

King Midas and His Many Extremely Young Species: Studies on Speciation in Cichlid Fishes in Nicaraguan Crater Lakes

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In 1984, now more than 25 years ago, I traveled to Nicaragua for the first time. I was then a young Ph.D. student from the University of California at Berkeley. The communist commander Daniel Ortega was in power in Nicaragua. A civil war was raging between Ortega's Sandinista army and the so-called Contras, who were backed by U.S. president Ronald Reagan. A strict U.S. embargo against Nicaragua inflicted even greater poverty on this already staggeringly poor country. It was logistically not easy, but I had to travel to Nicaragua, because I wanted to study a particular lineage of fishes that only live there. Other foreigners also visited this war-torn country at that time, but many of them were there to provide humanitarian aid to the Sandinistas and the suffering Nicaraguan people, whereas I was there to study special cichlid fishes for evolutionary research in the great lakes and crater lakes of Nicaragua (FIGURE 1).

At the time of my first trip to Nicaragua, there were only three species of cichlid fishes formally described that belonged to this lineage. They are *Amphilophus citrinellus* (FIGURE 2), *A. labiatus* (FIGURE 3), and *A. zalius* (FIGURE 4); then they were still considered to be members of the genus *Cichlasoma*. Both *A. citrinellus* and *A. labiatus* are geographically widespread and are found in the two largest lakes in Nicaragua, Lake Nicaragua and Lake Managua, and at least *A. citrinellus*, or one or more species that look much like it, is also found in several small crater lakes in Nicaragua. Already when I was still a student it was known that *A. citrinellus* and *A. labiatus* stand out owing to their unusual color variation (termed "polymorphism"). In most populations, about 90% of the fish have black-and-white stripes, the "normal" or "dark" coloration, but about 10% of the individuals lose their black color pigment once they have reached a length of about ten centimeters, at which point they turn bright yellow and are referred to as "golds" (FIGURE 2A and 3A). Because of this conspicuous color polymorphism, these fish are colloquially referred to as Midas cichlids, an allu-



FIGURE 1 Map of Nicaragua, showing the locations of the two great lakes and the crater lakes.

sion to King Midas in Greek mythology, who could turn everything he touched into gold. It is now known that this also occurs in some other species in some crater lakes in Nicaragua.

Amphilophus labiatus (from the Latin word *labia*, lips) has its name because of its conspicuously enlarged lips (FIGURE 3A and 3B), which it uses, it is believed, as a kind of seal when it tries to suck prey species, such as crabs, out of crevices between rocks. The third species that had been formally described as a separate species at the time of my first trip to Nicaragua is *A. zaliosus*, called the “arrow cichlid” because of its narrow, elongated body. It had been scientifically described less than 10 years before by my Ph.D. advisor, George Barlow, and one of his students (FIGURE 4). It is only found in one of the crater lakes (Lake Apoyo, FIGURE 1) in Nicaragua.

George Barlow (1929–2007) and his students had been studying both the aggressive behavior and the mating behavior of *Amphilophus citrinellus* from Lake Nicaragua and from a crater lake called Lake Masaya (FIGURE 1) since the

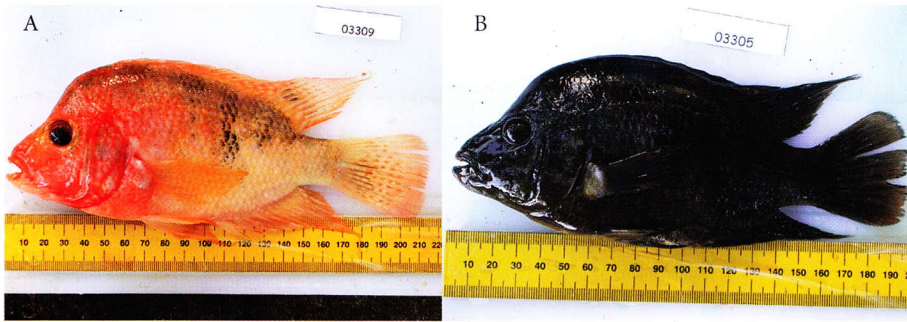


FIGURE 2 The “gold” and “normal” morphs of *A. citrinellus* from Lake Nicaragua.

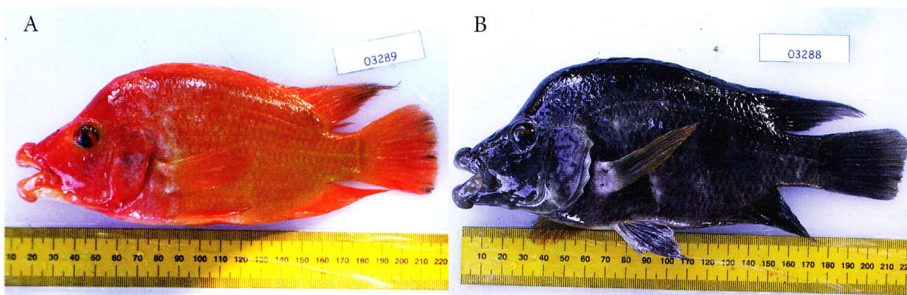


FIGURE 3 The “gold” and “normal” morphs of *A. labiatus* from Lake Nicaragua.

