small paleo-lakes during its history (Mourguiart 2000). Similarly, the geological history of the Caspian basin involves major transgressions and regressions followed by drastic changes in salinity regimes that triggered several well-documented episodes of mass extinction (Zenkevich 1963). Even Lake Baikal, the oldest, deepest and most stable of the ancient lakes, experienced notable environmental perturbations (Williams *et al.* 1997). Baikalian mollusc and diatom fossil data indicate that their evolution proceeded through several distinctive phases, each characterized by diverse and unique endemic faunas (Sherbakov 1999). Clearly, the fauna of ancient lakes must have been severely affected by the major changes in water levels which were associated not only with physico-chemical modifications but also with both biological stress and opportunities for population subdivision. These perturbations were often associated with major extinction waves, and it is likely that they played important roles in opening new ecological axes, making the lakes susceptible to new waves of colonization. How these factors influenced the pathway of diversification remains largely unexplored.

Colonization history, timing and tempo of radiation events

An understanding of evolutionary diversification processes benefits from clear knowledge of the evolutionary history of lineages that experienced radiations. Thus, particular consideration has been given to the ages of species flocks, the timing of their radiation, and the rate of speciation. The extremely young age of cichlid species in the African great lakes and their high speciation rate (Seehausen 2002; Won *et al.* 2005) has led some authors to speculate that many species flocks are very young. By contrast, there are also several assemblages, such as the gammarids, pulmonates, and possibly turbellarians of Lake Baikal, the gammarids and mysids of Caspian Lake, and the thalassoid gastropods and several cichlid tribes of Lake Tanganyika that contain lineages as old or older than the basin they inhabit, suggesting that ancient lakes may also act as long-term evolutionary reservoirs (Nishida 1991; Sher-

bakov *et al.* 1998; Salzburger *et al.* 2002b; Cristescu *et al.* 2003; Wilson *et al.* 2004). Assigning ages to particular group is not a trivial task, particularly when considering that most of these closely related lineages contain manifold clades of various ages resulting from a complex invasion history (Sherbakov 1999). Colonization histories of ancient lakes can vary from single events to pulse or continuous introductions over long periods of time. Continuous introductions are more probable for plankton and for benthic species with life stages adapted for high dispersal potential, whereas pulse introductions are more common for taxa with low dispersal ability. Regardless of dispersal strategy, species utilizing either continuous or pulse dispersal mechanisms have developed distinct species flocks. However, the complex invasion histories are potentially obscured even further by introgressive hybridization between closely related species (Seehausen 2004; Herder *et al.* 2006).

The intricate relations between the fauna of major ancient lakes and their surrounding watersheds have been examined in a historical perspective to infer the number of colonization events that have given rise to a particular species flock (Martens 1997; Macdonald *et al.* 2005; Elmer *et al.* 2009). For example, the cichlids of Lake Tanganyika are probably derived from multiple invasions that independently inaugurated radiations (Meyer 1993). Similarly, the recent Quaternary radiation of the Ponto-Caspian crustaceans (cladocerans, amphipods, mysids) that resulted in diversification at the genus level represents an extension of a diversification initiated earlier (during the Miocene, Pliocene, or Pleistocene) in the Sarmatian paleolake and seeded by multiple colonizations of both marine and freshwater ancestors (Cristescu *et al.* 2003; Cristescu & Hebert 2005). Examples strongly supporting monophyletic relationships indicative of one major colonization also exist. For example, large groups of cichlids in Lake Victoria and Malawi and of cottoid fishes in Lake Baikal are descended from one or a few ancestral species (Kontula *et al.* 2003; Verheyen *et al.* 2003). While the pace and geographical pattern of ancient lake radiations need to be confirmed for each studied flock, complex radiations of manifold clades suggestive of pulse diversification have been shown to be common.

The ecological and evolutionary factors that facilitate repeated colonizations followed by major, successive habitat transitions remain elusive. Kolar & Lodge (2001) propose an invasion model where species introduction and colonization face a series of selective filters based on both physical/chemical and biological characteristics of the habitat. Within this context, after a drastic ecological perturbation, an ancient lake would be less resistant to invasion with a high probability of new niches being

made available. Such environmental perturbations had the potential to facilitate new waves of colonization of ancestral lineages, while also advancing further diversification of surviving resident lineages. It is easy to imagine that severe environmental perturbations also facilitate massive hybridization waves.

Interestingly, hybridization itself could play an important role in facilitating repeated colonization and parallel evolution. For example, Schluter & Conte (2009) propose the 'transporter' hypothesis to explain the repetitive, independent origin of freshwater stickleback species from a marine ancestral ecotype, in one of the best-documented cases of parallel evolution. The authors suggest that the derived freshwater population contributes to the standing genetic variation in the marine (ancestral) population. In this simple scenario, alleles from freshwater-adapted populations are constantly exported back to the sea by hybridization followed by introgression. The freshwater-adapted genotype disintegrates slowly through recombination while supplementing the marine gene pool with novel alleles, providing increased invasive potential to their carriers and allowing the repeated colonization of new freshwater ecosystems. The model proposed by Schluter & Conte (2009) for postglacial stickleback evolution in North America could also explain the pattern of repeated invasions in ancient lakes if lake-adapted alleles are being transported outside the main lakes via hybridization and introgression, enhancing the invasiveness of populations inhabiting the surrounding watersheds. Although appealing, this model remains to be rigorously tested in other systems.

