

# Ecological genetics of freshwater fish: a short review of the genotype–phenotype connection

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## Abstract

*Ecological genetics of freshwater fish: a short review of the genotype–phenotype connection.*— Molecular ecology or ecological genetics is an expanding application of population genetics which has flourished in the last two decades but it is dominated by systematic and phylogeographic studies, with relatively little emphasis on the study of the genetic basis of the process of adaptation to different ecological conditions. The relationship between genotype and adaptive phenotypes is weak because populations are often difficult to quantify and experiments are logistically challenging or unfeasible. Interestingly, in freshwater fish, studies to characterize the genetic architecture of adaptive traits are not as rare as in other vertebrate groups. In this review, we summarize the few cases where the relationship between the ecology and genetics of freshwater fish is more developed, namely the relationship between genetic markers and ecological phenotypes.

Key words: Ecological genetics, Molecular ecology, Genotype–phenotype relationship, Adaptation, Landscape genetics, Species introduction.

## Resumen

*Genética ecológica de los peces de agua dulce: una breve revisión de la conexión genotipo–fenotipo.*— La ecología molecular o la genética ecológica es una aplicación de la genética de poblaciones que durante las dos últimas décadas ha sufrido un proceso de expansión. Sin embargo, en la ecología molecular predominan los estudios sistemáticos y filogeográficos, con relativamente poco énfasis en el análisis de la base genética del proceso de adaptación a diferentes condiciones ecológicas. Esta relación entre genotipo y fenotipo adaptativo es poco evidente, porque las poblaciones son difíciles de cuantificar y los experimentos son logísticamente complicados. Es interesante destacar que en peces de agua dulce estos estudios no son tan poco frecuentes como en otros grupos de vertebrados. En esta revisión, nuestra intención es resumir los pocos casos en los cuales la relación entre ecología y genética de peces continentales está más desarrollada, principalmente entre marcadores genéticos y fenotipos ecológicos.

Palabras clave: Genética ecológica, Ecología molecular, Interacción genotipo–fenotipo, Adaptación, Genética del paisaje, Introducción de especies.

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## Introduction

Ecological genetics or molecular ecology is the application of molecular genetic tools to ecological problems and the field has developed enormously in the last two decades. Molecular ecology is, however, dominated by systematic and phylogeographic studies, and there is relatively little emphasis on the study of the genetic basis of the process of adaptation to different ecological conditions (Bowen, 1999). In the words of Avise (2006), 'mechanistic connections between observable genotypes and ecologically relevant phenotypes often remain black boxes' and 'molecular ecology will become a more mature discipline when it also incorporates ecologically germane information on genotype–phenotype connections.' In this review we intend to summarize studies where the relationship between genotypes and phenotypes may be regarded as adaptive. We focus on genetic studies of ecologically important traits in freshwater fish.

The link between observable genotypes and ecologically relevant phenotypes is difficult to assess in most species. First, population sizes and demographic statistics are often difficult to quantify, and experiments are logistically challenging and often unfeasible. Second, although new molecular methods are becoming available (*i.e.*, RAD-tags), molecular markers or gene sequences may be difficult to obtain. And third, identification of ecologically relevant phenotypes is not easy. In most cases a trait is considered adaptive when it is suspected to improve survival of the individual or its fitness, but very few studies to date have focused on the effects that alternative phenotypes may have from an evolutionary point of view. Identifying the genetic architecture of such traits (and the causal mutations of the alternative phenotypes) allows detection of evidence for selection in the DNA, and it may therefore highlight the evolutionary importance of that genotype.

Interestingly, because in some freshwater fish species it is possible to study population dynamics as well as to breed several generations in a few years, studies to characterize the genetic architecture of adaptive traits are not as rare as those in other vertebrate groups. A review focused on these well described cases may thus provide useful information for other non-model organisms for which little previous data is available.

