

ADAPTIVE EVOLUTION AND EXPLOSIVE SPECIATION: THE CICHLID FISH MODEL

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The cost of DNA sequencing continues to fall, which makes it feasible to develop genomic resources for new model species that are well suited for studying questions in evolutionary biology. The thousands of closely related cichlid fishes in the lakes of East Africa are an ideal model system for understanding the genetic basis of vertebrate speciation. Genomic techniques are helping to integrate empirical and theoretical studies by identifying the genes that underlie the phenotypic differences among species.

SEXUAL ANTAGONISM

Where an allele is favoured in one sex and selected against in another.

ALLOPATRIC SPECIATION

Speciation that involves the differentiation of geographically separate populations.

SEXUAL SELECTION

Selection among individuals of one sex that is exerted through competition for mates, or the mating preferences of the opposite sex.

PHYLOGENY

The evolutionary relationships of a group of organisms, which are often depicted as a tree diagram.

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Charles Darwin named his 1859 book *On the Origin of Species by Means of Natural Selection*¹. Almost 150 years later, understanding the mechanisms by which new species are generated — the process of speciation — remains a central problem in evolutionary biology. We have learned a great deal about the evolutionary forces that change allele frequencies within populations, and developed an elaborate theory of population genetics to model changes in allele frequency. However, we still have only a rudimentary understanding of the interplay of evolutionary forces that lead to the origination of new species, and we can point to only a few genes that are directly implicated in the speciation of any group. Detailed knowledge of the genetics of speciation still eludes us.

For most of the last century, new species were thought to arise by the gradual development of reproductive isolation among populations with separate geographic ranges². If enough incompatibilities had accumulated, populations would fail to produce offspring when they came into contact, and they would be considered separate species. The original theory proposed that incompatibilities arose by random genetic drift, or as a pleiotropic effect of natural selection in each population. More recent work indicates that SEXUAL ANTAGONISM, the evolutionary ‘arms race’ between male and female reproductive systems, might be the primary origin of reproductive incompatibility³. Random silencing

of duplicated genes might also contribute to the accumulation of hybrid inviabilities⁴.

Although there is a wealth of evidence to support the idea that classic ALLOPATRIC SPECIATION has been important in the radiation of many groups², it would be a mistake to conclude that this mechanism explains the origin of all new species (BOX 1). Some proponents of the allopatric model actively discouraged the study of alternative mechanisms of speciation for more than 50 years. During this time, relatively little attention was paid to how selection might directly contribute to the emergence of new species. There is now growing evidence that indicates that natural selection has an important role in accelerating, or even precipitating, speciation⁵. SEXUAL SELECTION has also been recognized as a powerful force that can contribute to the origination of new species⁶.

There is also a shift towards identifying the genetic variation that is responsible for phenotypic differences among closely related species. Since the 1960s, most work in evolutionary genetics has focused on using neutral genetic markers to characterize population structure and identify the PHYLOGENETIC relationships among species. However, genomic approaches are beginning to filter down to non-traditional models. One vertebrate system in particular seems to be ideally placed to capitalize on our increasing ability to analyse in detail the genomes of new model organisms. Fishes

Box 1 | Speciation mechanisms

Speciation mechanisms have been classified in many ways, depending on the importance that is ascribed to different factors by particular investigators. Mayr proposed and promoted the traditional, geographic, classification². Allopatric speciation is the gradual divergence of populations with completely separate geographic ranges. Parapatric speciation is the divergence of populations with adjacent geographic ranges, and which occasionally exchange genes. Sympatric speciation, among populations in close physical contact, was thought to be unlikely because high levels of gene flow would prevent differentiation. These geographic categories are really surrogates to describe the different levels of gene flow owing to geographic barriers or physical distance.

Templeton⁶⁵ proposed a similar classification, but used more explicit population-genetic terminology. For example, he considered divergence among populations that are completely isolated, connected by low levels of gene flow, or that occupy separate habitats within the same geographic area. He added further categories involving various kinds of genetic transience — rapid shifts in genetic architecture arising from inbreeding or hybridization.

Recent classifications have focused on the selective forces that are responsible for the differentiation of populations, regardless of the levels of gene flow among incipient species. There is growing evidence that ecological selection on traits such as feeding morphology, environmental tolerance or reproductive timing, has an important role in the divergence of populations⁵. As sexual selection acts directly on mate-preference traits, it seems particularly likely as a candidate for promoting pre-mating reproductive isolation⁶⁶. There is also growing interest in genetic conflict, such as the co-evolutionary antagonism between the sexes, as a mechanism for promoting the divergence of incipient species³⁶.

of the family Cichlidae represent the most species-rich family of vertebrates. More than 3,000 species of cichlids are distributed from Central and South America, across Africa to Madagascar and southern India. Cichlids are a diverse fauna in each of these areas, and have repeatedly shown a capacity for rapid radiation and sympatric speciation^{7,8}. However, it is the rapid

radiation of cichlid fish species in the Great Lakes of East Africa that has attracted the most attention. In these lakes, almost 2,000 species of cichlids have evolved in the very recent evolutionary past. These species are a tremendous resource of phenotypic variation that is akin to a natural mutagenic screen. These mutants are amenable to genetic analysis because many of these species are reproductively compatible in the laboratory. Moreover, the thousands of species in these lakes provide, in one well-characterized system, a series of snapshots of the speciation process. The signs are good that the lessons that we learn from this system will be generally applicable. Studies of cichlids are beginning to provide important insights into the genetic basis of adaptive evolution and speciation. This system is likely to be even more useful as we start to apply the genomic tools that are now becoming available.