The Malili Lake system of Central Sulawesi (Indonesia) with its three hydrologically connected lakes (Matano, Towuti and Mahalona) and complex watershed provides a good setting for testing the 'transporter' hypothesis. For example, the sister species of the sailfin silversides of the family *Telmatherinidae* possess adaptive traits (skull and jaw morphology) specific to certain local shoreline habitats, suggesting strong local adaptation, as well as conspicuous male chromatic polymorphism indicative of sexual selection (Roy *et al.* 2007). However, based on a multilocus phylogenetic approach, Herder *et al.* (2006) concluded that the group shows strong signature of hybridization (conflicting cytonuclear and intranuclear phylogenetic signal in the multilocus dataset), suggesting that the spread of adaptive alleles across the population's distribution via introgressive hybridization may have contributed to this radiation. Interestingly, Herder *et al.* (2006) observed that recent hybridization occurred not only within one of the major lake clades, the *Telmatherina* sharpfins, but also between lake and stream *telmatherinids*. The number of stream/lake habitat transitions

(colonizations) that seeded the radiation of *telmatherinids* remains obscured by the recurring hybridization events between stream and lake lineage. Nevertheless, this finding opens the possibility that key adaptive alleles could be also transported outside the main lakes via hybridization and introgression, enhancing the capacity of stream individuals to explore or colonize lake habitats.

Perhaps, the most intriguing aspect of ancient lake evolution is the rapid nature of speciation in some flocks within these systems. There is strong molecular, fossil and biogeographical evidence that many adaptive radiations in ancient lakes have occurred at a very high rate. The mean times to speciation for amphipods and sculpins in Lake Baikal have been estimated at 5–7 and 0.6–0.9 Myr, respectively, while speciation intervals for cichlids range from 0.1 to 0.3 Myr in Lake Malawi and 0.7–1.1 Myr in Lake Tanganyika (reviewed in Turner 1999; Coyne & Orr 2004). Speciation intervals for cichlids in the crater lakes Nabugabo and Barambi Mbo (0.004–0.4 Myr) and Lake Victoria (0.001–0.2 Myr) appear to be much shorter (Schliewen *et al.* 1994; Seehausen 2002). Overall, these values are significantly higher than the mean diversification interval of 6.5 Myr calculated by Coyne & Orr (2004) for a representative number of plants, animals and protists. Based on a comparison of 33 intralacustrine radiations and 76 non-radiations in cichlids, Seehausen (2006) documented that young radiations and earlier stages of the older radiations are characterized by high speciation rates that start declining after at least 0.5 Myr since colonization. This evidence is consistent with mathematical models (Gavrilets & Vose 2005), suggesting that speciation rates are very rapid near the time of colonization, but begin to decline as the radiation ages. Gavrilets & Losos (2009) proposed several explanations for this pattern, including the concept of ecological opportunity or the availability of niches in a particular environment. It is generally thought that at the time of colonization, there is a wide range of niches open to utilization, triggering the rapid diversification of a species to occupy these spaces. As these niches are filled, the number of opportunities is reduced and the rate of speciation plateaus or declines. Additionally, as species become more specialized, there may be greater genetic and physiological constraints that hinder further divergence and thus limit speciation rates. However, as Seehausen (2006) points out, ecological opportunity alone is not sufficient to predict the occurrence of a radiation, and thus important intrinsic factors are probably involved. Additionally, cases of nonradiation in biologically similar colonization events suggest an element of chance as well, reminding us that evolution has also a contingent component.

Parallel evolution in ancient lakes

One considerable area of study has been whether ancient lakes radiations are predictable, that is, driven by common ecological and evolutionary processes. Although various taxonomic groups have undergone adaptive radiation in different lakes (e.g. cichlid fishes in the African Great Lakes Malawi, Tanganyika and Victoria; cottoid fishes and gammarid amphipods in Lake Baikal; thalassoid gastropods in Lake Tanganyika; oreitiid fishes and hyalellid amphipods in Lake Titicaca; telmatherinid fishes in the Malili lakes of Sulawesi), remarkable cases of evolutionary parallelism have been observed in unrelated species flocks from various lakes, suggesting that similar ecological and evolutionary drivers have triggered the accelerated cascade of diversification observed in many ancient lakes (Martens 1994; Fryer 1996). For example, the *Cyprideis* ostracods of Lake Tanganyika resemble morphologically the *Cytherissa* ostracods of Lake Baikal. Similar armature morphology has been observed in the acanthogammarids of Lake Baikal and the hyalellids of Lake Titicaca (reviewed in Martens 1997). The gastropods of the Malili lakes display many parallel shell and radula morphs in different lakes (von Rintelen *et al.* 2004). Morphologically similar species of cichlids have also evolved independently in different East African Lakes (Kocher *et al.* 1993; Rüber *et al.* 1999; Allender *et al.* 2003; Salzburger 2009).