mid-1970s. He was interested in finding out exactly how and why “gold” and “normal” black-and-white females prefer to breed with males of the same coloration and how this color polymorphism affects aggressive interactions. That color mattered in terms of sexual selection (that is, which mates are chosen for reproduction) could be seen in nature, where most of the breeding pairs (typically almost 95% or more) had the same body coloration. Also, when given a choice in the laboratory, females prefer males of the same color as themselves over males of a different color. My Ph.D. advisor’s interest was not so much in the evolutionary consequences of this assortative mate choice but, rather, was focused on understanding the behavioral mechanisms that led to it. By contrast, my main interest was in gaining a better understanding of the processes by which new species arise. Evolution by natural selection might lead to better ecological adaptations but it might also result in the evolution of reproductive barriers and, hence, to the evolution of new species. This could happen through assortative mating with individuals that resemble each other ecologically or that resemble each other in color.



FIGURE 4 *A. zaliosus*, a species endemic to Lake Apoyo.

It was also noticed that, in lakes other than Lake Apoyo, there were individual fish that appeared to be of the same species that were somewhat more elongated. Thus, not only was there variation in terms of the conspicuous color polymorphism, but there was also polymorphism in other characteristics that might have evolved repeatedly and independently in various lakes. This had to be explained. A number of hypotheses might explain this variation. One possibility was that what was initially thought to be one species was, in fact, several related species. This turned out to be the case. Now we know that there are about 12 (or perhaps even more) species of Midas cichlids.

Because they live in crater lakes, this group of species of cichlid fishes also seemed to be an ideal group about which to ask some intriguing questions: under what geographic circumstances do mating preferences evolve? Is ecological speciation or speciation through sexual selection more common, or faster, in bringing about reproductive isolation and new species? As it turned out, some of the species of Midas cichlids in Nicaragua that live only in particular crater lakes might have arisen through this process of ecological speciation under sympatric conditions without geographic barriers (in sympatry) to gene flow. Traditionally, it had been thought that geographic separation is necessary for new species to arise, but we could show that new species can arise also within a very small body of water such as a crater lake, that does not have geographic barriers that nascent species could not cross to find mates. But the Midas cichlids of the crater lakes in Nicaragua present an opportunity to study not only sexual selection—in other words, the emergence of new species through selective breeding—but also to test theories of ecological species formation in this special geographic setting.

The cichlid fishes from the great lakes of East Africa, where hundreds of endemic species of cichlids form so-called adaptive radiations, have also been

investigated extensively with molecular-genetic tools by my laboratory for the past two decades. Cichlids became, alongside the Darwin's finches from the Galápagos Islands and the *Anolis* lizards of the Caribbean, one of the best-studied model organisms for research on adaptation and speciation.

WHAT IS THE ROLE OF GEOGRAPHY IN THE ORIGIN OF SPECIES?

Under what geographic conditions can speciation happen? For Darwin, this question did not seem to matter all that much; at least, based on reading *On the Origin of Species*, he did not specify in what geographic settings new species should or should not arise. This led later generations of evolutionary biologist to interpret Darwin's writings to suggest that species could arise without geographic barriers that separated previously interbreeding populations. This might occur if natural selection were to favor extremely different organisms—say, those that are the smallest and largest—while putting intermediate individuals at a disadvantage. In theory, such “disruptive selection” might lead to the evolution of two distinctive species from a more variable ancestor. However, the idea that speciation could occur in situ by one species being separated into two within a locality (a process termed “sympatric speciation”) had increasingly fallen into disfavor since the late 1930s and 1940s. This was because the architects of the so-called “modern synthesis,” which combined the findings of various branches of evolutionary biology into a unified theory, focused on geographical conditions as the key precondition for speciation. They believed that geographic isolation was necessary so that genetic exchange was not possible, allowing two populations to diverge from each other and eventually to become different species. In such a geographic setting, natural selection played either no role or only a minor role, and speciation was seen as a mere by-product of geographic separation and not due to local adaptations to particular ecological conditions, as Darwin had thought speciation would work. Thus, natural selection came increasingly to be seen only as the key driving force on the path towards better adaptation within a population, but not as the mechanism for speciation.

During his career, the famous evolutionary biologist Ernst Mayr (1904–2005) was, for many decades, one of the most influential proponents for the process of speciation by geographic restriction of gene flow, also called “allopatric speciation.” (Allopatry means “living in different places.”) According to this theory of geographic speciation, populations that are physically isolated for many generations accumulate so many genetic mutations quasi passively that, if the geographic boundary between those populations ceases to exist, the individuals in those populations are no longer able to interbreed. In other words,

speciation occurs allopatrically, or alone owing to geographic isolation without the action of natural selection.