Here, I discuss the impressive recent progress that has been made in studies of adaptive evolution and speciation in cichlids, and highlight the potential that genomic approaches have to make this system a potent natural laboratory for evolutionary genetics. First, I explore in more detail the reasons why cichlids are such an interesting system within which we can address evolutionary genetic questions of general significance. I then summarize the complex interplay of selection pressures that are implicated in the rapid evolution of this species group. This is followed by a summary of the progress that has been made in using genomic tools to elucidate the genetic basis of adaptive evolution and speciation in cichlids. Finally, I discuss the future of studies of this system and a vision of how the very diversity of processes that underlie cichlid variation could be the system's greatest strength.

Box 2 | The nature of cichlid species

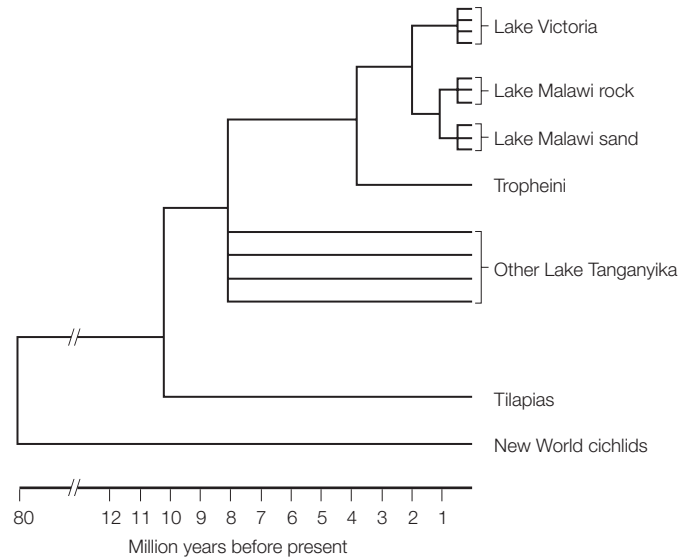
What is a species? Many students are taught Mayr's traditional species concept — which states that species are groups of organisms that do not interbreed with other such groups — a definition that focuses on reproductive isolation. In practice, however, we often use a morphological species concept, in which individuals are categorized by similarities in size, shape and colour. This definition works well, and we have no difficulty in recognizing discrete species of birds in our backyards, even though few of us have ever tested their reproductive compatibility. Cichlid species are no different. If we survey the organisms that are present at a particular location, we can usually sort them by colour pattern and/or morphology into discrete groups that we would all agree were unique species. We can also identify behaviours that are specific to particular taxa. For instance, some male cichlids build sandcastle mating bowers that have species-specific shapes.

The difficulty comes when we consider similar forms at different localities. We cannot easily assess whether forms that differ slightly in colour or morphology would mate if brought into physical contact. Mate-choice assays can be carried out in the laboratory, but if hybridization occurs skeptics can always dismiss it as an artefact of the simplified laboratory environment. So, we typically resort to a working hypothesis that is based on a morphological species concept. Allopatric populations that are as different as recognized sympatric species in colour pattern (or morphology) are generally accepted as distinct species. Genetic data usually corroborate these morphologically defined species, but fixed differences in allele frequency are rarely observed. Hybridization and gene flow might continue to occur among these forms.

The fact that many cichlid species are easily hybridized in the laboratory should cause no confusion. These are young species that have not accumulated a large number of genetic incompatibilities since they last shared a common ancestor. Most cichlid species are kept separate by pre-zygotic behavioural isolating mechanisms, more specifically by female mating preferences, which can be overcome if the females are not given an appropriate choice of mates. Although the cues that are used by females to identify males of the same species are not fully characterized, they are sufficient to prevent hybrid matings most of the time. Post-zygotic isolating mechanisms seem to be less common, but are sometimes manifested as embryonic lethality or highly skewed sex ratios in hybrid progeny. The frequent absence of post-zygotic isolating mechanisms and incomplete characterization of pre-zygotic isolating mechanisms means that debates on whether morphologically distinguishable but similar cichlids represent one or two species are still common. These debates, and the cichlid taxonomies they pertain to¹⁰, will probably continue to have a major influence on research in this system.

Box 3 | **Challenges in phylogenetic reconstruction**

The rapid radiation of cichlids in East Africa has posed a challenge to those attempting to reconstruct the historical relationships among species. The major lineages, and a time frame for the radiations, were easily established from the sequences of mitochondrial DNA⁶⁷ (see figure). The Lake Tanganyikan flock consists of several independent lineages that invaded the lake 7–9 million years ago. One of these lineages, the Tropheini, is sister to a group of fishes that dispersed through the rivers and lakes of East Africa^{68,69}. These fishes, the haplochromines, underwent several independent radiations after they colonized lakes Malawi and Victoria over the past 1–2 million years^{70,71}. Reconstructing the recent history of these radiations is complicated by the fact that many species still share the genetic polymorphisms that were segregating in their common ancestor^{72,73}. For this reason, the phylogeny that is inferred from the sequences of a particular gene might not accurately represent the history of individual species. The most extreme example of this is the retention of ancient polymorphisms in the major histocompatibility complex (MHC)⁷⁴. Only by averaging many individual gene trees can we infer the true relationships among species. Genomic techniques that survey polymorphism at thousands of sites across the genome are beginning to reveal the recent history of the species flocks^{64,75,76}.



Why study cichlids?

In historical terms, speciation is an infrequent event, which makes it difficult for relatively short-lived humans to study. Fortunately, a few groups of organisms seem to undergo speciation at unusually high rates, which allows us to see intermediate stages in the process. The most spectacular radiations of vertebrates are the species flocks of cichlids in the lakes of East Africa, where in the last 10 million years almost 2,000 unique species have evolved (BOX 2). In each of the major lakes in the region, one or a few species have initiated rapid adaptive radiations, which have culminated in flocks of several hundred closely related but phenotypically diverse species. Lake Tanganyika has a flock of at least 200 species, whereas Lake Malawi and Lake Victoria each contain more than 500 species^{9,10}. Few systems offer the opportunity to study so many recent, ongoing and replicate speciation events. Such a rapid radiation of species is a significant challenge for phylogenetic reconstruction. Nonetheless, significant progress has been made in determining the fine-scale phylogenies that are required to interpret adaptive evolution and speciation within these species flocks (BOX 3). These radiations have occurred in such a short period of time (and in spite of significant levels of gene flow) that classic models of speciation do not easily explain cichlid evolution. For these reasons, African cichlids have figured prominently in debates about speciation mechanisms.

