Ghost of speciation past

Thomas D. Kocher

Lurking in the rivers of Botswana are the remnants of a diverse flock of cichlid fishes, whose origins can be traced to a lake that vanished more than 2,000 years ago.

Just add water. A simple recipe, but when applied to cichlid fishes it reliably causes spectacular evolutionary radiations of hundreds of new species. Generalist riverine cichlids have given rise to flocks of highly specialized fishes in each of the major lakes in East Africa. Now, on page 90 of this issue, Joyce and colleagues[1] describe a previously unrecognized radiation of cichlids in the extinct Lake Makgadikgadi, whose descendants still haunt the rivers of southern Africa today.

Joyce and colleagues' evidence[1] consists of mitochondrial DNA sequences determined from fishes collected from rivers throughout southern Africa. Rivers in East Africa typically hold just one haplochromine cichlid species (haplochromine is the name given to one of the major lineages of cichlid fishes in Africa). But the Okavango, Cunene, Limpopo and upper sections of the Congo and Zambezi rivers in southern Africa each harbour several species of haplochromines. Evolutionary analyses show that these fishes share a common ancestor quite separate from that which gave rise to the better known radiations in lakes Malawi and Victoria (Fig. 1). The diversity of these riverine cichlids peaks in the Okavango delta, which geological evidence suggests was, until about 2,000 years ago, part of a lake larger than Switzerland.

The extent of this newly recognized adaptive radiation is truly impressive. The remnants of the Lake Makgadikgadi flock display a morphological diversity nearly as great as that of the extant cichlids in lakes Malawi and Victoria. The flock includes ambush predators with long heads and jaws, and snail crushers with deep heads and massive teeth. Only a few specialized morphologies are missing — such as those of pelagic plankton eaters and benthic algal scrapers — probably because these niches are not available in the remaining river habitat.

What is it about lakes that facilitates these rapid radiations? Certainly, large lakes provide a range of new ecological habitats not present in rivers. The still waters of lakes probably also reduce rates of gene flow between different groups of fish, enabling them to diverge genetically from each other and eventually to speciate. But other groups of fishes have not responded to these physical factors with similar bursts of speciation. It seems there is also something special about haplochromine cichlids that causes them to diversify in these large lakes. What are the key innovations that make them particularly prone to rapid adaptive radiation?

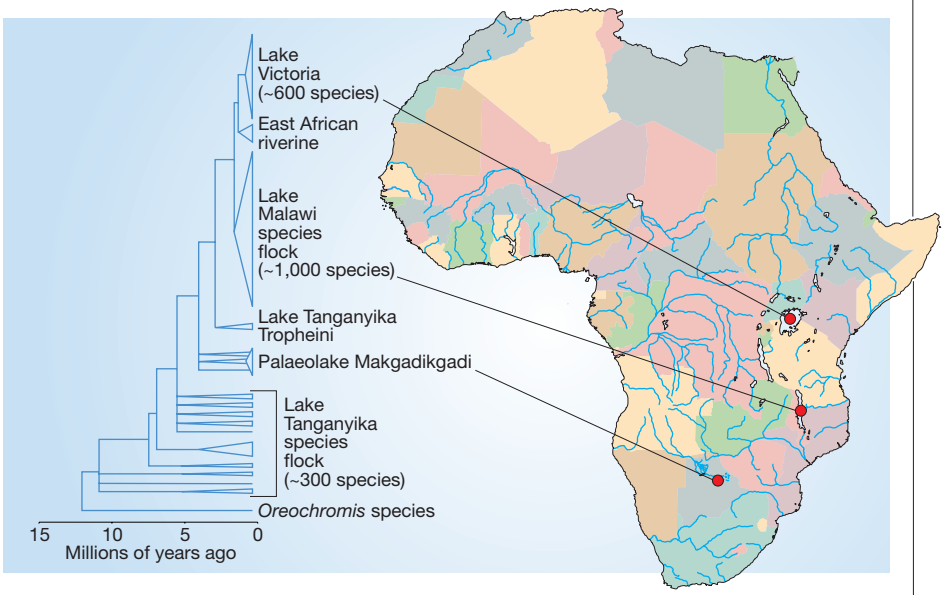

Figure 1 **Repeated diversification of cichlid fishes in southeast Africa. Mitochondrial DNA sequences reveal a radiation of cichlids in Lake Tanganyika beginning 7–9 million years ago. Over the past 3–4 million years, one of these lineages has spread through the rivers of southern and eastern Africa. These haplochromine cichlids gave rise to independent radiations in lakes Victoria and Malawi, and, as Joyce *et al.*[1] now describe, in the palaeolake Makgadikgadi. In Lake Tanganyika, these fishes also gave rise to a recent radiation of the Tropheini. The tree is rooted with tilapia (genus *Oreochromis*), a popular food fish that is closely related to the haplochromine cichlids of the region. (Tree modified from ref. 10.)**

