

6 ON THE VIRTUES OF INTERSPECIFIC RELATIONS

Wavuvi wazee husema kuna nyoka mkubwa sana katika ziwa, ana macho mekundu yapata kama futi 50. Huyu nyoka hui-buka juu baada ya miaka 50. Akiibuka tu, wavuvi huvua sana samaki.

The old fishermen say there is a huge snake, which has red eyes and only comes up every 50 years. When it comes up, fishermen catch a lot of fish.

—Traditional story (translated from Kiswahili) of Lake Victoria from Mbarika, Mwanza Gulf, Tanzania

A birder trying to add gulls to their life list will often need a good deal of patience, persistence, and tolerance of failure. The same goes for a hiker trying to identify the oak trees they happen across while walking through a forest in the eastern United States. The problem is that many individual gulls and oaks can never be identified to the species level, even with a specimen in hand and DNA sequence, let alone from a brief glance. This is because gulls of different species frequently mate with one another, yielding offspring that don't belong definitively to either parental species. Oaks do the same. Sometimes

these hybrid offspring go on to mate with other hybrids, or they may “backcross,” mating with individuals of one of the parental species; things quickly become messy.

Beyond birders and hikers, the issue of interspecific hybridization and gene transfer between species (technically known as introgression, different from hybridization in that it emphasizes the transfer of genes rather than the matings themselves) have attracted more widespread attention in recent years as evidence has emerged that billions of us are carrying DNA that originated with Neanderthals and Denisovans—which are generally considered different species from ours. Moreover, some of these introgressed genes can influence important traits such as how likely we are to contract serious diseases, and how such diseases affect us. For example, two different fragments of the Neanderthal genome, which many people possess, have been shown to be correlated with how severely ill a person is likely to become if infected with SARS-CoV-2, which causes COVID-19. On the other side of the disease coin, the exchange of genes between different strains of influenza has resulted in some of the worst flu pandemics.

These observations clearly pose a challenge to the biological species concept, and the issue is not new. Nevertheless, hybridization was long treated as anomalous by the mainstream of science and quietly neglected. With more and more genomes becoming available, including large samples of genomes from different individuals for some species, hybridization and introgression have become much easier to detect and study, and their evolutionary significance has become more apparent.

One consequence of extensive hybridization can be extinction. We explored earlier how human-caused changes to lake

water and light transmission can cause formerly well-defined cichlid species to collapse into a single gene pool. This can also result when people move animals into new locations where the new arrivals encounter populations or species with which they can interbreed, but from which they were previously separated by distance or physical barriers. For example, nonnative tilapia (*Oreochromis* sp.), escaping from aquaculture or released deliberately, with usually good intentions, are prone to mating with native wild fish and thus are putting native species at risk in some Tanzanian waters. The problem can be worse if the environment is disturbed in a way that makes interbreeding more likely or helps the hybrid offspring survive.

The effects of hybridization are not always so harmful, however. Hybridization can at times contribute to speciation, adaptation, and even adaptive radiation. In plants, the origin of new species through hybridization, or hybrid speciation, is surprisingly common, but involves genetic mechanisms that occur less frequently in animals. Nevertheless, young animal species with clearly hybrid origins have on occasion been documented. In chapter 2, we explored examples from ricefish and cichlids in which hybridization was involved in the formation of new species, including parallel hybrid speciation in *Pundamilia* from Lake Victoria. An additional pair of likely cases comes from Lake Victoria, this time involving “parental” species so distinct that they are in different genera—*Pundamilia*, once more, and the related genus, *Mbipia*.

Irene Keller and her colleagues in Ole Seehausen’s laboratory at the University of Bern collected genomic and other data from five members of these genera at a single site in Lake Victoria, Makobe Island. Their analyses suggest that two of

the species, one in each genus, are a result of recent hybridization between a species of *Pundamilia* and species of *Mbipia*. With such a messy situation, the existing classification scheme and genus names are in serious trouble, although to be fair, classification is generally a fraught enterprise in Lake Victoria, certainly when attempted above the level of species.

Hybridization can also contribute to adaptation without any need to form a new species. The steps and mechanisms involved may be straightforward or more subtle. In the simplest scenario, matings between species may enable one partner lineage to acquire a beneficial allele from the other. The benefit of the “imported” allele may, for example, be that it can replace a gene damaged by mutation. Or the new allele may be better suited to a changed environment; adaptation to higher temperatures comes to mind in our time. Introgression of this sort certainly occurs and may contribute to a commonly observed anomaly: analyses based on mitochondrial DNA often indicate different evolutionary relationships for a set of species than do analyses based on the remainder of the genome, which is generally referred to as the nuclear genome because it is found in the nucleus.

Mitochondria are key organelles in the cells of animals and plants, performing certain valuable metabolic reactions that cannot be completed without them. Mitochondrial DNA is separate from the rest of the cell’s DNA because mitochondria evolved from bacteria that were engulfed by our ancient single-celled ancestors. Over time, mitochondria came to live permanently inside our cells in a mutually beneficial arrangement: we got a more efficient metabolism, and they got a safe home stocked with all of their needs. Thus, each animal, each

of us, is the result of a symbiosis, which means organisms of different species living together. The organization, structure, and seeming unity of our bodies is a bit illusory—the result of an ongoing collaboration between two distinct genomes. This is one of those familiar facts of biology that can become remarkable again with a moment or two of reflection.