The radiation in Lake Malawi can be characterized in three historical stages (FIG. 1). The first stage, habitat divergence, is represented by the evolution of distinct rock- and sand-dwelling clades. The second stage is the elaboration of morphologically distinct genera, which is characterized by the differentiation of the feeding apparatus by natural selection. Finally, the most recently evolved species differ primarily in colour pattern, which indicates the action of sexual selection. Although it is clear that numerous selective forces function at each step,

their relative importance changes with each stage¹¹. Adaptation to different macrohabitats was important early in the radiation, but is no longer an important axis of differentiation as the most recent pairs of sister species are usually found in the same habitat. Ecological selection on TROPHIC MORPHOLOGY was probably important through the first two stages of the radiation, but recently derived species of rock-dwelling cichlids have essentially identical jaw and tooth morphologies. Sexual selection on colour pattern was probably important throughout the radiation, and could be the most important force in the third stage of the radiation. This ‘radiation-in-stages’ model is consistent with the patterns in the less marked radiations of sticklebacks, Arctic charr, Caribbean anoles, Galapagos finches and Hawaiian honeycreepers¹². The lessons that we learn about adaptive evolution and speciation from the extraordinary radiations of cichlid fishes are likely to be generally applicable.

The well-characterized population structure of cichlid species is also a major advantage in terms of understanding speciation at both the population and molecular level. Many cichlid species, particularly those that inhabit rocky habitats, show a high degree of population structure¹³. Microsatellite DNA markers reveal significant differences in allele frequencies among populations that are separated by only a few hundred yards^{14,15}. These differences are largely due to low levels of migration. Cichlids lack significant larval or juvenile dispersal and adults are highly philopatric. One study indicates that gene flow occurs primarily by migration of sub-adult males as they search for breeding territories, as females tend to stay in their natal area¹⁶. Although levels of gene flow are low, adjacent populations are estimated to exchange more than one migrant per generation, and therefore are not expected to diverge by solely neutral processes such as genetic drift¹⁷. Some form of selection is required for divergence, and understanding why and how this operates could go a

TROPHIC MORPHOLOGY
The morphological characteristics of an animal that relate to its intake of food.

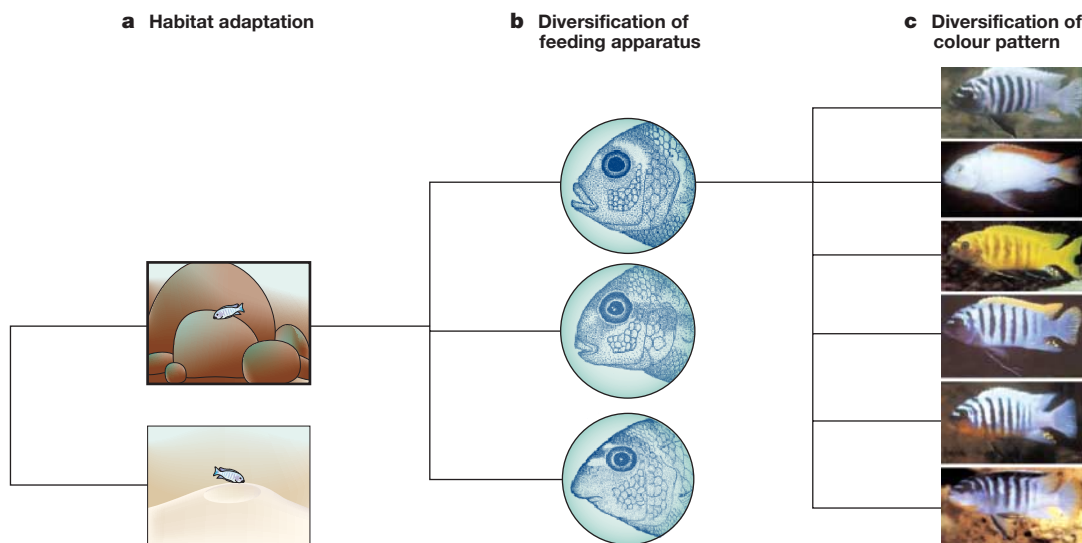


Figure 1 | **Three-stage radiation of cichlids in Lake Malawi.** **a** | The first stage of cichlid radiation involves adaptation to distinct rocky and sandy habitats in the lake. **b** | The second stage is a radiation of trophic morphologies within each habitat, which is represented by the jaws of *Metriaclima*, *Tropheops* and *Labeotropheus* (top to bottom, respectively). **c** | The third stage is a diversification of male colour patterns within each lineage, which is represented here by species of the genus *Metriaclima*. Modified from REF. 11 © (2001) Blackwell Scientific.

long way towards resolving the argument between the advocates and opponents of sympatric models of speciation. Moreover, the highly structured cichlid populations should make it much easier to map the genes that underlie reproductive isolation in these species (see below).

Selection pressures

As cichlid populations within each lake are not completely isolated by physical barriers to gene flow, other factors must be involved in the differentiation of new species. At least three major selective forces might have contributed to the divergence of cichlid species: selection on ecological traits, sexual selection and genetic conflicts.