Recent studies suggest that selection regimes alone owing to similar ecological context or similar female preference are not sufficient to produce these parallel morphologies (Salzburger 2009) and that intrinsic factors such as life history attributes (e.g. brooding, ovoviviparity, viviparity, breeding philopatry), developmental or genetic constraints (Albertson *et al.* 2003), as well as key morphological or physiological innovations (Liem 1973) could contribute to the observed directional diversification (reviewed in Martens 1997; Schön & Martens 2004). For example, the modification of the pharyngeal jaw apparatus (PJA) of cichlid fishes, which allows the specialized use of the oral jaw for prey capture and the pharyngeal jaw for food processing, is often regarded as an evolutionary key innovation that has played an important role in their trophic diversification (Liem 1973). However, recent phylogenetic analyses suggest that the accelerated rate of diversification in East African cichlids occurred within the last 2.4 Myr, well after the acquisition of the PJA (Seehausen 2006; Salzburger 2009). This significant time lag has led several authors to suggest that the high speciation rates in many labrid and cichlid clades are better correlated with the evolution of sexual characters (Alfaro *et al.* 2009; Salzburger 2009), suggesting a

predominant role of sexual selection in these diversifications. Interestingly, the ecologically important and plastic PJA has also allowed the evolution of mouth-brooding behaviour and the production of species-specific courtship sounds in male cichlids (Amorim *et al.* 2004), which have the potential to promote enhanced behavioural isolation. It is likely that this important evolutionary innovation might have served a twofold role: a historical role in trophic adaptation and a more recent (derived) role in sexual selection. Clearly, the role of the PJA in the radiation of cichlids remains open to investigation (Danley & Kocher 2001; Schön & Martens 2004; Alfaro *et al.* 2009). More generally, the interplay among shared ecological contexts, shared life history attributes and unique one-off innovations in promoting diversification and parallelism remains an intriguing area of ongoing research.

Processes of speciation in ancient lakes

The evolutionary forces responsible for the explosive speciation in ancient lakes have been intensely debated. It is generally agreed that among the most important forces involved in triggering speciation are the high intra- and inter-specific competition for limited resources and a high level of predation and parasitism correlated with spatial confinement. Selection is generally considered to be the driving force (Schluter 2000, 2001), although opportunities for geographical subdivision have been also discussed (Wilson *et al.* 2004). However, little is known about the reproductive barriers involved, the traits important in the speciation or the genes underlying the speciation traits.

Intralacustrine barriers and historical barriers to gene flow

Early models of speciation in ancient lakes were highly influenced by Mayr's (1942, 1963) view that speciation can only proceed if geographical barriers obstruct gene flow to a great extent. In allopatry, the speciation process is driven by stochastic events such as genetic drift or a mutation-order process. Different mutations become fixed in different populations as they drift apart or adapt to more or less similar habitats (Mani & Clarke 1990). Bateson, Dobzhansky and Muller envisioned a simple situation in which the evolution of reproductive isolation is a direct result of the gradual accumulation of genetic incompatibilities (see Orr 1996 for a review). Under this model, one population would become fixed for one allele at one locus, whereas the other population would become fixed for a second allele at another locus, and the two independently evolved alleles would cause inviability and/or infertility when co-occurring in

hybrid offspring (reviewed in Coyne & Orr 2004; Lynch & Force 2000). This simple and intuitive model has received wide acceptance and advanced allopatric speciation as the null model of speciation against which all alternative models should be compared (Orr 1996; Coyne & Orr 2004).

Given the broad acceptance of allopatric speciation, it is not surprising that for almost a century research on ancient lakes focused heavily on the biogeography of speciation and brought forward arguments to support vicariant and dispersal modes of speciation (Brooks 1950; Martens 1997). The evolutionary success of many benthic species flocks has been at least partially attributed to extrinsic factors such as habitat discontinuity within most ancient lakes (Brooks 1950). Intralacustrine barriers to gene flow have definitely shaped the population structure of many benthic or philopatric species such as the cichlids of the tribe Eretmodini (Rüber *et al.* 2001) and the lamprologine cichlids (Nevado *et al.* 2009) of Lake Tanganyika. Moreover, geographical separation of previously intralacustrine (interbreeding) populations likely occurred during intense periods of climatic changes or geological events (reviewed in Salzburger 2009; Kocher 2004). Geological evidence shows that water levels in most ancient lakes have risen and fallen repeatedly, creating isolated basins and satellite lakes (Owen *et al.* 1990; Williams *et al.* 1997; Mourguiart 2000; Nevado *et al.* 2009). It can be argued that the satellite lakes and rivers offered opportunities for population subdivision and repeated colonizations, as well as temporal refugia during periods of mass extinction. Moreover, these satellite habitats would have different characteristics compared to the original lake and would constitute areas of intense population bottlenecks and selection. To what extent these temporal restrictions in gene flow shaped the diversification of ancient lake lineages remains unclear for most taxa. Detailed population genetics analyses based on mitochondrial data suggests that philopatric populations of rock-dwelling cichlids of Lake Malawi experienced marked demographic expansion within the last 75 000 years (Genner *et al.* 2010), during the most recent rise in lake level. Water-level changes in Lake Victoria also left signatures of population bottlenecks at the molecular level (Rüber *et al.* 2001; Nevado *et al.* 2009). Although the contribution of allopatric or micro-allopatric models of speciation to overall ancient lakes diversification can only be evaluated with very good knowledge of the evolutionary history and ecology of numerous groups of species as well as the geological history of ancient basins, evidence suggests geographical isolation has occurred in some lineages. Geographical isolation can clearly facilitate local adaptation. However, secondary contacts of formerly isolated populations can promote speciation

via hybridization, chromosomal speciation and/or reinforcement of reproductive barriers that emerged during intermittent periods of isolation. Indeed, fine-scale phylogeographical studies have revealed not only patterns of historical geographical fragmentation or restriction in gene flow but also signatures of hybridization and introgression following secondary contact (Nevado *et al.* 2009). It is largely accepted that geographical separation alone cannot account for the enormous species richness that most of the ancient lakes harbour (Coyne & Orr 2004).