Housed inside the mitochondria themselves, mitochondrial genes are inherited almost exclusively through eggs (in animals), separately from an organism's other genes. Essentially clonal, mitochondrial genes do not take part in sexual reproduction. With these differences, it is not surprising that when we estimate evolutionary trees using the mitochondrial genes from a set of species or populations, the results sometimes differ from the trees suggested by the rest of the genome. Occasionally, though, the inconsistencies are major. Different species or subspecies sometimes share nearly identical versions of their mitochondrial genes despite substantial differences across the rest of the genome or for more visible traits. These inconsistencies can be explained by hybridization that is followed by selection favoring the new mitochondrial type, allowing it to sweep through the population. This may occur even if little mixing happens between the species for the rest of the genome. Selection is not the only explanation for such patterns, and more haphazard processes can also result in mitochondrial sweeps. For our purposes, it is the resulting discrepancy that is mainly of interest. These discordant patterns between mitochondria and the rest of the genome, which have been seen in several ancient lake radiations such as Sulawesi's telmatherinid fishes, were one of the first indications that hybridization may be more common during adaptive radiations than had been suspected.

THE TRANSGRESSIONS OF GENES

The newly mixed genes of two species can sometimes produce traits quite different from those present in either parent, and not simply in the sense of being intermediate or even combining traits previously found only in different species. On the surface, the parental species in this scenario may be outwardly similar to each other. Naively, we might expect that their hybrid offspring would be similar to both. Suppose, for example, that two species of lemur had evolved an elongated finger to help them catch insect larvae living in holes in trees (lemurs do such things, but the details in my example are hypothetical). If the superficially similar parental species mated, their hybrid offspring might be expected to have similarly long fingers, and if the hybrids continued breeding with one another we might expect things to stay about the same, generation after generation.

But depending on the genetics and details of how the long fingers of each species develop, things might be more complicated and more interesting. For instance, one species might achieve long fingers owing to a mutation favored by selection that causes an extra bone to be added. This is plausible since primate fingers already vary in the number of bones they possess, with the human thumb, for example, having lost one of the bones present in the other fingers.

In the second species with a particularly long finger, a single finger bone might grow longer, with no change in the number of bones. When genes for these different mechanisms end up together in hybrid offspring, the resulting finger lengths could be more extreme than those of either parent, depending

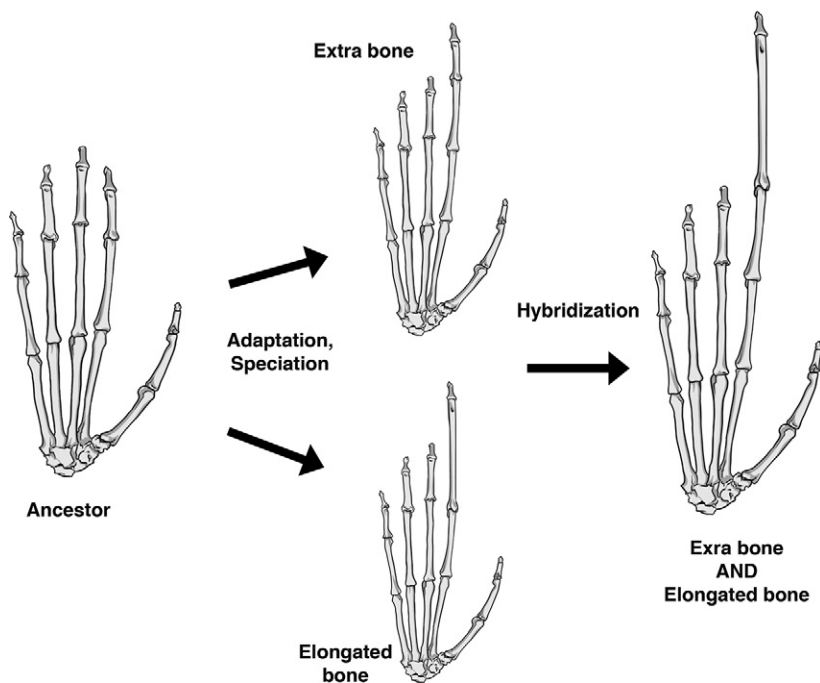


Figure 6.1

An ancestral lemur gives rise in different places to two new species, each having evolved a long finger to catch insect larvae in tree holes, but via different genetic changes. When they hybridize, the hybrid has a longer finger than either parent. *Source:* Haleigh Mooring.

on which alleles are dominant or recessive. For example, if both an elongated bone and the addition of a bone to each finger are dominant traits, hybrid offspring will have one notably long finger bone as well as an extra bone (figure 6.1). With two mechanisms of finger elongation both present in individual hybrid lemurs, those hybrids should have even longer fingers than either of the parental species—which already had

impressive digits. The outcome will be different if the genetic details are different, but as long as there are not too many genes involved and the hybrids keep interbreeding for a few generations, we expect to see more extreme finger lengths in some of these hybrid individuals (figure 6.1). The key point is that the variation in the hybrids is expected to *transgress* the range seen in the pure (nonhybrid) forms, leading to this phenomenon's technical name, *transgressive segregation*, or a little less formally, *transgressive variation*.