Ecological selection. African lake cichlids are a classic example of adaptive ecological radiation. Early studies focused on the obvious diversity of trophic morphology among species¹⁸. In a remarkably short evolutionary period, cichlids have produced a dazzling array of jaw and tooth morphologies that are adapted for different modes of feeding. Other axes of ecological divergence include habitat preferences and behavioural niche partitioning. In some cases, such as the cichlid flocks in the crater lakes of Cameroon, these differences have arisen in absolute sympatry¹⁹. Similar differences among species in the East African lakes might not have arisen in complete sympatry, but probably still evolved among populations that were connected by weak gene flow. For this reason, attention has focused on theoretical models of sympatric speciation.

The classic, single-gene models predict that sympatric ecological speciation can occur among populations that are connected by gene flow only if there is strong linkage of genes for an ecological trait and mate preference^{20,21}. However, two relatively recent studies

showed that a polygenic basis for these traits actually allows speciation to occur more readily^{22,23}. Although they used slightly different mathematical approaches, both studies found that disruptive selection on a quantitative fitness trait can recruit a second polygenic trait (for example, colour) as a marker for assortative mating. Speciation can occur very quickly once the genetic correlation between the fitness and mate-choice traits reaches a critical threshold.

These papers have removed a theoretical barrier to the acceptance of sympatric speciation. But, although it is clear that ecological selection has had an important role in the radiation of these fishes, it remains to be seen whether it is an important factor in the speciation of the most recent lineages, which generally show less divergence in trophic morphology.

Sexual selection. Female cichlids lay a small number of relatively large eggs, and care for their young for several weeks until the yolk is absorbed. Most of the African lake cichlids are maternal mouthbrooders — the females pick up the eggs immediately after laying and incubate them in their mouths for several weeks. In some species, maternal care continues for several weeks after the free-swimming juveniles are released. By contrast, males of most species contribute nothing but genes to their offspring. This asymmetric parental investment leads to strong sexual selection, sexual dimorphism and has further consequences for cichlid life history and dispersal.

The diversity of male colour patterns among rock-dwelling cichlids led to speculation that sexual selection was involved in speciation²⁴. McKaye²⁵ extended this idea to sand-dwelling species, noting the highly ornamental nature of male bowers (FIG. 2). These studies focused on Lande's model of divergence by sexual selection²⁶, in

which male traits evolve in response to female mate preferences. Lande's model predicts a rapid co-evolution of male traits and female preferences, especially if female preferences are open-ended (for example, for the brightest available male). This 'runaway' process could result in rapid divergence in male display traits among discrete populations. More recent theories indicate that sexual selection can also lead to sympatric speciation. Turner & Burrows²⁷ identified conditions under which an allele for reversed female preference (for example, for black instead of white males) could invade and cause rapid sympatric speciation within small populations. Payne and Krakauer²⁸ considered a model in which male dispersal depends on mating success, and leads to LINKAGE DISEQUILIBRIUM (LD) and an acceleration of FISHER'S PROCESS. Higashi and colleagues²⁹ found that a divergent runaway process could produce speciation, even when several genes control both the male and female mating traits. Finally, van Doorn and colleagues³⁰ found rapid sympatric speciation in a model that coupled sexual selection and NICHE DIFFERENTIATION.

These models show that sympatric speciation by sexual selection is theoretically possible if we accept certain assumptions about the genetic basis of female preferences. Experimental tests of these assumptions are difficult to carry out. Female preference functions are inherently difficult to measure, especially as it is hard to predict which of several possible male characteristics are

important to females. Despite these obstacles, several studies have shown pre-mating isolation to occur among colour morphs. Given unfettered access to males, females reliably choose the same species in the field³¹ and the laboratory³². This choice can be mediated by visual cues alone³³. Males can also recognize females of the same species using only visual cues, if the females of each species belong to different colour morphs³⁴.

Marked sexual dimorphism and the various male colour patterns are prima-facie evidence that sexual selection is a strong force in the evolution of these species flocks. Runaway divergence is clearly possible from a theoretical perspective, but it remains to be empirically tested. We need more quantification of female mate preferences to evaluate these models.

Genetic conflicts. Various molecular mechanisms allow genes to compete within the genome, sometimes to the detriment of organismal fitness. The most well-known examples are the 'selfish' genetic elements, such as transposons, that replicate to high copy numbers unless they are opposed by another form of selection. Conflicts between different elements of the genome are a powerful evolutionary force³⁵. The genes that are most often in conflict are those that reside in different cellular compartments (for example, cytoplasmic versus nuclear genes) or which are inherited asymmetrically (for example, autosomes versus sex chromosomes).