We also briefly discuss two important topics that, from our point of view, may provide valuable information on the genotype–phenotype relationship: landscape genetics and invasive species. Landscape genetics is a new discipline focused on how physical landscape influences genetic traits of the populations (Guillot et al., 2005). Although not directly related to adaptedness or phenotype modelling, such interactions could alter the viability of the population and thus have drastic evolutionary consequences. Furthermore, biological invasions exemplify the process of species adaptation to new environments, and its analysis can add novel information about the relationship between ecology and genetics.

## Genotype–phenotype relationships

Several freshwater fish species are used as models to study the genetic architecture of putative adaptive traits. Such traits are related to survival and thus fitness of individuals. Although some traits (*e.g.* coloration) can safely be assumed to reflect adaptation, in other cases it is not obvious whether a particular phenotype is adaptive. Genetic analyses focused on identifying the causal mutations of a phenotype can help to determine if the trait is really adaptive through a further detection of evidence for selection in the DNA sequence (Nielsen, 2005). In general, the most frequent strategy to undertake this approach requires fully interfertile diverse populations showing different phenotypic traits, development of molecular markers in the targeted species (or close relatives) and setting up experimental crosses, which allow the identification of quantitative trait loci (or QTL) and the actual causative mutations. All these requirements restrict the range of species being studied, although some models are now fully established.

### *Gasterosteus aculeatus*

The three-spine stickleback (*Gasterosteus aculeatus*) is a complex species that shows repeated episodes of colonization of freshwater habitats, including both lakes and rivers, from marine stocks (reviewed in McKinnon & Rundle, 2002). These colonization events have caused divergence in several traits (which could be adaptive) such as morphology, behaviour and physiology. The variety of environments inhabited by three-spine stickleback (ranging from fresh water to ocean) may explain the importance of behavioural and physiological traits that may be critical for adaptation to new physical and chemical conditions. In contrast, morphological traits have been linked to the different predators they might encounter (Marchinko, 2009).

Interestingly, all marine and fresh water forms are interfertile. An extensive set of microsatellite markers and over 45,000 single nucleotide polymorphisms (SNPs) are available (Hohenlohe et al., 2010) to carry out genome-wide typing assays. Several experimental crosses have been developed, yielding different QTLs with effects on gill structure, spine length, number of lateral plates (Peichel et al., 2001), armour (Colosimo et al., 2004, 2005) and pigmentation (Miller et al., 2007). In some of these studies, particular genes have been statistically associated with phenotypic changes, linking *Ectodysplasin* to changes in armour and *Kit ligand* to changes in pigmentation. The only phenotype with known causal mutations is pelvic size, which is modified by regulatory mutations in *Pitx1* (Shapiro et al., 2004).

Characterization of the genetic architecture of these traits has allowed novel approaches to confirm the traits as adaptations and to analyze the dynamics of such genotypes in populations (Kitano et al., 2008; Makinen et al., 2008; Marchinko, 2009; Chan et al., 2010).

QTL analyses in these species have also confirmed a role for sex chromosomes in speciation, related to the location of loci involved in behavioural

isolation along the X chromosome (Kitano et al. 2009). Other studies have focused on specific genotypes, using a candidate gene approach. Generally, following this strategy, the function of the targeted gene is known and it is considered to be adaptive. The analysis of sweet taste receptors (Hashiguchi et al., 2007) or immunity related genes (Reusch et al., 2001; Wegner et al., 2008) would fall within this category. More recently, the analysis of plasma level mRNA expression and the genomic signature for selection on one gene in the thyroid hormone signalling pathway, *TSH $\beta$ 2*, have shown significant differences between ancestral marine and stream-resident ecotypes (Kitano et al., 2010). These results suggest that evolutionary changes in hormonal signalling may have played an important role in the adaptive divergence of sticklebacks.

#### *Astyanax mexicanus*

The Mexican tetra (*Astyanax mexicanus*) has two forms, the surface and the cave-dwelling forms. Interestingly, the troglodyte form (or cavefish) presents some phenotypic traits shared with other animals adapted to the dark environment of caves: reduced eyes (or even complete regression) and loss of pigmentation, including albinism in some cases. Moreover, cavefish probably have more taste buds, larger jaw size and more fat reserves (reviewed in Jeffery, 2009). All these traits seem relevant for survival under some obvious cave conditions, especially absolute darkness and the low quantity of nutrients.