The opportunities for transgressive variation can be surprisingly extensive. In our imaginary lemurs, there are other ways fingers could elongate. Considering just one bone, it could end up longer by either growing faster or for a longer period—processes that could easily involve different genes. Or instead of the final finger bone lengthening, it could be the middle bone that elongates or the first bone at the base of the finger. Combining these different bones with the alternatives of faster- or longer-duration growth, the possibilities start to add up fast. And we have not even thought about what could happen at the levels of cells, molecules, and biochemical pathways. In addition, transgressive variation can arise through other processes subtly different from the example here. There are a lot of possibilities.

Conceptual illustrations are helpful for thinking through and explaining the possibilities, but what evidence is there that transgressive variation has contributed to evolution? One of the first and most influential studies in this area, helping set the stage for a long series of ancient lake studies, involved fruit flies. It was conducted by a giant of population genetics and one of its more memorable personalities, R. C. “Dick” Lewontin.

I knew him a little bit because I took his class in population genetics while a PhD student. He left a strong impression. Lewontin was famously a Marxist and interested in student learning but disinterested in grades, so he let us know at the course's start that the default plan was for everyone to get a B+. A charismatic and slightly eccentric lecturer, he almost always dressed in a near uniform of denim shirt and khaki pants, and addressed the class with some of the quirky mannerisms you might include if you were designing an old-school professor from scratch. He was also generous with students, at one point initiating a weekly reading group, almost a course, in an area he was not enthusiastic about because students were interested.

Lewontin's seminal publication on the role of hybridization in adaptation continues to be cited annually in dozens of scientific papers, including articles on ancient lake radiations, more than fifty years after it first appeared. Together with collaborator L. C. "Charles" Birch, he studied a late nineteenth- and twentieth-century expansion in the geographic range and temperature tolerance of an Australian fruit fly, *Bactrocera tryoni* (then called *Dacus tryoni*), an important agricultural pest. Based on observations of apparent hybrids in nature, they inferred that interbreeding with a sister species had occurred, possibly as a result of human changes to their habitats. They hypothesized that the introgression of genes into *B. tryoni* had facilitated adaptation to a new temperature range not formerly tolerated by either species.

To test important assumptions of this hypothesis, they collected the two species from their natural distribution and started hybrid and pure *B. tryoni* populations in the laboratory at a range of temperatures. The flies are small and short-lived

enough that one can use them to study the process of evolution experimentally, allowing different populations to evolve over several generations. Initially the hybrids did not survive and reproduce as well as the pure populations, but as time passed the hybrids improved, and also came to resemble the *B. tryoni* in appearance. At moderate, relatively optimal temperatures, the later generations of hybrids roughly matched the performance of the pure *B. tryoni*. But at the most extreme temperature, 31.5°C, the hybrids were superior. This is a remarkable finding. That temperature is clearly beyond the optimal range for the sister species as well as *B. tryoni*. Somehow the combination of genes from the two species substantially improved survival and reproduction *beyond* the usual tolerance of either “parental” species. In this system, therefore, there was transgressive variation for biological fitness at high temperatures.

Such multigenerational experiments have not been performed yet with ancient lake species, but a notably original study of the performance of hybrids in novel ecological situations was conducted by Oliver Selz. Working with Seehausen in Kastanienbaum, he conducted this “proof-of-concept” experiment using African cichlids. I met Oliver before he performed these experiments, when he visited my laboratory early in his graduate studies to discuss a possible internship. Even just starting his PhD, he was an impressive scholar as well as delightful guest (even bringing fondue ingredients with him from his native Switzerland).

The transgressive segregation study began with Selz and Seehausen setting up matings between a Lake Victoria *Pundamilia* cichlid and two other species, each with a different ecology. The *Pundamilia* sp. had a narrow, specialized diet of

zooplankton, the tiny crustaceans and other animals that circulate in the open water of the lake. One of the species with which the *Pundamilia* were mated was a specialist feeder that scrapes algae from rocks; it is also native to Lake Victoria. The third species used in the crosses was a generalist, with broad, flexible feeding habits and tolerances, and native to Lake Malawi. The familiar part of the study involved measuring the feeding success of each species and their hybrid offspring on the prey types typical of those eaten by the two specialist species. The ecological speciation hypothesis predicts that the specialists should be most successful on the diets to which each has been adapting for many generations, compared to the other pure species or the hybrids. The more noteworthy part of the study involved presenting the pure parentals and hybrids with food items distinct from those of the parental specialist species. The novel foods were a freshwater shrimp and gammarid crustacean (the latter distantly related to the gammarids that radiated so spectacularly in Baikal), which are similar to prey eaten by some other species of fish, including other cichlids. If hybridization and transgressive variation give rise to novel forms with the ability to colonize new ecological niches, the hybrids should be superior on at least some of the novel prey. It was a potentially risky test of a bold hypothesis.