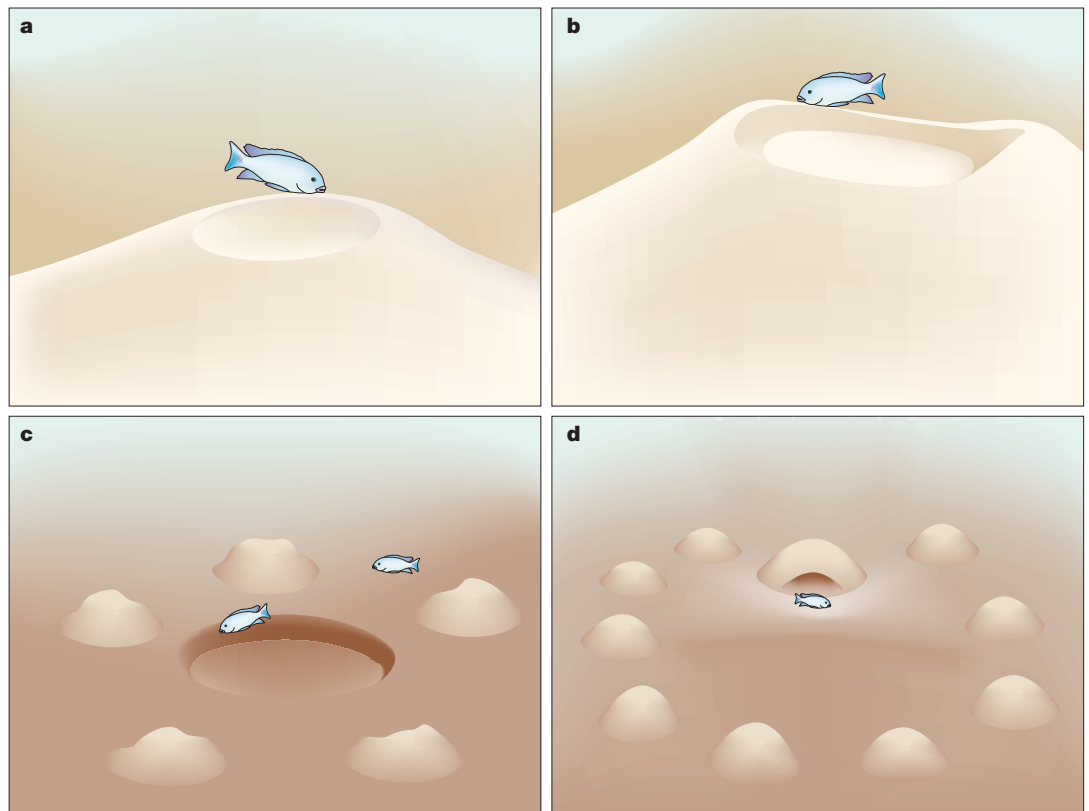


Figure 2 | **Diversity of bower form in Lake Malawi cichlids.** Males of many sand-dwelling species of Lake Malawi cichlid build elaborate sandcastle bowers, the shape of which varies greatly among species. These bowers are a focal point for male courtship displays. Mating takes place in the bower, after which the female leaves with the fertilized eggs in her mouth. Bowens of **a** | *Copadichromis* sp **b** | *Tramitichromis* sp **c** | *Lethrinops lethrinus* and **d** | *Lethrinops auritus*.

LINKAGE DISEQUILIBRIUM (LD). A measure of genetic associations between alleles at different loci, which indicates whether certain allelic combinations are more common than expected.

FISHER'S PROCESS
A process of runaway evolution of male traits and female mating preferences under sexual selection, which Ronald A. Fisher is credited with recognizing.

NICHE DIFFERENTIATION
The tendency for co-existing species to differ in their niche requirements.

Box 4 | **Speciation through genetic conflict**

Genetic conflicts between maternal and zygotic genes over the sex ratio of offspring could contribute to speciation. The cycle of sex-chromosome evolution and speciation would occur in stages (see figure).

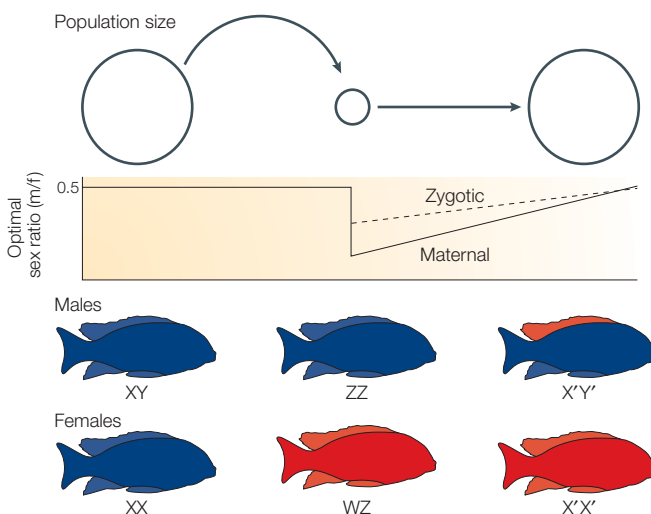
Stage 1

Owing to changes in lake level, a new habitat becomes available, and a small group of fish establishes a new population there. In this small population brothers will frequently compete for mates, so selection favours mothers that produce a high proportion of females. This population is therefore open to invasion by a dominant male-repressor (*W*; not necessarily allelic with the pre-existing XY system), because it increases the proportion of females. A new colour mutation (for example, orange-blotch) arises in tight linkage with the female-determining gene on the *W*. Males that recognize these new females as mates receive a selective advantage, which creates a correlation between the female trait and male preference that contributes to a runaway fixation of the new colour variant in the population. The result is a new, female heterogametic (WZ), system of sex determination.

Stage 2

As the population grows, the probability of inbreeding declines, and the optimal sex ratio returns to 0.5. Under these conditions, a new male-determining gene (*Y'*; not necessarily allelic to either the previous XY or WZ systems) can invade the population because it increases the proportion of males. A new colour mutation (for example, red dorsal fin) arises in tight linkage with the male-determining gene on the *Y*. Females that prefer these new males receive a selective advantage because it returns their offspring to an optimal sex ratio. The result is fixation of the new male determiner and convergent evolution of a male heterogametic (*X'Y'*) sex-determining system.

This cycle is especially likely to occur in association with the founding of new populations, but it could also be maintained by a tension between maternal and zygotic genes in mildly inbred populations. The predictions of this model include differences in the sex-determining mechanism of closely related species, and the linkage of colour polymorphisms to sex-determining genes. Both of these predictions have been confirmed in East African cichlids^{40,77,78}. A similar model has been proposed by Lande and colleagues⁴¹.



Conflicts can also arise between genomes (for example, maternal versus paternal or parental versus zygotic). Intersexual conflict, or sexual antagonism, is thought to be responsible for the rapid evolution of proteins in the male and female reproductive tracts, and to thereby cause both pre- and post-zygotic hybrid infertility among closely related species^{33,36}. These particular kinds of conflict probably do not have an important role in cichlid evolution, because cichlid eggs are fertilized externally, and because the egg MICROPYLE limits the opportunity for conflicts arising from POLYSPERMY³⁷.