At least thirty different caves in Mexico have been noted to harbour cave forms of *A. mexicanus*, and there is evidence of parallel evolution and several independent origins for the cave forms (Borowsky, 2008). Blind *Astyanax mexicanus* from different caves are already used as a model organism to study molecular pathways of eye development (Jeffery, 2008). In addition, several experimental crosses have been designed to identify the genetic architecture of other interesting traits. Protas et al. (2006) identified cave-specific mutations of *OCA2* causing albinism, thus demonstrating evolution by convergence. Interestingly, although pigmentation loss is usual in cavefish, albinos are not present in all caves. Some depigmented (or brown) phenotypes have been found to be caused by two different mutations of *MC1R* (Gross et al., 2009), although other brown mutations are likely to exist. As well as confirming convergence this pattern indicates that certain genes are frequent targets of mutation, at least in regressive phenotypes.

Other traits that have been analyzed in experimental crosses include eye size and development of the jaw, teeth and taste buds (Protas et al., 2007) as well as total length and metabolism (Protas et al., 2008). Although the causative mutations for these phenotypes have not been identified and only QTLs have been located, these results represent a first approximation to describe pathways of molecular evolution. Moreover, they suggest some hypotheses about the evolutionary mechanisms behind the phenotypes, such as genetic drift and indirect selection through pleiotropy.

#### *Xiphophorus* spp.

The genus *Xiphophorus* includes two of the most popular aquarium fishes: swordtails and platyfishes. Both these fishes, and their intercrosses were used in early genetic mapping studies of pigmentation (Gordon, 1931), which have since allowed the identification of at least 33 loci linked to coloration (reviewed in Basolo, 2006). However, no causal mutations have been identified so far, and some specific (adaptive?) functions of color patterns are still not fully understood (Price et al., 2008).

Interestingly, the analysis of pigmentation led to the genetic characterization of melanoma formation. The mechanism involves the interaction of two loci, Tu and R, (Baudler et al., 1997), and while the Tu locus has been identified as the *Xmrk* gene (Wittbrodt et al., 1989), the R locus remains unknown. As hybridization is the trigger for melanoma appearance in *Xiphophorus*, these genes may have had a role in early speciation as a postzygotic isolation mechanism (Schartl, 2008). Sexual selection, based on female preference for the color phenotype linked to melanoma (Fernandez & Morris, 2008), larger males carrying the melanoma-related allele (Fernandez & Bowser, 2010), and intrasexual selection (Fernandez, 2010) have also been suggested to be the main forces maintaining oncogenes segregating in natural populations.

Swordtails and platyfish are also model organisms in the study of sexual behavior, but information about the genetic basis of such traits is limited (Rosenthal & Garcia de Leon, 2006). Interestingly, *Xmrk* males display increased aggression in mirror image trials, and thus may experience a competitive advantage over wild-type males (Fernandez, 2010). Moreover, there is strong evidence for some reproductive tactics being inheritable (Zimmerer, 1989) and the P locus, affecting sexual maturity, fecundity and size (Kallman and Borkoski, 1978) has been linked to *MC4R* (Lampert et al., 2010).

The sword of swordtails is a classical example of a sexually selected trait because females (as well as predators) prefer larger swords (Basolo, 1990; Rosenthal et al., 2001). Through the analysis of gene expression in developing and regenerating caudal fins of swordtails and platyfish, the main signalling pathway (involving the *fgfr1* gene) has been identified (Offen et al., 2008). However, there is no information available on the effects of this sexual selection at a molecular level.