In their initial analyses, Selz and Seehausen confirmed the long-standing prediction that pures, when feeding on their usual prey items, should generally outperform hybrids and specialists adapted to other prey. For example, algae scrapers fed more successfully on algae (actually a laboratory approximation) than did the zooplankton feeders or the hybrids. Thus, the baseline results were largely as expected. But on

novel prey, things were more interesting. On the unfamiliar prey items, the hybrids always did at least as well as the parental pures, and in some cases they did better. Specifically, the hybrid offspring of the algae scrapers and zooplankton feeders were the best at catching a novel and ecologically distinct prey species—freshwater shrimp. Similarly, in a different crossing in which the zooplankton eater, the generalist, and their hybrid offspring were each given an alga-like prey item, quite unlike either parental's usual foods, the hybrids again had the greatest success. The authors interpret their results cautiously and present a number of caveats. Yet their evidence that hybrids can surpass their parents in some novel ecological contexts argues that hybridization, possibly involving transgressive variation, has at least the potential to play an important role in adaptive radiation.

Speciation typically involves barriers to mating too. In a related study, Selz and several collaborators asked if hybridization might initiate such mating isolation. They asked if hybrids between cichlid species might sometimes mate more often with each other, preferring the same sort of hybrids, than with either of the parental species. As in the feeding study, they again began by crossing a set of Lakes Victoria and Malawi cichlid species with each other, generating sets of hybrid offspring from three pairings of parental species. These were raised apart from their mothers to avoid imprinting effects. When females of each of the parental species were allowed to choose among males of their own species, male hybrids, or males of a different species, they always showed a strong preference for males of their own species, mating with them almost exclusively. This was not a surprise, but much more difficult to predict was what

the hybrid females would do. One commonality was that all three sets of hybrid females rejected the males of at least one parental species, and all mated fairly readily with hybrid males. The most startling and intriguing finding, though, was that hybrid females of a pair of parental species from Lake Malawi strongly preferred hybrid males, rejecting both types of “pure” male. Hence partial premating isolation arose in a single generation between hybrids and their parental species, and nearly complete premating isolation in some instances. When Selz and colleagues analyzed color variation in the Malawi pair, their results suggested that hybrids were transgressive for color. And since the transgressive color patterns were preferred by hybrid females but rejected by pures, it follows that the preference could also be considered transgressive.

Even with strong premating isolation between the first generation of hybrids and parental species, it is unlikely that a new species would be instantly established by the hybrids. The problem is that first-generation hybrids do not usually “breed true,” to use the language of animal breeders, showing the same traits one generation after another. Rather, when the hybrids interbreed, their offspring will generally exhibit more of the variation present in the pures, with different combinations in each individual second-generation hybrid. Still, if both sexual and natural selection were acting to favor hybrid traits, a new species could emerge over time.

In a quite different but notably detailed example from a small radiation, the *Cyprinodon* pupfish of the Bahamian island of San Salvador, hybridization and introgression are hypothesized to have facilitated both ecological innovation and speciation. Although not located in an ancient lake, these pupfish

have strong links to ancient lake systems. They are members of the same family that radiated in Lake Titicaca and include a scale eater, reminiscent of Lake Tanganyika, but in this case with apparently no right or left lateral twisting.

Emilie Richards, Chris Martin, and colleagues, working mainly at the University of North Carolina and University of California, have found that different populations and species of *Cyprinodon*, distributed across the Caribbean and nearby land-masses, almost invariably have a simple diet of algae and detritus (i.e., bottom muck). However, in San Salvador's lakes, which are less than about 10,000 years old, a species of scale-eating specialist (or even two) has evolved as well as a snail-eating specialist. Overall, the San Salvador pupfish miniradiation is not especially genetically variable, but is noteworthy for possessing unusually large amounts of adaptive genetic material from distant Caribbean islands—though from generalist species—and these genes are disproportionately associated with the newly evolved specialist feeding habits. Thus, “ancient” alleles present in generalists from different localities have been reassembled through hybridization, introgression, and natural selection into new adaptive combinations, reminiscent of the fruit flies studied by Lewontin and Birch, though more extreme in their innovations.

HYBRIDIZATION AND INTROGRESSION COULD HAVE ACCELERATED ADAPTIVE RADIATION. . . . DID THEY?

Several lines of evidence suggest that ecological opportunity, combined with the capacity to evolve reproductive isolation

quickly through sexual selection, are important for explaining the most extreme ancient lake radiations such as the cichlids of the African Great Lakes. But are they really enough to account for the almost-surreal pace of diversification seen in the most extreme cases? Some models as well as intuition suggest there must be more going on. We have seen evidence from relatively small-scale studies that hybridization is a plausible candidate through the additional variation it can provide. The challenge, a daunting one, has been to identify unique, testable predictions about what patterns should be seen if hybridization and introgression have indeed played important roles in accelerating major adaptive radiations in ancient lakes. The largest genomic data sets currently available, with the sort of data essential for addressing such questions, are from the cichlids of the African Great Lakes.

A first step is to ask if there is evidence for hybridization at the start of a radiation to seed it, and in addition, ask if hybridization was ongoing as diversification proceeded. Lake Victoria, the youngest of these lakes, has almost certainly experienced the highest sustained pace of speciation and thus is the greatest challenge to explain. As more and better genome sequences accumulate, a process that is itself accelerating, research findings will surely grow more definitive, but important results have already appeared.