However, intragenomic conflict over sex ratio might cause rapid evolution of sex-determining mechanisms in sexual species³⁸, which might contribute to speciation. Both zygotic genes (for example, sex chromosomes or CYTOPLASMIC FACTORS) and parental genes (for example, MEIOTIC DRIVE, GAMETIC IMPRINTING and maternal mRNAs) can influence the sex of the offspring. Under some circumstances, such as inbreeding, the optimal sex ratio for mothers and their offspring can differ, which results in a co-evolutionary race over the mechanism of sex determination³⁹. Consistent with this idea, Seehausen and colleagues⁴⁰ observed sex-ratio distortion in crosses between sympatric, partly assortative mating, morphs of a Lake Victoria cichlid. They built on these results to propose two models of sympatric speciation⁴¹. Each model incorporates sex-linked colour polymorphisms that become the basis for sexual selection and assortative mating among the newly evolved species. A 'speciation engine' that is

driven by repeated cycles of genetic conflict over the sex ratio might contribute to speciation in highly structured populations of cichlids (BOX 4).

Evolutionary genomics

Ultimately, these various models of speciation are about selective pressures on particular genes. These genes are the link between theoretical models and empirical observations of speciation. The large gap in our understanding of speciation is that we have not identified many of these speciation genes. Genomic technologies promise to close this gap, by accelerating the discovery of speciation genes in traditional laboratory models. Decreases in the cost of genome sequencing are slowly undermining the dominance of traditional model organisms, and allowing the emergence of new model systems that are selected specifically for their ability to elucidate evolutionary mechanisms.

East African cichlids offer many advantages as a model system for studying the genetic basis of evolutionary change. The diverse species of cichlids can be viewed as the products of a natural mutant screen for various morphological and behavioural phenotypes. The study of these natural mutants can provide an important counterpoint to laboratory mutagenesis. These natural variants will provide insights into the function of many vertebrate genes that have escaped analysis in traditional models. Cichlid mutants are readily accessible to genetic analysis because most interspecific hybrid crosses are fully fertile.

MICROPYLE

An opening in the egg capsule through which spermatozoa enter.

POLYSPERMY

The entry of several sperm into one egg.

CYTOPLASMIC FACTORS

Genes in host organelles such as mitochondria, or in intracellular parasites such as *Wolbachia*, that are typically passed from mother to offspring through the cytoplasm.

MEIOTIC DRIVE

The preferential transmission of one gamete genotype over another genotype, in which the genotypes in question might derive from the same meiosis.

GAMETIC IMPRINTING

The persistent differential methylation of parental genes that results in the expression of the allele from only one parent in the offspring.

Various genomic tools have been developed for work on East African cichlids (see online links box). These include genetic maps of the tilapia⁴² and Lake Malawi cichlid⁴³ genomes. The co-linearity of these maps, and the broad phylogenetic utility of the microsatellite markers on which they are based, allow a consensus cichlid linkage map to be used to study some 2,000 species of East African cichlid. A physical map that is based on the restriction fingerprints of 35,000 large-insert clones has also been assembled to facilitate positional cloning. A suite of bioinformatic tools is being developed to link these maps to the relatively complete genome sequences of other fish species, such as zebrafish, medaka and pufferfish. Other groups are developing cDNA-based microarrays for the study of jaw development and brain function. These genomic tools allow several complementary approaches to the identification of speciation genes.

The most direct route to identifying the genes underlying the phenotypic differences among recent species is genetic mapping. It is now practical to map chromosomal regions that are responsible for essentially any quantitative trait in East African cichlids. Positional cloning to identify the particular genes that are responsible for the phenotypic differences among species has already begun.

Jaw shape. Phylogenetic evidence indicates that trophic adaptation was particularly important in the second stage of the radiation in Lake Malawi. Albertson and colleagues⁴⁴ studied the genetic basis for differences in jaw morphology between a highly specialized algal scraper (*Labeotropheus fuelleborni*) and a more generalized suction feeder (*Metriaclima zebra*). Comparison of the phenotypic variance in F_1 and F_2 hybrids indicated that between 4 and 11 genes affected the shape of individual skeletal elements. Mapping of these quantitative trait loci (QTLs) suggested that some of these genes might have pleiotropic effects on several traits⁴⁵. The distribution of alleles in the two species indicates that there is a history of consistent directional selection on jaw morphology.

A chromosomal region that is marked by polymorphism in the bone morphogenetic protein 4 gene (*bmp4*) contributes to differences in the shape of both upper and lower jaw elements⁴³. *Bmp4* is known to have an important role in the development of the jaw in mice⁴⁵. Terai and colleagues⁴⁶ found an elevated rate of amino-acid substitution in the pro-domain of *Bmp4* in East African cichlids. *bmp4* is therefore an interesting candidate that might explain some of the divergence of jaw morphology by ecological selection.

Tooth shape. Tooth shape is highly correlated with the feeding ecology of cichlid fishes. For example, *Metriaclima zebra* adults have a bicuspid dentition that is suited to its generalist feeding habits, whereas *Labeotropheus fuelleborni* have densely packed tricuspid teeth that efficiently shear attached algae. This marked difference in tooth shape seems to be controlled by a major gene that might simultaneously affect both tooth shape and tooth number⁴⁷. These observations

have established cichlids as a new model system for understanding the genetic control of tooth shape in vertebrates.

Colour pattern. Closely related species are most easily distinguished by differences in colour pattern, and these differences are important in mate choice^{33,48}. Despite the importance of both male and female colour pattern in theories of speciation by sexual selection, relatively little attention has been focused on the genes that underlie these differences.