Founder effect or bottleneck-induced speciation

As the colonizers of many ancient lakes are expected to have experienced reduced population size, founder effect and/or bottleneck-induced speciation has also been viewed as a possible mechanism for species diversification in ancient lakes, at least early on in the history of species flocks. Theory predicts that the small effective population size of founding populations or severe bottleneck in population size can lead to important genetic consequences (e.g. change in allele frequencies, linkage disequilibrium, new gene combinations), which, in concert with the novel environment experienced by founder or bottlenecked populations, could accelerate speciation (Gavrilets & Boake 1998). The general view today is that founder event speciation represents "one extreme of a continuous range of possibilities" (Barton & Charlesworth 1984) and experimental and theoretical support for these models is weak.

The very few studies that have attempted to infer the potential role of founder effects during the colonization stage of ancient lakes do not support the premise that speciation by founder effect triggered radiation in these habitats. For example, studies on the intraspecific variability at the major histocompatibility complex (MHC) suggest that the cichlid speciation in Malawi Lake was not because of a founder effect as no major population bottleneck occurred (Klein *et al.* 1993). However, the scarcity of empirical studies that have screened for the genomic signature of founder events or population bottlenecks associated with the radiation of species flocks (Moran & Kornfield 1995; Elmer *et al.* 2009) leaves a deficit of knowledge regarding the occurrence and contribution of founder effects in radiation events. A systematic evaluation of bottleneck signatures—across many ancient lake taxa—can provide insight into the importance of founder effects in the speciation process (Genner *et al.* 2010). Recent advances in estimating both long-term and the ancestral effective population size (N_e and N_a) from DNA polymorphism in extant populations as well as from interspecific polymorphism could shed light on the potential role of founder effects

or population bottlenecks in speciation (Walsh *et al.* 2005).

Speciation with gene flow: on the role of introgressive hybridization in ancient lake speciation

Despite being theoretically problematic, sympatric speciation has almost certainly been involved in the formation of several species flocks in ancient lakes (Schliewen & Klee 2004; Barluenga *et al.* 2006). The strongest evidence comes from examples of diversification in areas in which it is hard to envision a physical barrier to gene flow. The most convincing case studies are the cichlid flocks found in the small and environmentally homogeneous crater lakes Barombi Mbo and Bermin (Schliewen *et al.* 1994; Schliewen & Klee 2004). Based on a comprehensive phylogenetic study, Schliewen & Klee (2004) concluded that hybridization between ancient cichlid lineages likely acted as a driving force for speciation that influenced both the speed and direction of evolution.

Although radiations in ancient lakes provide several uncontested cases of hybrid speciation in sympatry, the premise that hybridization could be an important evolutionary force has been highly debated (reviewed in Barton 2001; Mallet 2007; Seehausen 2004). Two main problems have historically been raised. First, if hybrids enjoy good fitness, they are likely to be involved in backcrossing and introgression. As a result, hybridization has been viewed as a force counteracting divergent and disruptive selection and therefore a major impediment to species divergence in sympatry (Mayr 1963). Alternatively, low fitness of the hybrids would render them evolutionary dead ends and of no significant importance in speciation. The solution to these problems lies in the fact that hybridization and introgression have the potential to increase genetic variance which, in turn, can prompt ecological transitions and create new ecological opportunities unavailable to parental species (Rieseberg *et al.* 2003; Grant *et al.* 2005; Baack & Rieseberg 2007; Nevado *et al.* 2009). For example, hybridization frequently results in transgressive segregation, the appearance of extreme phenotypes in hybrids, which facilitates the utilization of new niches (Rieseberg *et al.* 1999). Although it seems intuitive that only "a minority of the gene combinations formed by the hybridization of species might be fit, perhaps fit enough to spread onto as yet unoccupied adaptive peaks" (Dobzhansky 1970), the wide genetic constellation of the hybrids and their propensity to invade new ecological niches, coupled with the high rate of hybridization among closely related species, suggests that hybridization could have led to rapid speciation, especially after ecological perturbations that have opened new niches either by extinction or habitat shifts.