#### Other species

Even though zebrafish (*Danio rerio*) is a model species in genetics and development, natural variation has not been analyzed thoroughly. To our knowledge, only one experimental cross between wild and lab strains has been carried out (Wright et al., 2006b) to detect QTLs affecting anti-predator behavioural and morphological differentiation. Due to the design of the cross, these traits might be more related to domestication than to adaptation to natural environments. The same experimental cross has also been used

to detect epistatic regulation acting on these traits (Wright et al., 2006a). Interestingly, the comparison of colour and stripe pattern development between different species of the genus *Danio* has shown the importance of genes such as *kit* or *fms* in the evolutionary change of adult phenotypes (Quigley et al., 2004; Mills et al., 2007).

African cichlid fishes (family Cichlidae) are a classical example of adaptive radiation, and there are probably more than one thousand species in just three lakes in East Africa (Lakes Malawi, Tanganyika and Victoria) (Turner et al., 2001). They have been used to investigate the molecular mechanisms of adaptation and speciation, focusing on traits that may have relevant roles in diversification. The huge variation in their colour patterns is a central feature in the behaviour and evolution of these species and may have contributed to their explosive speciation. Although the full genetic architecture of coloration is not known, the orange blotch (OB) phenotype, associated to one single QTL near the *c-ski1* gene (Streelman et al., 2003), is due to a cis-regulatory mutation of the *Pax7* gene (Roberts et al., 2009). It has recently been suggested that sensory adaptation could be a key feature in the radiation of these species, and the genetic causes of visual pigment diversity have been identified (Hofmann et al., 2009; Carleton et al., 2010). Interestingly, regulatory changes (and not structural mutations) have been linked to the parallel evolution of fish visual systems in Lake Tanganyika and Lake Malawi (O'Quin et al., 2010).

Other interesting traits are related to morphological variations. Cichlid fishes forage by different modes, which are related to the functional design of the feeding apparatus. Thus, QTLs under directional selection related to jaw and teeth development have been described, and *bmp4* has been suggested as a causative gene (Albertson et al., 2003, 2005).

Several other freshwater fishes are currently cultured to supply both ornamental and food market demands. Although these domesticated populations do not provide information about the genetic architecture of natural variation or adaptation, they have been used in several experimental crosses. Tilapia (*Oreochromis* spp.) crosses have allowed identification of a genetic mechanism for sex determination (Shirak et al., 2006) and characterization of QTLs related to immune response and growth (Cnaani et al., 2004); medaka fish (*Oryzias latipes*) have been used to characterize the role of *tyr* gene in albinism (Inagaki et al., 1998; Koga et al., 1995; Tsutsumi et al., 2006); and multiple experimental crosses of rainbow trout (*Oncorhynchus mykiss*) have been carried out to focus on the genetics of a variety of traits, including the immune response to pathogens (Nichols et al., 2003; Johnson et al., 2008), maturation (Haidle et al., 2008) and smoltification (Nichols et al., 2008).

Other farmed fishes have also been used to characterize immune responses because pathogens can be an issue in intensive production systems. Although most works have focused on seawater species, some freshwater studies are worth mentioning, such as the genetic characterization of brook charr (*Salvelinus*

*fontinalis*) resistance to *Aeromonas salmonicida* (Croisetière et al., 2008), and the herpesvirus resistance of common carp (*Cyprinus carpio*) (Rakus et al., 2009). These domestic populations are also providing valuable information about the effects of captive breeding. Strikingly, wild-born offspring of captive *Oncorhynchus mykiss* show a rapid fitness decline (Araki et al., 2007). If these results are confirmed in other organisms, they could seriously compromise some conservation strategies of endangered species.

### New molecular tools

The recent development of high throughput genetic techniques has made genomic information accessible and affordable for a great number of organisms through next generation sequencing and DNA microarrays. In general, these new DNA sequencing technologies require a reference genome to align the massive number of short sequences that they produce. This alignment will yield high quality results only when the sample genome does not differ from the reference genome (Frith et al., 2010). However, as only a few fish species have annotated genomes (five in the ENSEMBL database, <http://www.ensembl.org/index.html>) these techniques have limited applications, but they have a very promising future in the field of comparative genomics. Genomes have been analysed in attempts to identify regions of synteny in some species, such as African cichlids, where low-coverage genomes of five phenotypically and ecologically diverse Lake Malawi species have been compared (Loh et al., 2008), and also in *Danio rerio* and *Astyanax mexicanus*, for which no genetic map was available until recently (Gross et al., 2008).