One particularly enigmatic pattern, orange-blotch (OB; FIG. 3), is observed in numerous species from Lake Malawi and Lake Victoria. On the basis of an extensive series of crosses, Seehausen and colleagues⁴⁰ proposed that OB is produced by an X-linked locus that is modified by a second, autosomal locus in Lake Victoria species. A similar polymorphism is found across several genera of Lake Malawi cichlid and maps as a dominant allele on linkage group 5 (REF. 49). Comparative mapping with other fish species has identified a region of CONSERVED SYNTENY around OB and identified several candidate genes. Analysis of allele frequencies in a natural population showed a strong association with a marker (*cski*) approximately 2 cM from OB. This result indicates that a high degree of population structure might allow the use of LD MAPPING to narrow QTL intervals and speed up the positional cloning of other traits in Lake Malawi cichlids.

There is considerable interest in using a candidate gene approach to identify the genes that are responsible for other colour patterns. Terai and colleagues⁵⁰ identified an accelerated rate of evolution in *hagoromo*, a gene that is implicated in the development of pigment patterns in zebrafish. This gene also has a complex pattern of alternative splicing, which produces numerous splice variants in each species⁵¹. These splicing patterns seem to be species-specific, but the differences in splicing have not yet been related to gene function or to differences in colour pattern among species. Most of the colour-pattern phenotypes in cichlids have no direct counterparts in either mammals or zebrafish, so the study of cichlid colour patterns will provide new insights into the developmental regulation of neural crest cell lineages.

Visual sensitivity. Variation in opsin visual pigments might be expected to alter visual sensitivity, and ultimately mate preferences. Carleton and colleagues⁵² showed that some Lake Malawi cichlids have an ultraviolet (UV)-sensitive cone pigment, which is able to detect the UV reflectance that is common to many blue cichlids (BOX 5). Further research has shown that differences in visual sensitivity among Lake Malawi cichlids are largely due to changes in which of several opsin genes are expressed⁵³.

Changes in visual sensitivity can also arise through amino-acid substitutions that tune the absorption spectrum of the opsin protein. Two studies have detected the signature of positive selection in the evolution of the rhodopsin and long-wavelength sensitive (LWS) opsin genes^{54,55}. Cichlid visual pigments might

CONSERVED SYNTENY
Conservation of the linkage relationship among genes between two species.

LINKAGE DISEQUILIBRIUM MAPPING
The analysis of single-nucleotide-polymorphism alleles in population-based studies to identify loci that are associated with a particular phenotype.

have evolved rapidly to adapt to differences in water quality in different habitats. Whereas Lake Tanganyika and Lake Malawi are among the clearest lakes in the world, Lake Victoria and the rivers that feed it are quite turbid, and have a very different spectrum of light available for visual communication. These differences in visual sensitivity have influenced the evolution of colour patterns and mate preferences in these species flocks⁵⁶.

The way forward

Traditional vertebrate laboratory models are ill suited for studies of how organisms adapt to their environment, and therefore several new model systems are being developed to promote the fusion of ecology, evolutionary biology and functional genomics⁵⁷. In addition to cichlids, attention has turned to natural populations of pocket mice⁵⁸, sticklebacks⁵⁹, salmonids⁶⁰ and cave-fish⁶¹. Among these, cichlids are remarkable for their combination of laboratory tractability and the breadth of the morphological, physiological and behavioural phenotypes that are available for study. The diversity of cichlid species in East Africa provide an extraordinary resource for studying the genetic mechanisms that underlie vertebrate adaptation and speciation.

Research on speciation has moved well beyond a categorization of mechanisms that is based on the geographic distribution of populations. The focus is now on the various forces that contribute to phenotypic divergence, regardless of the level of gene flow between incipient species. We have a well-developed theoretical base

that can integrate the forces of selection, gene flow and drift on individual genes, to predict whether speciation is likely⁶². Unfortunately, we still lack even a rudimentary description of the genes that underlie most phenotypes. Genes will be the common currency that allows theorists and empiricists to discuss the importance of different evolutionary forces during speciation.

Some small steps have been made towards identifying the genes that underlie divergent trophic morphologies in cichlids. The results indicate that ecological selection has been strong, and has acted on a relatively small number of genes. These studies have been conducted using crosses between different genera, and might reflect processes that were important early in the radiation of the Lake Malawi flock. Analysis of other interspecific crosses would help to identify the full set of genes that are responsible for the trophic radiation of East African cichlids.

Despite the probable importance of pre-zygotic isolation to cichlid diversification, we are largely ignorant of the genetic basis for mate preferences. Good progress has been made in developing phenotypic assays for mate choice, and there is evidence to suggest that mate choice is largely based on visual cues. It would be useful to carry out unbiased surveys of the genome to identify other male traits that females use to select mates. The identification of these traits might make it feasible to design sensitive phenotypic assays for the mapping of mate-preference genes.

In general, the candidate gene approach seems risky, because we are still largely ignorant about how most traits are coded in the genome. Developmental pathways involve many genes, and even relatively simple traits can have a complex genetic basis. Divergence in morphological traits seems likely to be controlled by differences in the timing or strength of gene expression in complex tissues. It might be difficult to apply current techniques for quantifying gene expression to this important problem. And for behavioural traits such as mate preference, which are of the greatest interest with respect to speciation, we have no idea what the candidate genes might be. Genetic mapping will be the primary tool for identifying the genes that are important to speciation.

It is also time to bring to bear new analytical methods in population genetics. There should be an exciting interplay between QTL mapping in laboratory populations and ASSOCIATION STUDIES in natural populations. As cichlid populations are highly structured, LD mapping is likely to be an important adjunct to positional cloning. Once the genes that underlie particular phenotypes are identified, it will be interesting to examine the COALESCENT HISTORY of alleles at these genes to reconstruct the history of selection and population structure in recently derived clades. Although these methods are relatively well developed, they have so far been applied only to anonymous genetic markers⁶³.