It has been noted that ecological conditions that are conducive to adaptive radiation, such as geographically confined zones and the presence of closely related species competing for limited resources, are also likely to promote hybridization (Seehausen 2004; Mallet 2007). In ancient lakes, hybridization can occur not only between sister species incompletely isolated by habitat or by geography but also between lacustrine (resident) lineages and recurrent (nonresident) colonists. This two-fold hybridization stage has the potential to increase the strength of reticulate evolution via the repeated input of novel genetic variation. Indeed, recent multilocus phylogenetic analyses have revealed significant conflicting signals among loci, suggesting that interspecific hybridization played an important role in the evolution of several major species assemblages, such as the sailfin silversides in the Malili Lakes of Sulawesi (Herder *et al.* 2006), the cichlid fishes of Lake Victoria, Lake Tanganyika and Lake Malawi (Danley & Kocher 2001; Salzburger *et al.* 2002a; Smith *et al.* 2003; Won *et al.* 2005; Nevado *et al.* 2009), the cichlids in Cameroonian and Nicaraguan crater lakes (Schliewen *et al.* 1994; Schliewen & Klee 2004; Barluenga *et al.* 2006), and potentially the sculpine fishes of Lake Baikal (Kontula *et al.* 2003). Introgressive hybridization has been suggested to have played a key role in the radiation of these ancient lake species flocks by significantly increasing the genetic diversity of the resident populations (Rüber *et al.* 2001; Salzburger *et al.* 2002a; Smith *et al.* 2003; Herder *et al.* 2006). Unfortunately, the potential role of hybridization in driving the radiation of invertebrate flocks remains largely unexplored. The vast majority of phylogenetic studies are based on a single locus, generally mitochondrial (Hidding *et al.* 2003; Audzijonyte *et al.* 2006; Gerner *et al.* 2007; von Rintelen *et al.* 2007). Although these studies are essential in revealing general patterns of diversification, they cannot detect the signature of hybridization: i.e. the potential cytonuclear or nuclear discordance affecting major branching patterns across phylogenies of major invertebrate radiations.

Even with multilocus studies, documenting the process of reticulate evolution is inherently difficult because of the great amount of genetic variation that closely related sister species share and their high level of inherited polymorphism (Seehausen 2004; Grant *et al.* 2005; Won *et al.* 2005; Herder *et al.* 2006; Baack & Rieseberg 2007). Comprehensive phylogenetic studies on ancient lakes radiations based on multiple nuclear and mitochondrial markers, coupled with population genetic studies and morphological and ecological data, have the potential to discriminate between phylogenetic incongruence because of true hybridization versus ancestral polymorphism and to demonstrate the extent to which hybridization and introgression between sister

species acted as a driving force for rapid speciation in ancient lakes.

Approaches for studying speciation in ancient lakes

Historically, ancient lake research was inspired by the opportunity to examine some of the oldest habitats and most species-rich endemic assemblages in the world. Brooks' (1950) classical seminal review on Lake Baikal inspired the research of ancient lakes for more than half a century. His fine-scale comparative biogeographical approach on species flocks with contrasting life history attributes and evolutionary histories established the basic conceptual model for ancient lake research, directing attention towards specific geographical and geological settings with emphasis on intralacustrine restrictions to gene flow. This original research programme was extended by Martens' (1997) integrative approach of contrasting patterns and processes of speciation across lakes with various geological histories and ecological conditions. Current research in ancient lakes takes advantage of the new environmental tracers available in limnology, emerging ecological genomics approaches, next-generation sequencing technology and new analytical techniques to further advance the field of speciation.

The central goal of speciation research as originally proposed by Dobzhansky (1970) and elegantly reiterated by Coyne & Orr (2004) is identifying the reproductive isolating mechanism(s) and evolutionary forces responsible for building isolating barriers. To date, only a handful of studies (outside ancient lake systems) have employed a comprehensive dissection of both prezygotic and postzygotic reproductive barriers and directly measured their relative and absolute strength. Collectively, these few studies (Coyne & Orr 1989; Shemske & Bradshaw 1999; Ramsey *et al.* 2003; Gow *et al.* 2007) suggest that a complex of reproductive barriers operate in many sister species and that identifying their historical succession and pinpointing the factors that initiated the speciation process is inherently problematic (Coyne & Orr 2004). Moreover, very little is known about the traits involved in reproductive isolation or the genes underlying the key speciation traits. The problem of speciation research stems from the longevity of the process that spans thousands of generations and its gradual progression through a succession of reproductive mechanisms governed by a complex of intrinsic and extrinsic forces (Coyne & Orr 2004). Understanding the evolutionary forces driving large-scale radiation events pose a much harder problem as these radiations involve tens or hundreds of speciation events as well as many extinction events that remain largely undocumented.

Nevertheless, ancient lakes provide an incredible setting as natural experiments and have the potential to reveal the evolutionary forces responsible for inaugurating or driving the diversification, the reproductive barriers involved at different time stages of a particular speciation process, the traits responsible for reproductive isolation, and the genes underscoring the traits. Several emerging methodological approaches advance ancient lakes as attractive model systems for speciation research.

Bridging sister-clade and sister-taxon studies with population studies: a window in time

The presence of species flocks of various sizes, evolutionary ages and degrees of reproductive isolation offers the opportunity to investigate the reproductive barriers that initiate speciation as well as the mechanisms that intensify species demarcation or complete the speciation process. This setting offers the prospect to investigate the genetic architecture of a radiation through time, from the sister-clade or sister-species level to population (ecotypes) level through a snap-shot research approach.