Mayr was also responsible for defining the biological species concept, which stipulates that interbreeding defines species as reproductive units and separates them from other species. Mating choices—and thus, inversely, breeding barriers—are the key factor from the point of view of the biological concept of species. Only members of the same species should be able to breed with each other and produce fertile offspring. Surely, that definition does not always hold, since hybridization between species can sometimes result in fertile individuals as well. Not least because of the influence of Ernst Mayr have evolutionary biologists been focusing, therefore, on the geographic setting of the process of speciation and the origin of so-called isolating mechanisms that prevent individuals to mate across species boundaries. Traditionally, geographic isolation (allopatry) has been seen as all-important during the initial stages of species formation, and it was thought that, without such barriers (i.e., in sympatry), it is extremely difficult, or nearly impossible, for new species to arise.

As a result of this kind of thinking, the origin of new species has been seen almost exclusively as a nonselective process—at least natural selection took on a subordinate role in this theory of speciation since differences between populations and isolating mechanisms are thought to accumulate passively over time and not due to selection forcing populations to become different. For decades, not least due to Ernst Mayr's influence, this model was perceived as the dominant mechanism by which new species come into being. On the other hand, sympatric speciation, the emergence of new species from a single population due to ecological selection without geographic barriers to gene flow, was seen as an impossible mechanism, or at least as a highly unlikely mechanism, for the emergence of new species, the reason being that genetic exchange between diverging populations would tend to homogenize them, preventing them from becoming distinctive species. This view is still pervasive today, and, possibly because of this, only very few empirical examples exist that show that species arose without geographic barriers to gene flow.

THE ROLE OF NATURAL SELECTION DURING ECOLOGICAL SPECIATION

However, this perception of adaptation and selection having only a minor role in speciation has changed during the last 10 to 15 years as more empirical examples have surfaced that support the idea that selection to different ecological niches can lead to the origin of species in a process that is now referred to as “ecological speciation.” It was recognized that Darwin, who had initially proposed that geography might not matter all that much, might

be correct after all. Darwin postulated that many, if not most, species might be arising by means of divergent natural selection that would favor the more extreme individuals, and that the average individual might be at a competitive disadvantage when compared with individuals at both extremes. How can such divergent selection lead to speciation? The idea is that ecological speciation, as it's now called, can lead to reproductive isolation between populations, or between portions of individual populations, through adaptations to different ecological niches or to different environments. If such individuals that are adapted to the ecological extremes mated only among themselves, then two new species might arise through this process of ecological adaptation and through mating assortatively within one's type.

As I will detail at greater length below, the Midas cichlids provide compelling empirical examples that ecological sympatric speciation has led to the origin of new species in Nicaraguan crater lakes. We found that sympatric speciation can occur both through ecological speciation by natural selection acting through adaptations to different ecological niches and by sexual selection. The latter mechanism might work in sympatry, leading to the origin of new species, if animals mated only with animals that looked like them and not with other animals that, for example, had a different color, but still belonged to the same species.

THE SECOND JAW OF CICHLIDS

All cichlids possess a functional second jaw, known as the pharyngeal jaw, which enables them, with the teeth on these jaws, to process sources of food that remain inaccessible to other fish. These jaws developed evolutionarily from the fifth bony gill arc, which still holds gills for breathing in many species that diverged early in fish evolution. The evolutionary innovation of this “second jaw” probably contributed toward making the family Cichlidae, containing a total of probably more than 3,000 species, such an evolutionary success. It is thought that, by possessing the “normal” jaw with particular types of teeth as well as their functionally second jaw, cichlid fishes can be particularly efficient in feeding, as well as using ecological resources (such as hard snails that only they are able to crack) that are not available to other families of fishes. Therefore, the evolution of such jaws, coupled with other factors (such as their conspicuous coloration, which might play a role in sexual selection and speciation through mate choice) contributed to the evolutionary success of cichlid fishes.

During my Ph.D. work, I noticed that the individuals of the presumed single species *A. citrinellus* not only differed in color, but also in some specific morphological aspects—in particular, in such ecologically highly relevant structures as their so-called “pharyngeal jaws” (FIGURE 5). There was also pronounced

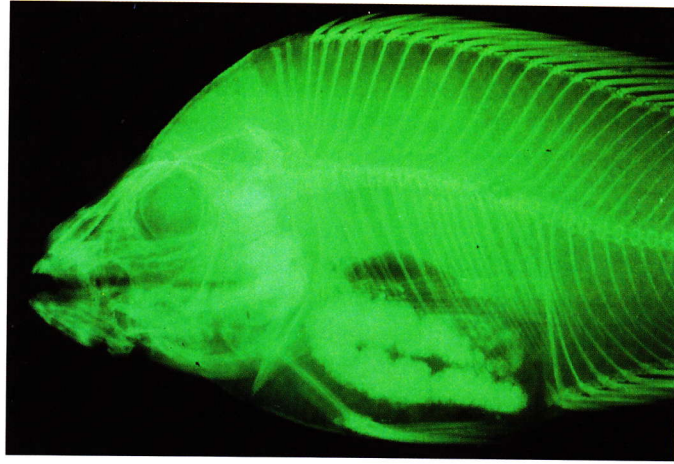


FIGURE 5 An x-ray image of an *Amphilophus citrinellus* from Lake Nicaragua with pronounced (molariform) pharyngeal jaws. Note that this fish has cracked and ingested many snail shells, as can be seen in high contrast in its intestine.

variation in body shape within and between the populations of Midas cichlids of any single lake, with particularly marked differences in the shape of this “second” jaw, but also in other characteristics. Some Midas cichlids are able to grow very strong “molariform” pharyngeal jaws (FIGURE 6), which carry very strong “molar teeth” that enable them to break hard snail shells, while others have “papilliform” pharyngeal jaws with small, sharp teeth with which they can macerate efficiently such soft foods as insect larvae. However, the Midas cichlids with papilliform jaws are unable to break snails’ shells. Thus, there were clear ecological differences that were caused by these differences in the teeth of the two populations of Midas cichlids. It seemed feasible to propose that this variety of forms associated with feeding may be one of the decisive factors contributing to the emergence of new species, possibly through ecological speciation.