Substantial evidence does indeed indicate that hybridization between long-separated lineages of cichlids occurred during the early stages of the adaptive radiation in Victoria and associated lakes. The cichlids of these lakes, which include Lakes Edward, Kivu, and Albert along with Victoria and a few smaller bodies of water, are often referred to as the Lake Victoria Region

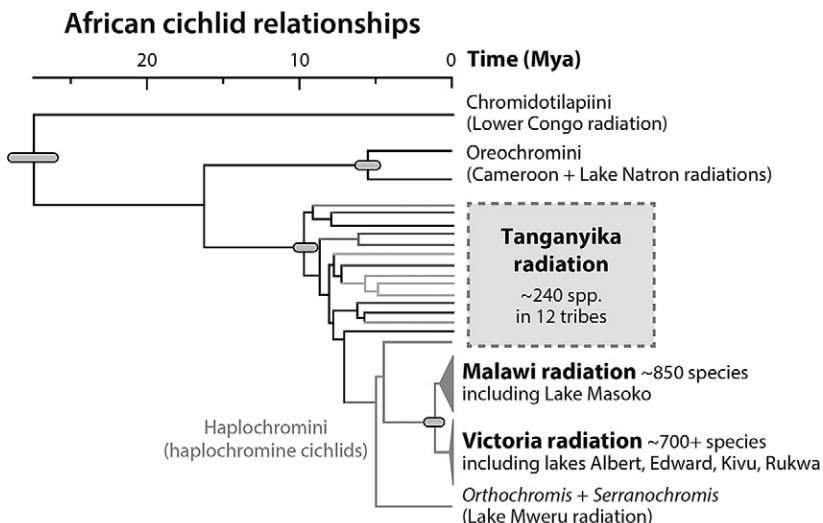


Figure 6.2

The relationships among African cichlids (hybridizations not illustrated), with particular reference to the African rift valley lakes. *Source*: Reprinted with minor modifications with permission from Springer Nature, Ronco et al., “Drivers and Dynamics of a Massive Adaptive Radiation in Cichlid Fishes,” *Nature* (2020) as modified by Svoldal et al., “Genetic Variation and Hybridization in Evolutionary Radiations of Cichlid Fishes,” *Annual Review of Animal Biosciences* (2021).

Superflock (figure 6.2). One of the first hints of hybridization at the start of this radiation was the inconsistency between the results from analyses of mitochondrial DNA and genes from nuclear DNA, specifically for Lake Victoria species. The mitochondrial data suggest an extremely young and genetically homogeneous species group, whereas some of the nuclear data reveal more variation and hence an older diversification. This pattern could be explained if hybridization and introgression had occurred early on.

More extensive analyses of nuclear sequences sampled from across the genome of a wider range of African cichlid species have provided further support for the hybridization-at-the-start hypothesis. Joana Meier, who led this study with a group of collaborators from Bern, found that the nearest relatives of the species flock came from the Congo drainage, but not all genes told the same story. About 20 percent of the genome suggests a closer relationship to an upper Nile lineage. Their interpretation of this pattern is that substantial hybridization occurred between Congo and Nile lineages at the start of the radiation—a result in part of the active geology of the region and changes in connections between rivers. Critically, the patterns in the DNA are different from what would be seen if separate Congo and Nile lineages simply diversified alongside one another with a hybridization now and then. Rather, the analyses by Meier and her colleagues reveal that genes of Congo and Nile origin are often interspersed throughout a single individual's genome. Further, the proportion of Nile-origin genes is about the same across all the lakes, indicating that hybridization occurred in the common ancestor of the superflock and the introgressed genes persisted as the radiation proceeded. Because there was substantial evolutionary divergence between the Congo and Nile lineages prior to hybridization, the probability of transgressive variation in the hybrids is considerable.

One of the best-studied individual genes in the system, for the long wavelength-sensitive opsin, appears to have one major set of alleles with a Nile origin and another with a Congo origin. Differences between the opsins similar to those between *P. pundamilia* and *P. nyererei* pop up time and again throughout the Lake Victoria radiation. Generally, the

shorter wavelength-sensitive form of the opsin gene is originally associated with a Congo lineage, whereas the longer (redder) wavelength-tuned allele has a mainly Nile history. In Lake Victoria, the longer wavelength-sensitive allele is generally found in species living in more turbid and/or deeper waters, just as in *Pundamilia*.

Matt McGee, Seehausen, and a large group of international and Bern collaborators, including Meier and others mentioned previously, have looked further into hybridization and diversification in Victoria and beyond, assembling and analyzing several large cichlid data sets in a landmark study. We have already reviewed some of their findings from an evolutionary tree that included sequence data from every species of cichlid with regard to ecological opportunity and sexual selection. They also conducted innovative analyses of 100 complete genomes sampled strategically from the more than 500 cichlids native to Lake Victoria and ecologically divergent pairs of species native to a set of lakes of varying ages that contain cichlid radiations.

One of their main efforts focused on genome evolution in Lake Victoria species for which conventional evolutionary trees could not be considered a given. Rather, these analyses assumed a network of species exchanging alleles with each other. If hybridization is frequent enough, this makes sense, since different parts of the genome will not have descended from the same ancestor, as in the branching pattern of evolution that is usually assumed. Each genome will instead be a mishmash of genes that came together at different times, some perhaps early in the radiation and others quite late, through a recent hybridization event. In these analyses, species with similar diets and habitats still shared more genetic material than

expected by chance. Yet the results for stretches of DNA associated with color patterns and presumably carrying genes for color suggested that closely related species more often differ for color genes. This is consistent with the hypothesis that sexual selection and color divergence have played an important role in cichlid speciation in Lake Victoria.