Large-scale phylogenetic reconstructions are particularly useful for identifying not only the number of colonization events that seeded a particular radiation and the age of the radiation, but also the general pattern of cladogenesis—the sister-clade relationships. For example, based on a comprehensive phylogenetic history of the cichlid fishes of Lake Malawi, Danley & Kocher (2001) suggest that this diversification involved three distinct waves of radiations governed by different evolutionary forces. The first diversification resulted in the differentiation of two major ecological lineages, the sand- and rock-dwelling forms. This original ecological isolation set the stage for the second major radiation that involved the trophic specialization of rock-dwelling cichlids. The third and most explosive wave of diversification entailed the evolution of male nuptial colouration. Danley & Kocher (2001) proposed a verbal speciation model of divergence with gene flow based on an early model developed by Rice & Hostert (1993) to explain the observed pattern of diversification. The authors suggest that diminished gene flow in combination with strong, divergent selection pressure at multiple loci can work in conjunction to promote and speed up speciation. This general model is relevant to cichlid diversification because it predicts the emergence of a positive feedback loop in which cladogenetic events can potentially lead to subsequent speciation episodes (Danley & Kocher 2001).

Experimental approaches focused on ecologically divergent species pairs can also greatly advance our understanding of the components of reproductive

isolation between closely related species. Such approaches have been often employed in cichlid fishes in an attempt to identify traits (e.g. mouth morphology, body shape and size, coloration, behaviour) that are relevant to their evolutionary success (Albertson *et al.* 2003; Salzburger 2009). Similarly, the holarctic three-spine stickleback species complex that includes limnetic–benthic, lake–stream and anadromous–freshwater sister pairs has long been established as an advanced model of speciation research (McKinnon & Rundle 2002; Gow *et al.* 2007). Although the geographical context and natural history of these two model systems is contrasting, both systems provide the advantage of the replicative nature of evolution, a well-understood ecological setting, and the potential to advance generalizing knowledge of speciation.

While phylogenetic studies and species pair experiments have often been involved in speciation research to reveal the tempo and pattern of divergence, population genetics studies are less often employed. However, Via & West (2008) suggested that as speciation is a long-term process that generally spans thousands of generations and will ultimately result in differences in numerous loci affecting isolation, the investigation of emerging reproductive barriers within lineages has to begin within species, at the population level. The common concern with this approach is that not all diverging populations will complete the speciation process and become reproductively isolated species (Coyne & Orr 2004). Nevertheless, studies at the population level can be used to infer the genetic architecture of incipient reproductive isolation (Butlin 2010).

Within the past decade, several analytical methods that rely mostly on the analysis of multilocus genetic data have been developed or improved, greatly facilitating the investigation of fine-level population genetic structure, restrictions to gene flow as well as other processes relevant to speciation research such as population bottlenecks, admixture, or hybridization (for a review see Manel *et al.* 2005). Generically classified as ‘assignment methods’, these approaches can be used to group individuals (without prior population information) in genetic clusters and therefore identify cryptic population genetic structure (e.g. STRUCTURE, Pritchard *et al.* 2000; BAPS, Corander & Marttinen 2006) or test the belonging of a genotype to predefined populations and quantify contemporary dispersal (e.g. GENECLASS, Piry *et al.* 2004; BAYESASS, Wilson & Rannala 2003). In addition, the recent program, MIGRATE (Beerli & Felsenstein 1999), is useful for estimating effective population sizes and historical migration rates between subpopulations. The core algorithms of these programs are generally similar, but the underlying assumptions (e.g. Hardy–Weinberg or linkage equilib-

rium) differ slightly, and thus each model is best applied under particular circumstances (reviewed in Excoffier & Heckel 2006). These applications have proven valuable for inferring population structure, admixture and demographic history of ancient lake populations. For instance, using STRUCTURE and BAPS, Walter *et al.* (2009) identified distinct genetic clusters in the telmatherinid fishes of Lake Matano, Indonesia, despite relatively large and uniform dispersal rates, suggesting cryptic population structure.

From a single-locus approach to genome-wide scans: developing new model systems of adaptive radiation

Assessing the congruence among independent markers has become an important approach in both phylogeographical and phylogenetic studies (Avice 2000). Concordant results across markers generally reflect shared evolutionary history, while patterns of incongruence can reveal the role of introgressive hybridization and selection in evolution (Lu *et al.* 2001). An increasing number of studies in cichlids of African lakes are inferring relationships among populations or species based on combined analysis of different DNA regions (Schlieven *et al.* 1994; Won *et al.* 2005; Barluenga *et al.* 2006). Where more traditional studies with single or few markers and chromosomal comparisons have fallen short, genomics approaches have already proven to be extremely powerful in speciation research (reviewed in Butlin 2010). However, given that multilocus or genome-wide studies are rare outside the cichlid fish model (but see Macdonald *et al.* 2005), there is a clear need for developing new invertebrate model systems of radiation research.

Advances in next-generation sequencing technologies associated with a decreasing sequencing cost enable the development of genomic resources for new model species. Such resources would enable not only multilocus phylogenetic analyses but also large-scale comparative genomic approaches. For example, comparative mapping has the potential to reveal chromosomal rearrangements (e.g. deletions, insertions, inversions, translocations) between sister taxa. Changes in chromosomal colinearity can act as barriers to gene flow between diverging populations or sister species, creating islands of divergence within regions of low recombination. It has been demonstrated that genome-wide scans for neutral or quantitative trait loci (QTL) divergence (Rieseberg *et al.* 2003; Rogers & Bernatchez 2005) can reveal the genomic architecture of the speciation process.