THE GEOGRAPHIC SETTING AND THE COLONIZATION OF THE NICARAGUAN CRATER LAKES

The western part of Nicaragua is underlain by one of the world’s most volcanically active areas. Known as the Central American Volcanic Arc, it is part of the “Pacific Rim of Fire.” After a volcano cools, the typically cone-shaped crater fills with groundwater and rainwater and becomes a crater lake. The crater

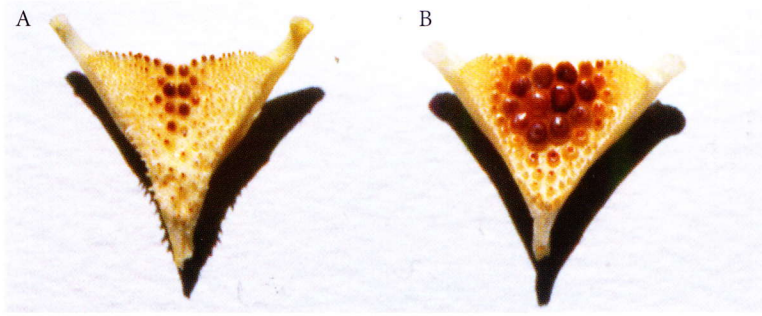


FIGURE 6 Specimens of “papilliform” and “molariform” lower pharyngeal jaws. These kinds of morphological differences allow the fishes to exploit different trophic resources differentially in their environment. Fishes with strongly enlarged teeth are able to crack hard snails, a food source that is not available to the individuals with the much weaker teeth (papilliform morph), but the latter are more efficient in feeding on softer types of prey.

lakes in Nicaragua are characteristically oligotrophic—that is, they contain only few nutrients in the water for primary production by algae, and they do not have any inflows or outflows. Typically, after some time, such crater lakes are colonized by aquatic organisms, including cichlid fishes. At least eight Nicaraguan crater lakes have been successfully colonized by Midas cichlids (FIGURE 1, TABLE 1). It is not known, for each case, how or when those colonizations occurred, but it is generally thought to have been through such natural phenomena as hurricanes, “fish rains,” or by fish having been dropped inadvertently by fish-eating birds, such as cormorants, bald eagles, or ospreys, although some lakes may have been stocked with fish by humans. (“Fish rains” are rare meteorological phenomena caused by waterspouts, which have been reported to be able to pick up large quantities of water from bodies of water, including the resident fish, and to drop this water elsewhere.) Initially, the crater lakes provided empty spaces that, once the lakes had been colonized, were quickly filled by newly arriving species. Because of their geographic isolation and known geological history, crater lakes are excellent natural systems for studying the evolution of species both in allopatry and in sympatry.

The two largest and oldest lakes in Central America are the great lakes Managua and Nicaragua. These date to the early Pleistocene Epoch, and together they cover approximately 9,000 km². Lake Managua lies at an elevation 7 or 8 m higher than the elevation of Lake Nicaragua (FIGURE 1). Connectivity between these two lakes has varied, depending on the fluctuating level of the water in the lakes: River Tipitapa rarely but periodically connects Lake Managua to Lake

Table 1: Geological and biological information on the Midas cichlid species complex from the Nicaraguan lakes