McGee and colleagues also used analytic methods that enabled them to extract more information from the genome sequences than has often been the case in studies based on genome comparisons. They were able to more fully consider not just the sequences of the nucleotides that comprise DNA but large-scale insertions and deletions of DNA sequences too. Insertions and deletions are important and informative because they are usually a distinctive, even unique type of mutation, and large ones are infrequent. In general, the presence of the same major insertion or deletion in two species argues that either each inherited it from a common ancestor or there was a recent exchange of genes between the species, such as through hybridization. Because they are so distinctive, insertions and deletions can act like tags with which we can follow a chunk of DNA as it passes through a radiation, much as we might follow a radio-tagged bear across a landscape. In both cases, it is not the tag that is informative but rather what it is attached to.

Unlike a radio tag, an insertion or deletion will not always be attached to something of interest, but with large samples of such markers, some inevitably will be, and their distinctiveness will allow their histories to be traced. McGee and his collaborators treated insertions and deletions as indicators of relatively old genetic variation since they originate infrequently and should accumulate slowly. Sets of species with a great deal

of such variation separating them are presumed to have been diverging for a long time or have obtained their ancient, divergent alleles in some other way, such as hybridization with other species. Applying this reasoning, McGee and colleagues calculated how many large insertions and deletions were present in the genomes of the ecologically divergent species they analyzed within each lake relative to the time separating the species sampled from that lake's radiation. They then compared their measure of insertion and deletion "enrichment" to a separate calculation of the rate of speciation in each lake. In the lakes with the most rapidly evolving radiations, especially Victoria and Malawi, there was more evidence of old genetic markers separating species than in the lake lineages with less rapid diversification, resulting in a positive correlation overall. The sample size of lakes is small, and avoiding spurious results can be challenging in such analyses, but it will be fascinating to see if the positive correlation they observed persists as data sets expand and the analyses are refined.

McGee and colleagues next concentrated on insertion and deletion variation within the 100 sequenced species from Lake Victoria. They emphasized old variants—the ones that also differed between lineages thought to be representative of the ancestors of the Victoria radiation. These markers were often correlated with particular ecological traits within the Victoria species, which would be expected if some of the individual insertions and deletions were located close to, or even within, genes that were adapted to particular ecological functions in the ancestral species. Presumably they were utilized in similar ways in Victoria cichlids, facilitating rapid adaptation and diversification. One particularly noteworthy association involved a

region on chromosome 9 harboring a small set of markers. This region was “fixed”—that is, the only form present—in all fourteen of the Lake Victoria cichlids (in this study) that ate fish, an unusual diet for cichlids. It was also fixed in a predatory cichlid from Lake Kivu and a more distantly related lineage of predatory cichlids from southern Africa, *Serranochromis*. Given the relationships among these species, the allele may have originated as long as ten million years ago, possibly in fish eaters in a lake that has since disappeared. Exactly what role it plays in fish eating, however, remains to be worked out.

The genomic results for Lake Victoria may help to resolve the puzzle of how a lake that is relatively young (though its basin is a little older) can be home to a cichlid radiation comparable to those in the more truly ancient lakes of Malawi and especially Tanganyika. It appears that much of the variation underlying the ecological diversity of Victoria’s cichlid radiation is in fact very old, and hybridization at the start of the radiation and subsequently has allowed it to be remixed into a wide variety of ecological types. The rapid speciation involved may have been facilitated by both ecological opportunity and strong sexual selection, with the latter linked to environmental variation as well.

Large-scale genomic analyses of the Lake Malawi cichlid radiations have also been conducted, led by Milan Malinsky, Hannes Svartal, and an international group of collaborators based at Cambridge University as well as several other institutions. Some Malawi lineages have diversified almost as fast as Lake Victoria’s, and there are even more species in Lake Malawi than in the Victoria region superflock. The radiation is somewhat older too.

The Malawi radiation appears, again, to have involved hybridization at the start, in this case of lineages estimated to be separated by more than three million years of evolution, even more than for those founding the Lake Victoria radiation. The Malawi radiation is dominated by DNA closely related to that of a lineage that includes Lake Victoria superflock cichlids as well as a group of widely distributed riverine cichlids. The second lineage that contributed to the Malawi radiation is today represented by a river-dwelling species of the genus *Astatotilapia*, which has only an informal name, “Ruaha blue” (based partly on where it was collected). One of the more surprising observations that emerges from this work is that this hybridization could easily have been missed. The Ruaha blue lineage is known only from one species first collected in 2012, at a site a considerable distance from Lake Malawi. If the lineage had gone extinct or been overlooked, detecting and making sense of the hybridization would have been problematic. Thus, an immense quantity of genome sequences may be required to get a complete picture of hybridization history.

The Malawi data also suggest that basal hybridization, at the beginning of the radiation, was important to diversification within the lake. Svoldal and colleagues evaluated genetic differences between species that live and forage along the bottom versus those that do so in the open water environment—a form of ecological divergence known to be ubiquitous in freshwater systems and crucial in the early stages of the Malawi radiation. They found that the genetic differences between the bottom-living and open water species disproportionately involved Victoria-associated DNA in one and Ruaha blue DNA in the other. They then looked throughout the genome at those genes

for which a Ruaha blue version was present in some species and a Victoria variant in others. As for Lake Victoria, genes for opsins, which underlie color vision and are implicated in both adaptation to different lake environments and mating preferences, were particularly common among such genes.