A long-standing hypothesis is that cichlids have a malleable feeding apparatus that can be quickly altered to take advantage of new feeding opportunities[2]. Cichlids have a second set of jaws in the back of the throat — the pharyngeal jaws — that process food before it enters the gut. The oral jaws are therefore relatively free to evolve specializations for acquiring food. This decoupling of functions has clearly contributed to the rapid evolution of feeding specializations in each of the species flocks that inhabit lakes.

Another key innovation is maternal mouth-brooding. Most non-haplochromine cichlids lay large numbers of small eggs on the lake or river bottom, and both parents cooperate in the care of the eggs and fry. In contrast, female haplochromines lay small numbers of large eggs, and carry the eggs in their mouths for several weeks until the young are released to fend for themselves. Haplochromine males provide no post-spawning care of their offspring.

This unequal parental investment results in strong sexual selection. Males must compete for females, and as a consequence have evolved an astonishing variety of nuptial colorations and behaviours to attract mates. These differences in colour are often enough to prevent interbreeding (hybridization) among existing species[3]. But it is not clear whether sexual selection on male traits is sufficient to drive speciation in the first place. Other genetic conflicts, including those between females and their offspring over the sex ratio, could also be important[4].

Joyce and colleagues[1] suggest that hybridization may itself have played a part in the origin of the Lake Makgadikgadi species flock. This lake was probably colonized by distantly related cichlids from previously unconnected rivers. If these lineages hybridized, the resulting pool of genetic diversity might have facilitated the generation of new morphological or behavioural characteristics, enabling the colonization of newly available ecological niches in the lake[5]. This hypothesis is difficult to prove, but may be testable through analysis of variation

in the genes underlying important adaptive traits.

The present situation in the Okavango delta may also provide some context for discussing the origin of today's Lake Victoria flock. Although some geological data suggest that the Lake Victoria basin was completely dry 15,000 years ago, molecular evolutionary studies generally suggest that the flock is much older (100,000 years)[6,7]. As with Makgadikgadi, it seems likely that several genetically and morphologically diverse cichlids survived the drying of Lake Victoria, and have been able to re-radiate into more than 500 species during the 15,000 years since the lake refilled[8].

The high rates of speciation observed in these African cichlids are almost beyond belief[9], but the evidence is clear. The discovery of yet another species flock[1] emphasizes the importance of the phenomenon, and reinforces the utility of these fishes for studying evolutionary mechanisms. African cichlid fishes represent about 5% of all vertebrate species. A synthesis of the mechanisms responsible for their spectacular radiation is essential if we are to fully appreciate the origins of vertebrate diversity. ■

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1. Joyce, D. A. *et al. Nature* **435,** 90–95 (2005).
2. Liem, K. *Syst. Zool.* **22,** 425–441 (1974).
3. Knight, M. E. & Turner, G. F. *Proc. R. Soc. Lond B* **271,** 675–680 (2004).
4. Kocher, T. D. *Nature Rev. Genet.* **5,** 288–298 (2004).
5. Seehausen, O. *Trends Ecol. Evol.* **19,** 198–207 (2004).
6. Seehausen, O. *et al. Proc. R. Soc. Lond. B* **270,** 129–137 (2003).
7. Verheyen, E., Salzburger, W., Snoeks, J. & Meyer, A. *Science* **300,** 325–329 (2003).
8. Seehausen, O. *Proc. R. Soc. Lond. B* **269,** 491–497 (2001).
9. Coyne, J. A. & Orr, H. A. *Speciation* (Sinauer, Sunderland, MA, 2004).
10. Salzburger, W. & Meyer, A. *Naturwissenschaften* **91,** 277–290 (2004).

Developmental biology

Morphogens hitch a greasy ride

Richard S. Mann and Joaquim Culi

Morphogen proteins guide the development of many tissues in animals, but how are these insoluble proteins ferried around the body? A well-known group of lipid transporters might be the answer.