Lake	Natural history	Minimum age (years before present)	Lake surface area (km ²)	Midas cichlid species
Crater Lakes				
Apoyeque	The name "Apoyeque" means "salty water" in the Náhuatl language; it is so-named because of its high mineral content, as is characteristic of Nicaragua's crater lakes. Recent research indicates there are two ecological morphs of Midas cichlid in the lake and some authors have called these different species.	1,900	2.50	<i>A. citrinellus</i>
Apoyo	This is the largest and oldest crater lake and is the most Midas cichlid species rich. <i>A. zuliae</i> , and likely other endemic species, arose by sympatric speciation. Apoyo is under pressure from lakeside development and introduced or cultivated exotic fishes.	23,890	21.10	<i>A. astorquii</i> , <i>A. chancho</i> , <i>A. flaveolus</i> , <i>A. zaliosus</i>
Asososca León	Little is known about this small lake's volcanic history or age. The lake is home to a large population of introduced African tilapia.	4,500	0.81	<i>A. citrinellus</i>
Asososca Managua	This crater lake is the water source for the city of Managua. It is probably the youngest crater lake. There are ancient paintings on the rocks, indicating that it was historically a sacred site.	1,245	0.74	<i>A. citrinellus</i>
Masaya	This lake is where Midas cichlid expert George Barlow collected most of his lab stocks. The lake originated ca. 6,000 years ago in the San Antonio eruption. Ca. 2,120 and 1,800 years ago there were major eruptions at one end of the lake: fish may or may not have survived.	6,000	8.38	<i>A. citrinellus</i>
Monte Galán	This is probably a young lake, formed by recent volcanic activity of nearby Momotombo.	?	0.79	<i>A. citrinellus</i>
Tiscapa	Located in the city of Managua, this tiny lake is surrounded by a touristic nature park. The lake is contaminated from channelling activity and local pollution.	?	0.13	<i>A. citrinellus</i>
Xilolá	This lake is located just above great lake Managua and beside crater lake Apoyeque. Lake Managua water level has historically periodically risen (e.g. 9 m high in last eruption), resulting in exchange between Xilolá and Managua.	6,100	3.75	<i>A. amarillo</i> <i>A. sagittae</i> <i>A. xilolaensis</i>
Great Lakes				
Managua (or Xolotlán)	The lake drains intermittently to Lake Nicaragua by the river Tipitapa.	Early Pleistocene	1,053	<i>A. citrinellus</i> <i>A. labiatus</i>
Nicaragua (or Cocibolca)	This is the largest lake in the western hemisphere south of the North America Great Lakes and north of Lake Titicaca. It is connected to the Caribbean Sea by the San Juan river.	Early Pleistocene	8,143	<i>A. astorquii</i> <i>A. chancho</i>

Nicaragua when water levels are unusually high. The great lakes are exceptionally shallow (mean depth around 8.6 m for Lake Managua and 12.4 m for Lake Nicaragua) and have gently sloping basins, allowing wind to stir the sediments. This and a high prevalence of phytoplankton make the water of the great lakes very turbid. Thus, this habitat is very different from the clear, deep waters of the crater lakes.

THE TAXONOMY AND DIVERSITY OF THE MIDAS CICHLID LINEAGE

The taxonomy of these cichlids from Nicaragua had been difficult since the start of their biological investigation. They were first studied at the beginning of the twentieth century by the ichthyologist Seth Eugene Meek of the Field Museum in Chicago. He lamented that he could not decide whether he was dealing with only two species or with up to 6 different species because the fish from different lakes looked so different, but they also had commonalities across lakes. For example, cichlid fish with enlarged lips are found not only in the two big lakes but also in some of the small crater lakes. But it was not clear to Meek whether he should consider those fish to be the same or different species. This conundrum is still not yet solved completely. Today, however, it is clear, based on the comparative genetic data that we have collected in all of these populations, that the two great lakes are the ancient source of the population of Midas cichlids. From these, the much younger crater-lake populations were seeded, and several new species arose, each of which lives in only a single crater lake in Nicaragua.

We could show, based on several lines of genetic evidence, that Midas cichlids from single crater lakes are genetically more similar to each other than they are to the Midas cichlids in any other lake. At present, there are nine species described in the Midas species complex (TABLE 1). Based on rapidly evolving genetic markers (such as so-called microsatellite DNA) of the type that are also used in paternity analyses and in the analysis of DNA from crime scenes, one can now distinguish many of these species genetically, although not all of the species and crater lakes have been investigated in sufficient detail so far. It is likely that, in the near future, still more species from the crater lakes will be described taxonomically and that the validity of the original species *A. "citrinellus"* will need to be revisited—surely it is composed of more than one species. Therefore, it is best to refer conservatively to Midas cichlids found in multiple crater lakes as *A. cf. citrinellus*.

Based on a large amount of genetic work, we ended up concluding that each of the crater lakes in Nicaragua, even those that are only 1,000 to 23,000 years old (TABLE 1), contains its own set of endemic species of Midas cichlids. It is clear from our genetic data that many, if not all, of these species must have

arisen through speciation in sympatric geographic settings, since the crater lakes in Nicaragua do not have any geographic barriers that would prevent fish within one crater lake from interbreeding.

EVOLUTION REPEATS ITSELF IN THIS HIGHLY VARIABLE LINEAGE OF CICHLID FISHES IN BODY FORM, LIPS, SECOND JAWS, AND COLORATION

The Midas cichlids are highly variable for such ecologically relevant attributes as body shape, pharyngeal-jaw apparatus, and hypertrophied lips. Moreover, it is interesting that evolution apparently repeated itself, since some types evolved more than once. This applies to the so-called “limnetic” species that tend to live more in the open water and to have slender, elongated bodies, to deeper-bodied “benthic” species, and also to species with hypertrophied big lips.