The Malawi results also confirm the role of ongoing gene flow throughout the radiation. By comparing evolutionary trees suggested by different portions of the Malawi cichlid genomes, the research team inferred that after initial divergence into three main lineages, hybridization continued in Lake Malawi. Some hybridization was of intermediate age, not at the start of the radiation, but between the ancestors of multiple modern species. Recent hybridizations between sometimes distantly related species are suggested by their analyses too. Variation in lake levels and conditions may have contributed to intermittent periods of elevated hybridization, as different species and groups were brought together by declining water volumes and reduced water clarity increased interspecific mating.

I have so far said little about hybridization in the most ancient of the African Great Lakes, Tanganyika. Fabrizia Ronco and colleagues in Walter Salzburger's laboratory at the University of Basel have investigated this topic in the most comprehensive genomic analysis to date of the cichlids of an African Great Lake. They sampled 240 species, or nearly every species in the radiation. I saw her present the initial results of this study at a conference on the shores of Lake Victoria in 2018, and the thoroughness of the work was impressive and memorable. Ronco and colleagues observed considerable hybridization between species within major branches of the Tanganyika radiation, known as "tribes," though little between

major branches. This is not so surprising given the great age of the major branches of the Tanganyika tree, but contradicts some earlier findings that suggested hybridization early in the Tanganyika radiation.

In a test of hybridization's potential role in mediating diversification rates among these branches, there was no clear effect. The speciation rate was not correlated with the hybridization rate. A different, potentially related pattern was present, however: the branches containing species with the highest average levels of heterozygosity—that is, genes with two different alleles—were the most rapidly speciating. Since hybridization would be expected to elevate heterozygosity and teasing apart these effects is difficult, a consequential role for hybridization in the radiation remains a possibility.

Considering the African Great Lakes together, there is considerable and growing evidence that hybridization at the beginning of the two younger radiations, in Victoria and Malawi, was important to their dazzlingly rapid diversification. Hybridization continued in each of the lake radiations and has likely made ongoing contributions to diversification.

Genomic data on the scale necessary to look reasonably comprehensively at the role of hybridizations in ancient lake diversifications only started to become available in the 2010s, and as I write, the African Great Lakes data sets are almost unique among large radiations. Still, the findings to date on the Telmatherinids of Sulawesi's Malili Lakes, ricefish speciation in the Malili Lakes and Poso (reviewed in chapter 2), and pupfish of Titicaca all support the concept of extensive hybridization in ancient lake adaptive radiations. I read almost daily about new initiatives to expand the breadth and depth of genome sequencing across the

tree of life, so we can anticipate the proliferation of vastly larger data sets with which to explore more powerfully, extensively, and decisively the role of hybridization in adaptive radiation. In the meantime, a different, younger set of freshwater systems provide complementary data on the roles that hybridization and standing genetic variation can play in speciation and adaptation, and merit a short foray out of the oldest lakes.

THE TRANSPORTER HYPOTHESIS

Studies of smaller, younger bodies of water have advantages as evolutionary systems owing to the replication they sometimes provide and tractability of their smaller scales. Research on one inhabitant of such habitats, the threespine stickleback, is generating results that notably reflect and complement those emerging from ancient lakes. Stickleback lived in the drainage ditch in front of the house I grew up in, and I later did my PhD work with them, so I have a particular fondness for these prickly little creatures and their frantic, jerky courtship dances. Today they are probably the most extensively studied vertebrate in evolutionary biology even though, unlike the cichlids, they never form large species flocks.

What is special about the stickleback is that it has done certain things, including colonizing fresh water from the ocean, over and over and over again in thousands of different streams, rivers, and lakes on similarly extraordinary numbers of landmasses, from continents to tiny islands. In doing so, sticklebacks have evolved in parallel on a hemispheric scale, typically in response to lakes and streams opening up as glaciers retreated, but in a few instances finding newly available

habitat when earthquakes moved coastal landscapes up and down. I have studied these charming fish in many locations, and one of my most memorable experiences was to see stream-resident stickleback in Gifu, Japan, for the first time. I knew that between British Columbia and Gifu, the stickleback of the Pacific Ocean were larger, bonier, longer spined, and more silvery than the dull little freshwater-resident fish scooting about the shallow streams of my hometown. Yet the Gifu fish, which my friend and collaborator Seiichi Mori showed me, could have been mistaken for the stickleback from the ditch in front of my parents' house. They were marvelous.

Considering freshwater sticklebacks from Japan, Europe, Alaska, and other distant locales, one might have guessed that the adaptations related to each freshwater colonization involved mainly different mutations, maybe even at different genes. A team led by Pam Colosimo in David Kingsley's lab at Stanford, collaborating with Dolph Schluter and his group, tested this possibility for one of the most conspicuous traits that often distinguish freshwater and marine sticklebacks: the bony plates along the side of the body. Marine stickleback have no scales, but possess prominent bony plates extending from just behind the head all the way to the tail. They look like the armored steeds of twelfth-century Europe, whereas the freshwater fish, especially in western North America, usually have just a few of these plates toward the head. Differences in predators and the relative cost of producing the plates probably contribute to selection favoring fewer plates in fresh water; the details are still debated somewhat, but what selection generally favors in each environment is clear. What is also clear is that plate genetics are unexpectedly simple.