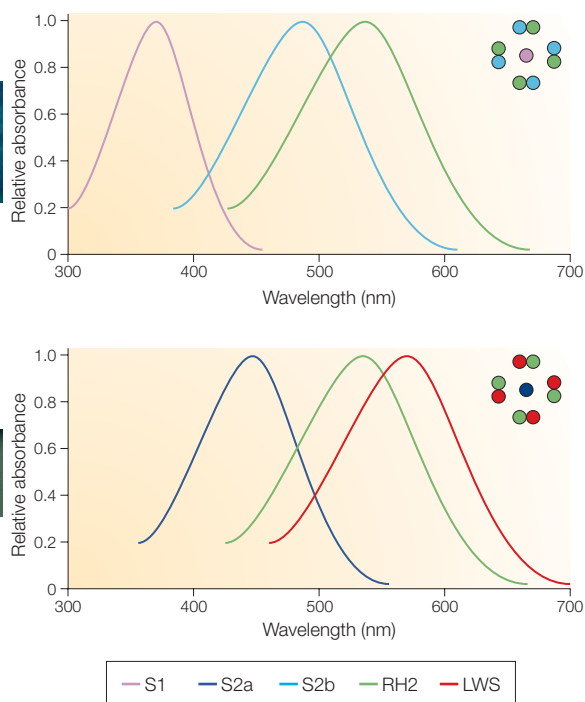
Finally, we need to study the fixation of alleles for different phenotypes in a phylogenetic context. The application of genome-scale amplified fragment-length polymorphism (AFLP) methods for phylogenetic



Figure 3 | **Intraspecific colour polymorphisms.** The lower fish is a male *Labeotropheus fuelleborni* showing the blue-with-black-bars pattern that is typical of both the males and females of many rock-dwelling cichlids in Lake Malawi. The upper fish is a female *Metriaclima zebra* that shows the orange-blotch pattern, a polymorphism that is found in many species from lakes Malawi and Victoria and that is due to a dominant allele on linkage group 5.

Box 5 | Evolution of visual sensitivity

The spectral response of the rod and cone cells of the retina is altered by changes in the amino-acid sequence of opsin, which is the protein component of the visual pigment. Humans have only three kinds of cone opsin (red, green and blue), each of which is encoded by a different gene. By contrast, cichlids have five major classes of cone opsin gene, including red (long-wavelength sensitive; LWS), green (RH2), blue-green (S2b), blue (S2a) and ultraviolet (S1)⁵². Individual fish typically express only three opsins in cone cells, which are arranged in a 'square-dance' mosaic pattern in the retina. Single short-wavelength cones (expressing either S1 or



S2a) are surrounded by four pairs of cells (double cones) that express longer-wavelength opsins. Spectral tuning of the cichlid cones is accomplished by two major mechanisms. Small shifts of 5–10 nm in the wavelength of peak absorbance are usually due to the substitution of one or a few amino acids in the corresponding opsin. More marked differences in visual sensitivity are due to changes gene expression: some species express the red, green and blue genes (for example, *Dimidiochromis compressiceps*, lower panel), whereas others express the green, blue-green and ultraviolet opsins (for example, *Metriacilima zebra*, upper panel)⁵³. Changes in visual sensitivity are of particular interest because they are likely to directly affect mate preferences. The evolution of female mate preferences might be responsible for the spectacular variation in male colour pattern that is characteristic of the cichlid radiations.

reconstruction provides new opportunities to study character-state evolution in these species flocks⁶⁴. Are the genes for trophic divergence fixed before or after the establishment of behavioural isolating mechanisms? Do changes in female mate preferences precede or follow divergence in male colour pattern? How often is divergence in sex determination associated with speciation?

Conclusions

Research on speciation has often drifted from one model to the next, and the history of research on cichlid speciation is no exception. The sequence of explanations might look like a search for a magic bullet — a single novel mechanism that could explain the diversity of cichlids in East African lakes. It is time for a more pluralistic approach. These extraordinary radiations are probably due to a remarkable confluence of several factors that also underlie the speciation of other groups of organisms. We should be working to establish the range of selective pressures that interact during the formation of new cichlid species, and to identify the commonalities with the mechanisms of speciation in other groups.

Cichlid species are separated mostly by pre-mating isolating mechanisms, or by reduced fitness of hybrids under ecological selection. In this respect, studies of cichlids differ from other studies of speciation, which typically focus on intrinsic post-mating barriers to fertilization or acute hybrid inviability. Further studies of cichlids could lead to a more general understanding of speciation, because they have diversified along different axes and under different selective pressures.

The tools of high-throughput genomics are particularly important to the future development of this field. By identifying the genetic variation that is responsible for phenotypic differences among closely related species, we can begin to understand the selective forces that have created and shaped these new species. In the near future, this research promises to unify statistical genetics (quantitative and population genetics) with mendelian genetics (the molecular genetic basis of adaptive variation). Nowhere is this unification needed more than in the field of evolutionary biology, which for too long has relied on correlative studies of neutral or linked variants to make inferences about adaptive evolution and speciation.

ASSOCIATION STUDIES
An approach to gene mapping that looks for associations between a particular phenotype and allelic variation.

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- Expression analysis reveals the genetic basis for the dramatic differences in visual sensitivity among Lake Malawi cichlids.**
- A phylogeny based on amplified restriction fragment polymorphisms demonstrates the repeated parallel evolution of male colour patterns in Lake Malawi.**
- Mapping of quantitative trait loci for jaw and tooth morphology reveals the imprint of consistent directional selection on the trophic apparatus.**
- Results from crosses among Lake Victoria cichlids stimulate new models of speciation that involve the evolution of colour patterns and the sex-determining genes.**
- Comparative mapping among fish species identified candidate genes for the OB polymorphism that is common among species from lakes Malawi and Victoria.**

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Competing interests statement

The author declares that he has no competing financial interests.

 **Online links**
FURTHER INFORMATION

Thomas D. Kocher's laboratory: <http://hcgs.unh.edu>

Cichlid Genome Consortium: <http://hcgs.unh.edu/cichlid>

Cichlid fishes of Lake Malawi:

<http://www.malawicichlids.com/>

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