Every once in a while, a scientific discovery results in the marriage of two previously disparate fields. On page 58 of this issue, such a romance is suggested by Eaton and colleagues[1]. On the one hand, there are morphogens — signalling proteins that play an essential part in animal development by inducing specific cellular fates in a concentration-dependent manner[2,3]. How these proteins move from the cells that synthesize them to neighbouring cells to create a concentration gradient is an area of intense debate. On the other hand, there are lipoprotein particles, most famous for their role in lipid transport and heart disease. The marriage, witnessed by intriguing experiments described in the new paper, raises the possibility that lipoprotein particles carry morphogens as they move through tissues during development.

Although biologists have had much success in measuring the biological effects of morphogens, understanding how these proteins spread through tissues to generate a concentration gradient has proved more challenging. This is in part because many morphogens are highly insoluble, which hampers their diffusion through the hydrophilic environment of tissues. In particular, two morphogens, the Hedgehog (Hh) and Wnt/Wingless (Wg) proteins, are attached to palmitic acid, and Hh is also covalently linked to cholesterol[4]; these lipids would promote the association of the two proteins with cell membranes.

Several models have been proposed to explain how morphogens move through tissues. Some suggest that they are actively transported by cells — through long cellular extensions called cytonemes, for example, or by being passed from cell to cell through cycles of secretion and uptake[2,3]. Although these models satisfactorily deal with morphogen insolubility, it has been difficult to confirm their role in morphogen spreading and activity. In contrast, accumulating biological evidence and mathematical modelling favour the idea that morphogens diffuse passively along the surface of cells, aided by local interactions with other molecules[2,3]. For such 'restricted diffusion' to be possible, lipid-modified morphogens must somehow acquire a relatively soluble form.

One potential solution, suggested previously by the Eaton group[5], posits that morphogens move in membranous vesicles called argosomes that are derived from morphogen-producing cells. Now the Eaton and Thiele labs[1] have revised this model by suggesting that morphogens move via large particles better known for their role in transporting lipids — the lipoproteins (Fig. 1). If they are right, this connection would contribute significantly to our understanding of morphogen spreading, and suggests a new role for lipoproteins during animal development.

Lipoproteins are large, globular macromolecular complexes composed of a central core of lipids surrounded by an outer layer of polar phospholipids, cholesterol and specialized proteins called apolipoproteins[6]. Most lipoproteins are synthesized in the liver and intestine of mammals and in the fat-body of insects, from where they move to peripheral tissues through the blood and lymph (the haemolymph in insects) to regulate lipid levels throughout the body.

Using their lipid adducts as anchors, Wnt and Hh proteins could in principle easily reside in the outer phospholipid layer of lipoproteins. Indeed, using biochemical fractionation of fruitfly (*Drosophila*) tissues, Eaton and colleagues[1] show that, although most Wg and Hh proteins co-fractionate with cellular membranes, a significant percentage partitions into the lipoprotein fraction. Moreover, lipoproteins can be co-immunoprecipitated with Wg and Hh, suggesting a tight association.

So, lipoproteins and morphogens have been seen together, but is this relationship a meaningful one? Eaton and colleagues suggest that it is, because lipoproteins seem to be required for the accurate establishment of morphogen gradients and activity in *Drosophila* larvae. The authors used a process called RNA interference (RNAi) to decrease apolipoprotein synthesis in the *Drosophila* fat-body — thus decreasing lipoprotein concentrations throughout the animal. This reduced the range of action of Hh and Wg in the imaginal discs, where morphogen activity is best characterized. Moreover, Hh accumulated at higher than normal levels in cells near the Hh source, perhaps as a consequence of reduced diffusion.

Although these findings are intriguing, the systemic way in which lipoproteins are synthesized and circulated, combined with their role in lipid metabolism, makes it difficult to design a watertight experiment. The authors describe two controls that help strengthen their conclusions. First, incubating the RNAi-treated discs with purified lipoproteins at least partially restored the long-range signalling activity of Hh. Second, growing larvae on a reduced-lipid diet did not affect morphogen activity or diffusion. Both experiments support the idea that the RNAi-induced defects are due to lower numbers of lipoprotein particles and not to a secondary effect such as reduced lipid levels.

Beyond providing a new mechanism by which lipophilic morphogens might spread through tissues (Fig. 1), the marriage between lipoprotein metabolism and animal development, if correct, hints at additional relationships. For example, the diffusion of most morphogens also requires heparan sulphate proteoglycans (HSPGs) — large molecules consisting of protein and carbohydrate that are found on the cell surface[7]. Lipoproteins are already known to interact