In a series of experiments and comparative studies, Colosimo and her colleagues showed that a single gene, known as *Eda*, is responsible for much of the variation in bony plates. And to the surprise of many of us, the very same *Eda* allele reappears in freshwater lakes and streams all over the Northern Hemisphere, especially in Pacific-connected streams and lakes. The explanation is that the recessive low-plated alleles are at low frequency and thus almost always in a heterozygous state in the ocean, paired with a dominant full-plate allele that causes the heterozygotes to look much like every other fully plated marine fish. The current thinking is that the low-plate allele is advantageous in fresh water, but occasional hybridization with marine, specifically anadromous, stickleback results in hybrids and “leakage” of low-plate alleles back into the marine population. The result is that when marine stickleback colonize a new freshwater habitat, the low-plate allele, now favored, can increase in frequency and the low-plate form can reappear. Colonizations of fresh water, both experimental and accidental, have been watched in real time, and the low-plated form has been seen to reemerge from marine colonists, rising to almost 100 percent in just a few decades. This process, in which low-plated fish seem to pass invisibly from one freshwater site to another, reminded Schluter of how a person in *Star Trek*’s transporter room would dematerialize only to reappear in identical form in a new locale, leading to the irreverent name for this hypothesis (figure 6.3). It is now clear that many other freshwater genes also leak into fresh water and are reused, so *Eda* is easy to detect but not unusual.

Much like genomic regions associated with Lake Victoria’s fish predation niche, which originally evolved for that role in a

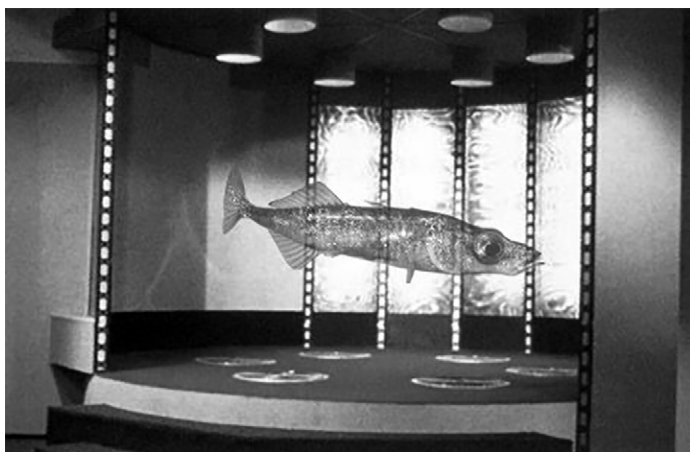


Figure 6.3

The transporter hypothesis of threespine stickleback evolution. *Source:* James Vaughan, under a Creative Commons CC BY-NC-SA 2.0 license (<https://creativecommons.org/licenses/by-nc-sa/2.0/>), with modifications by Dolph Schluter and the author.

different system and place, adaptation in stickleback frequently makes repeated use of the same genetic material in different freshwater systems. As a result, the low-plate *Eda* allele, like other freshwater-adapted alleles, is much older than the freshwater populations in which it often appears. Hence adaptation and even speciation do not always rely on new mutations but instead can make use of standing genetic variation, much of which has been around for a long time. It is interesting to speculate that the availability of more extensive, ecologically diverse ancient genetic variation may be a crucial difference between the tremendous breadth of Lake Victoria's radiations relative to the presence of just one or at most two species of sticklebacks in so many Northern Hemisphere lakes. Exploration of the

planet's biodiversity at the genomic level has only just begun, and the data are accumulating at a breathtaking rate. More surprises are surely forthcoming.

* * *

We are accustomed to thinking of hybridization as an occasional problem for identifying animals in the field and a brake on biodiversity. But the jumbling of genetic material that occurs with hybridization may result in novel traits and lead to diversification along new ecological paths. Hybridization may have been an especially important yet long underappreciated factor in accelerating diversification in the most extraordinary ancient lake radiations. The reuse of ancient variation through hybridization may also help to resolve the enigma of Lake Victoria's cichlid radiation and how this young lake has come to show diversity more typical of truly ancient systems. Evolution may make use of standing genetic variation, some of it quite old, more frequently than was suspected and occasional exchanges of genes between species will facilitate such reuse.

This is a section of [doi:10.7551/mitpress/13625.001.0001](https://doi.org/10.7551/mitpress/13625.001.0001)

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Citation:

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DOI: 10.7551/mitpress/13625.001.0001

ISBN (electronic): 9780262373524

Publisher: The MIT Press

Published: 2023



The MIT Press

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Grant funding provided by Furthermore: a program of the J. M. Kaplan Fund.



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The MIT Press would like to thank the anonymous peer reviewers who provided comments on drafts of this book. The generous work of academic experts is essential for establishing the authority and quality of our publications. We acknowledge with gratitude the contributions of these otherwise uncredited readers.

This book was set in Adobe Garamond Pro by New Best-set Typesetters Ltd.

Library of Congress Cataloging-in-Publication Data is available.

ISBN: 978-0-262-04785-2

10 9 8 7 6 5 4 3 2 1