Clearly, ancient lake research can take advantage of the accelerated developments in speciation research from other terrestrial or aquatic systems. For example, several well-documented genomics studies suggest that

many ecological species maintain their ecological attributes and distinctness in the face of high rates of hybridization and that hybrid speciation could be more common than we thought (reviewed in Hey 2006; Baack & Rieseberg 2007; Mallet 2007). Genome-wide analyses of introgression patterns find that different parts of the genome vary in introgression rates, with regions of low recombination rates showing reduced introgression (reviewed in Baack & Rieseberg 2007). Furthermore, other genomic studies demonstrate that genetic divergence between ecologically divergent sister species can increase at loci under strong divergent selection even if there is significant gene exchange and homogenization across the rest of the genome (Rogers & Bernatchez 2007; Via & West 2008). To explain the observed 'islands of differentiation' between host races of the pea aphids *Acyrtosiphon pisum*, Via & West (2008) introduced the mechanism of 'divergence hitchhiking'. The authors suggest that differential gene flow across the genome of diverging populations is responsible for the build-up of linkage disequilibrium between remote loci, advancing further increases in reproductive isolation. Theoretical work confirms that divergence hitchhiking can generate large regions of differentiation, but the conditions under which this occurs (e.g. small effective population size, small migration rates) are considered limited (Feder & Nosil 2010). These comparative genomics techniques make feasible the identification of genetic variation underlying phenotypic differences between diverging populations or sister species (Kocher 2004). With the availability of many radiations of varying ages in ancient lakes, we have the opportunity to take multiple "snapshots" of the genomes under divergence at various levels of divergence, adding an entirely new dimension to the study of speciation.

Comparative approaches

Ancient lakes offer also the opportunity to contrast patterns of speciation in species flocks with contrasting life history attributes: benthic and limnetic groups, vagile and poorly dispersive species, adaptive and nonadaptive radiations. While African cichlids are emerging as a powerful model system in adaptive radiation research (Kocher 2004; Seehausen 2006), a large number of invertebrate radiations (both large and small radiations) remain overlooked. Highly species-rich flocks, such as the amphipods of Lakes Baikal, Titicaca and the Caspian Lake, the ostracods of Lakes Baikal and Tanganyika, and the gastropods of Lakes Malili and Tanganyika offer the possibility to employ a large-scale comparative approach and explore replicated 'natural experiments' across clades. Less species-rich or conspicuous flocks of young age, such as the calanoids of The Malili Lakes

and the onychopods and mysids of the Caspian Sea, are also important for conducting deep-level molecular and experimental studies on the speciation process in ancient lakes. Patterns of multiple colonization and introgressive hybridization are easier to depict in a radiation with fewer hybridization nodes.

Furthermore, the Malili Lakes, being the only hydrologically connected system of ancient lakes, provide an opportunity to study dispersal, colonization success and the effects of hybridization within a 'replicated' ancient lake system. Species flocks of diatoms, shrimps, gastropods and fishes (von Rintelen *et al.* 2004; Herder *et al.* 2006; Roy *et al.* 2007; Bramburger *et al.* 2008) suggest that invasion potential alone does not regulate adaptive radiation within these groups among the different lakes where there is considerable gene flow within the connecting rivers (Schwarzer *et al.* 2008; Walter *et al.* 2009). Intra-lake processes such as resource partitioning, hybridization and introgression dominate the formation and maintenance of lake-specific species flocks.

The need for large-scale comparative studies of adaptive radiations has long been recognized. Bernatchez (2004) points out that there are still only a handful of comprehensively studied adaptive radiations, and most comparative studies have tested only a subset of the theoretical predictions. Additionally, Seehausen (2006) reminds us that most studies neglect many cases where adaptive radiations failed to take place despite favourable ecological settings. It is also possible that much could be learned from the relative abundance of the more cosmopolitan species that inhabit ancient lakes. Often, the percent endemism is quoted for ancient lakes as a descriptor of its relative importance with respect to biodiversity. However, in most ancient lakes, cosmopolitan species account for at least 50% of the species richness observed, although this value is well below 10% in the Malili Lakes of Indonesia. Certainly, the co-occurrence of cosmopolitan generalist and endemic specialist species can be an important ecological factor that needs to be further evaluated.

An integrative approach for the study of speciation

Understanding how species originate requires detailed knowledge of both extrinsic and intrinsic factors that limit gene flow between species and the specific conditions under which reproductive isolation originates as well as the way isolation progresses to completion (Coyne & Orr 2004). Because no single system can give us the entire road of the speciation process, comparative approaches (across lakes and across taxa) have often been proposed as a solution. However, the search for common patterns and processes has been greatly

impeded by the complex nature of these radiations. We can no longer hope to find *the* powerful evolutionary force or mechanism that would explain all these radiations. Many recent studies recognize the complexity of the process and the need of crossing traditional conceptual boundaries, such as the artificial division between allopatric and sympatric speciation. Instead, integrative research approaches that bring together the biology and genetics of speciation into an ecological and geological context appear to be more conducive to break-through discoveries. The traditional methodological approaches (e.g. phylogenetics, phylogeography, genomic screening and morphometric analyses) often appear alone or in pairs in the scientific literature, and as such, we continue to make small advances in our understanding of speciation. Future “integration” of multidisciplinary data into large-scale analyses is essential for making larger steps towards understanding complex evolutionary processes. This would involve large-scale multidisciplinary