Such limnetic species as *A. sagittae*, which is endemic to Lake Xiloá, and *A. zaliosus*, which is endemic to Lake Apoyo, are elongated in their body form, and have papilliform pharyngeal jaws, while such benthic species as *A. astorquii*, *A. chanco*, *A. flaveolus* in Lake Apoyo, and *A. amarillo* and *A. xiloaensis* in Lake Xiloá (TABLE 1) are more high-bodied—more closely associated with the benthos—and tend to have molariform pharyngeal jaws. *Amphilophus labiatus* has a slightly more elongated body, a more pointed snout, more lateral compression, and, most obviously, dramatically hypertrophied lips when compared with *A. citrinellus* from the two large lakes (FIGURES 2 and 3). Interestingly, a similarly thick-lipped morph is found at moderate frequencies (approximately 26%) in Lake Apoyeque, where it occupies an ecological niche that is distinct from that of the more abundant thin-lipped morph; big-lipped fish are also found, albeit much more rarely, in crater lakes Masaya and Xiloá. Those are potentially novel species that are still awaiting taxonomic description.

Other species in the Midas cichlid species complex than the previously mentioned *A. cf. citrinellus* and *A. labiatus* (FIGURES 2 and 3) from the large lakes are polychromatic. The more common morph is the grayish “normal” (or “dark”) morph with spotted, striped, and barred patterns, while the “gold” morph is uniformly orange, yellow, or even white. All of these fish begin life “dark” and some of them later lose their melanophores (the skin cells that cause the dark color) and become “gold” (or, more rarely, even completely white, lacking the cells that cause both dark and yellow color) permanently. Gold morphs are found in moderate to low frequencies (e.g., <20% in *A. xiloaensis* and <7% in *A. sagittae*) in crater lakes Xiloá, Masaya, Asososca León, and Asososca Managua, and in great lakes Managua and Nicaragua, where about 8–10% are gold.

SEXUAL SELECTION DUE TO ASSORTIVE MATING CAN BRING ABOUT NEW SPECIES IN SYMPATRY

Strong assortative mating based on color has been shown in the field and laboratory. We have shown, in Midas cichlids from Lake Xiloá, that such assortative mating may be resulting in speciation in sympatry, since gold and dark morphs of the Lake Xiloá are endemic species *A. xiloaensis* are already notably genetically differentiated, due to strong assortative mating based on coloration. Pairs in which both the female and the male are of the same color are found much more commonly than would be expected by chance. Therefore, we found that fish of the same color more often chose to mate with each other. Because they breed more often with their own kind, populations of the two color morphs do not often exchange genes and therefore their genetic differences tend to accumulate. It is interesting that, in contrast with this finding, we discovered that gold and dark morphs of *A. cf. citrinellus* and *A. labiatus* from the great lakes show little or no genetic differentiation, although these are surely older species. We don't know whether or not these species of the large lakes mate so strongly according to color. Unfortunately, because the water in large lakes is very cloudy, behavioral observations of the breeding behavior of Midas cichlids are not possible there.

Disruptive selection based on color polymorphism might contribute to the diversification of the cichlids of the Great Lakes of Africa as well, since many species from there are also known to be polymorphic with respect to color and because females often strongly prefer to mate with one type of male over another. Previously, this mechanism had not been convincingly demonstrated to act under sympatric conditions for species that are polymorphic for coloration. Assortative mating, changes in selection, and selection based on the frequency of one type or another by predation or intrasexual competition are believed to be able to contribute to the persistence of genetically different color morphs within one population.

However, sexual selection is also likely to play a role in the sympatric origin of at least some of the other species or species-pairs that differ in their coloration. This can happen through assortative mating. Breeding of each species often occurs in close proximity in these lakes; sometimes two different species breed even within a meter or two of each other. That they do not interbreed argues for strong selection for fast evolution of mechanisms for prezygotic reproductive isolation. Sympatric speciation "in the making" by sexual selection based on color (either gold or dark) was discovered by us just recently for *A. xiloaensis* from crater lake Xiloá. This was inferred from observational field studies and also from the analysis of genetic data that show that they can already be distinguished from each other genetically. Theoretical verbal and

mathematical models had before suggested that such speciation is possible, but evidence from nature had been wanting.

PROCESS OF SYMPATRIC SPECIATION IN NICARAGUAN CRATER-LAKE CICHLIDS

Many theoretical insights about how new species can evolve without geographical barriers have been put forward. However, empirical examples for speciation in the face of potentially continuous homogenizing gene flow owing to a lack of geographic barriers remain scant. Theory suggests that speciation could occur if a characteristic of a species is simultaneously affected by the same genes that are responsible both for mating with one's kind and for natural selection by ecological specialization. It is thought that isolated habitats, such as islands and crater lakes, are the most promising places to identify diversification in sympatry because, if new species exist there and only there, they probably arose in that small, geographically isolated locality. Therefore, some of the most compelling cases of speciation without geographical barriers to gene flow come from cichlid fishes from crater lakes, where it is believed that speciation is driven by disruptive natural selection for ecological traits.

Because crater lakes are small, geographically isolated settings, they are ideally suited as biological systems for the study of sympatric speciation. For two different crater lakes, we believe that we have found evidence that speciation can occur in sympatry, but through two different mechanisms—once through ecological speciation (in the case of *A. zalius* from Lake Apoyo) and through assortative mating based on a color polymorphism (in the case of *A. xiloensis* from Lake Xiloá).

How exactly could this have happened? In all cases where multiple Midas cichlid species have so far been recognized within a crater lake (TABLE 1), they have different ecologies and morphologies, which is strongly suggestive of the effect of disruptive natural selection to exploit different intralacustrine niches. Cichlid populations that inhabit a single crater lake find different sources of food at different locations within a lake, as we could show based on the analysis of their stomach contents.

These observations, combined with molecular and comparative morphometric analyses, led us to suggest that the mechanism of speciation in the case of *A. zalius* in Lake Apoyo is through ecological speciation in sympatry. The new species would be adapted to feed on different diets in different parts of the lake and might also there find its mates. The Arrow cichlid is only found in this crater lake, which is completely isolated from other lakes and rivers, just as are all of the other crater lakes in Nicaragua. We have studied its origin in greater detail in recent years using genetic, morphological, and ecological methods. It

turned out that the Arrow cichlid not only differs from the original species of Midas cichlid that colonized Lake Apoyo in terms of appearance and genetics, but that it also probably originated in this lake less than 10,000 years ago. It only breeds with other members of its own species, which has also been demonstrated by preferential partner selection in the laboratory, and it relies on other sources of food and more often lives at greater depths and farther from the shore than the originally colonizing species.

This type of speciation probably best explains the evolution of several more species of morphologically and therefore ecologically different endemic species of Midas cichlids in the Nicaraguan crater lakes. Even when speciation is still incomplete, there can be intralacustrine ecological differentiation, as we recently showed for the extremely young (TABLE 1) Lake Apoyeque (it is less than 1,800 years old), where we found that about 20% of the individuals have pronounced lips, as do individuals of *A. labiatus* from the large lakes. But, even based on a large set of quickly evolving microsatellite markers, we could not distinguish the lipped fish from the unlipped fish within Lake Apoyeque statistically. Hence, ecological differentiation can occur much faster than even rapidly evolving genetic markers can track; thus, the absence of genetic differentiation between ecotypes does not refute ecological speciation. Alternatively, we might be dealing with only a polymorphism in a population without assortative mating and, hence, not with genetic differentiation. Underwater observations would be necessary to show whether individuals with big lips prefer to mate with each other.

THE CRATER LAKES OF NICARAGUA PROVIDE NATURAL REPLICATED EXPERIMENTS IN ULTRAFAST SPECIATION

Comparative morphological analysis of all the species in the Midas cichlid species complex across six crater lakes and both great lakes in Nicaragua showed that the Midas cichlid from each lake has its own characteristic body shape that distinguishes it from other Midas cichlids from other lakes. Also, the body shapes of the Midas cichlids from the great lakes are distinct from those inhabiting the crater lakes. Such a pronounced variation in body shape between the Midas cichlids in the great lakes and those in the crater lakes is not unexpected because the clear, deep waters of crater lakes are quite different from the shallow and turbid environment in the great lakes of Nicaragua. Each lake is different from all other lakes, whether they be crater lakes or great lakes.

The populations of Midas cichlids from the crater lakes Asososca Managua and Apoyo were found to be the most divergent in body shape. However, the shapes of some individuals from some crater lakes overlap (particularly in indi-

viduals from lakes Asososca León and Apoyeque, and Xiloá and Apoyo), and the shapes of some individuals from the great lakes was also found to overlap. This observation suggests that similar body shapes independently arose in species of Midas cichlids that inhabit those pairs of lakes. Nonetheless, some crater lakes are very young, especially Asososca Managua (which only formed roughly 1,200 years ago) and Apoyeque (which formed roughly 1,800 years ago (TABLE 1). Therefore, the change in body shape that comes with colonization of a new crater lake must occur exceedingly fast. We estimate that some of these morphological changes and speciation events took place within only a few hundred or a few thousand generations. This, we think, may turn out not to be unusual, but rather we assume that future research will find other examples of extremely fast evolution. This may turn out to be more the rule and not an exception.

The greatest changes in the evolution of body shape across the entire Midas cichlid species complex occurred in the shape of the head and mouth, the size of the head, the depth and length of the body, and the width of the tail region. These are also body-shape characteristics that have been found, in other cichlid studies, to relate to trophic niche and ecological selection as they relate to how and where the fish forage. Thus, these findings, interpreted in light of previous research on ecomorphology in the species complex, suggest a strong role for ecological selection and adaptation in the diversification of body shapes in Midas cichlids.

Some morphologies are repeated across lakes (e.g., lipped fish that most likely arose several times independently in Lake Nicaragua and the crater lakes Apoyeque, Masaya, and Xiloá). The limnetic, open-water species *A. zaliosus* from Lake Apoyo and its ecological equivalent species, *A. sagittae* in Lake Xiloá, arose through ecological speciation under sympatric conditions more than once. These large-scale morphological changes are expected to arise, and they will permit the initial macrohabitat shift, or colonization of a new environment within the crater lake, and they will be the first evolutionary events to occur during the formation of an adaptive radiation. Founding events, in that only a portion of the genetic variation of the source population is transferred with the ancestral population of a small crater lake, may also be important factors contributing to speciation, since only few individuals are likely to colonize a new crater lake. This, too, will influence or limit future directions of morphological innovation or speciation in particular species assemblages. Local adaptations and ecological character displacement are two additional factors that are very likely to interact strongly in crater lakes that contain multiple ecological species (such as Lake Apoyo, Lake Xiloá, and possibly also Lake Apoyeque).