studies (e.g. geological, paleolimnological, ecological, morphometric, phylogenetics and genomics) as well as a strong interplay between theory and experimental approaches (Fig. 2). The necessity of merging the geography, ecology, biology and genetics of speciation has already been proposed in many systems (Bernatchez *et al.* 1999; Via 2002; Bernatchez 2004). However, ancient lakes provide an incredible opportunity to integrate geological and climatological data with ecological and genomics data (Sherbakov 1999). Given the relatively well-known geological history of most ancient lakes and a reliable fossil record for groups such as molluscs and ostracods, ancient lakes are ideal settings for molecular-clock calibrations and for investigations of the adaptive reoccupation of ecological niches and species turnover after major ecological disturbances (Schultheiß *et al.* 2009). Advances in the ecology and genetics of speciation can also result from the interplay of theoretical and experimental work (Gavrilets & Losos

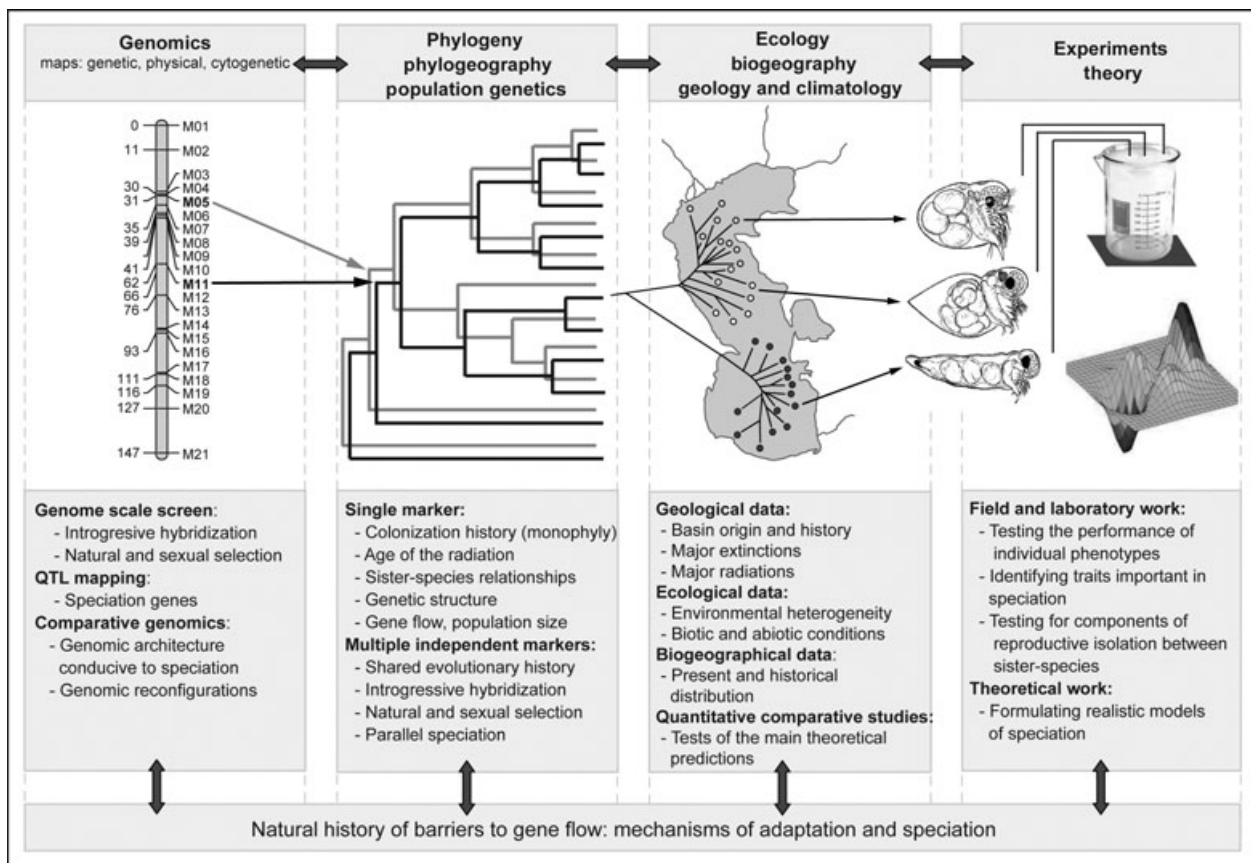


Fig. 2 An integrative approach for the study of speciation involving genomic, phylogenetic, phylogeographical, population genetics, morphometric, ecological and geological data and by integrating experimental and theoretical work. Double-headed arrows indicate cross-disciplinary approaches. Although such a synthetic approach could be applied to speciation research in general, it is especially suitable to ancient lakes research because of (i) the opportunity to investigate the sequential build-up of reproductive isolation at different stages of the speciation process from the population to sister-species levels, (ii) the relatively long historical record and (iii) the replication provided by the many examples of parallel evolution.

2009). While theory inspires empirical approaches by suggesting assumptions and parameters to test, empirical estimates (e.g. natural selection, genetic drift, population size, strengths of reproductive isolation, genetic architecture) are crucial for formulating realistic theoretical models (Via 2002). Overall, ancient lakes provide excellent settings for developing comprehensive studies of speciation and large-scale comparative approaches of the mechanisms underlying adaptations, diversifications and evolutionary innovations.

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