The crater lakes of Nicaragua are a natural experiment in the sense that every one of the lakes (at least as far as we are able to determine) was independently

seeded from one or more of the large lakes. This raises the question of whether evolution repeats itself. Repeated or parallel evolution—similar solutions to the same ecological challenge—would imply that there is predictability and, therefore, that there are rules of diversification to be discovered. Moreover, those rules would then presumably apply not only to the evolution of the Midas cichlid adaptive radiation across time and space, but would be expected to apply more generally.

An analysis of body shape with ecological niche and inferred adaptation across lakes would be important in order to clarify whether similar or different selection regimes act in each of these crater lakes. But the exact role that ecological selection and adaptation played in driving the evolution of morphological variation and speciation in the Midas cichlid species complex still needs to be examined in more detail.

ARE THERE OTHER SPECIES THAT ARE YET TO BE DESCRIBED?

Comparative morphological analysis showed that the populations of Midas cichlids in the large lakes, as well as those in each of the crater lakes, look slightly but distinguishably different. Therefore, based on morphology alone, this would argue that all populations of Midas cichlids are different from each other and should be considered to be different species.

Particularly for crater lakes with one described species, it is quite possible that there are still new species to be described. For example, the crater lakes in which *A. cf. citrinellus*—Apoyeque, Asososca León, Asososca Managua, and Masaya—is supposed to live may actually contain several novel species that are, as of yet, undiscovered or in need of formal taxonomic description. For example, the morphs with hypertrophied lips that occur in lakes Apoyeque and Masaya might need to be considered to be new species. In Asososca Managua, we collected a relatively elongated *A. cf. citrinellus* at low frequencies, and future research may identify it as an incipient limnetic ecotype or even a new species. Asososca Managua has a large water volume, the smallest littoral zone of all crater lakes, and clear water; thus, it is feasible that rapid speciation to exploit the open-water limnetic niche might have occurred there. Lake Masaya is maximally 6000 years old. Given the examples of other crater lakes in the region of similar age, Lake Masaya might be expected to harbor multiple species, though they have not yet been described. However, the lake has experienced frequent volcanic activity even as recently as 1,800 years ago. It is unknown to what extent such eruptions might affect a crater lake's fauna, but it is conceivable that they could have eliminated or decimated the entire fish population repeatedly. Additionally, historical documents indicate that there were no large fish,

such as Midas cichlids, in Masaya 450 years ago, which raises the interesting possibility that the Midas cichlid population in Lake Masaya is only a few centuries old. Either of these scenarios could explain both the low genetic diversity we have recorded and the apparent paucity of morphological variation of Lake Masaya's Midas cichlid fauna.

MORE WORK REMAINS TO BE DONE

The understanding of the processes that might lead to the origin of new species has changed in the past decade, partly through the study of cichlid fishes from the crater lakes in Nicaragua. The geographical aspects as well as the roles played by natural and sexual selection in speciation have been revised recently. Under certain circumstances, ecological specialization can indeed result in new species evolving very quickly and within a single population. If selection is sufficiently strong, this can happen even without geographical barriers to prevent the movement of genes. This type of selection works, just as Darwin envisioned it, against average individuals and in favor of extreme specialists—in the case of the Midas cichlid, for example, those individuals with particularly molariform or papilliform jaws, or those with especially long bodies, which are able to move more efficiently in open water, or others whose body shape enables them to maneuver particularly well close to the shore.

The Midas cichlid species complex has provided us one of the few examples that are known to date of the emergence of new species without geographical barriers—of sympatric speciation in nature. According to these findings, at least one new species (*A. zaliosus*) has come into being through ecological speciation within a few thousand generations in crater Lake Apoyo and another (*A. xiloaensis*) equally fast through assortative mating and sexual selection in crater Lake Xiloá. We don't know yet for sure which type of speciation is faster or more common.

In order to understand how new species emerge at a genetic level, we are now seeking to identify the genes that control the morphological and ecological differences between these young species. This also entails identifying how many genes are involved in shaping particular aspects of their morphology and which types of mutation caused the phenotypic differences between those species. It will be particularly interesting to work on species-pairs that evolved their similar morphologies independently to ask whether evolution repeats itself also at the molecular level or whether evolution has found independent genetic and developmental ways to respond to similar ecological challenges in different crater lakes.

Methodological developments, such as new techniques and machines for sequencing entire genomes quickly, now allow for the large-scale projects that

are necessary to accomplish these tasks. We would then hope to learn which genes—and how many mutations, and of which type—bring about the differences between species. One could then hope to learn whether evolution repeats itself also at the level of the gene or only in the phenotypes through so-called convergent evolution. These are not trivial problems, and it will take many more talented students to reach an understanding of these fundamental issues in evolutionary biology. Nevertheless, we have already learned a lot about these fish in this stunningly beautiful country, where the people are so friendly and where Daniel Ortega is President once again. This time around he was democratically elected and did not have to forcefully overthrow a dictator as he had to the first time I visited Nicaragua more than 25 years ago.

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