Genomic analyses have also been carried out in *G. aculeatus* using next-generation sequencing of RAD markers (Baird et al., 2008; Hohenlohe et al., 2010), a very promising approach. This methodology requires the construction and massive sequencing of a RAD tag library, with DNA fragments having a restriction site at one end and randomly sheared at the other. Without using any previous genomic information, this arrangement reliably identifies new SNP markers. To date, these markers have been used in genetic mapping, phylogeography, population genomics and even whole-genome sequencing (reviewed in Rowe et al., 2011). Although they have been used in only one freshwater fish species, *G. aculeatus*, they may yield very interesting results in other non-model species.

The lake whitefish species complex, *Coregonus clupeaformis*, is another case study for adaptive radiation (Bernatchez et al., 1999) and for which several genomic tools have been developed, including cDNA microarrays (Rise et al., 2004). Experimental crosses have already been used to identify QTLs affecting reproductive isolation (Rogers & Bernatchez, 2006), and to characterize loci under parallel selection within the complex (Rogers & Bernatchez, 2005). Genome-wide expression patterns have also been described using microarray technology, resulting in the first transcriptome analyses focused on speciation. This

technology provided the first evidence that parallel phenotypic evolution in *C. clupeaformis* also involves parallel transcriptional changes (Derome et al., 2006). This has been confirmed in another species of the same genus, *C. artedi* (Derome & Bernatchez, 2006), and in African cichlids (O'Quin et al., 2010), stressing the importance of gene regulation in rapid phenotypic divergence. However, one study focusing specifically on transcriptional divergence of a set of six candidate genes in three species of the genus (*C. clupeaformis*, *C. artedi* and *C. albula*) points out that parallelism on gene expression is not preserved among species (Jeukens et al., 2009). The availability of this microarray has also allowed the characterization of expression QTLs (eQTLs), showing a sex bias in the transcriptional genetic architecture of lake whitefish (Whiteley et al., 2008). Future research in this field may also help to disentangle whether adaptation is mainly driven by cis-regulatory mutations or by polymorphisms in coding regions.

### Landscape genetics

Neutral molecular markers are of little relevance for the study of adaptive processes. However, the integration of population genetic data obtained from those markers with landscape ecology in the new emerging field of landscape genetics (Manel et al., 2003) represents a new way to integrate ecology and genetics (Holderegger & Wagner, 2006; Sork & Waits, 2010). Understanding how landscape variables and environmental features explain gene flow and genetic discontinuities between populations contributes to our understanding of biological processes such as metapopulation dynamics or speciation (Meeuwig et al., 2010). Such studies are also of applied conservation value in identifying current anthropogenic barriers that reduce gene flow and genetic diversity, in predicting the effects of proposed management alternatives on genetic variation and population connectivity, and in identifying potential biological corridors to assist with reserve design (Storfer et al., 2006, 2010). In fish species, for example, such studies have provided evidence for the role of contemporary landscape features in shaping the observed patterns of genetic diversity at smaller geographic scales in *Salvelinus alpinus* (Castric et al., 2001) and *Oncorhynchus clarki* (Neville et al., 2006). Other studies have also shown constrained gene flow due to local adaptation in *Salmo salar* (Dionne et al., 2008) and selection gradients as responsible for cryptic population divergence in *Gasterosteus aculeatus* (McCairns & Bernatchez, 2008).

### Species introductions

Invasive species are a leading cause of biodiversity loss and global change, particularly in freshwater ecosystems (Mack et al., 2000; Clavero & García-Berthou, 2005), and they are also an underappreciated tool to study ecology and evolution at large spatial and

temporal scales (Rice & Sax, 2005; García-Berthou, 2007). Invasive species are a unique model to characterize adaptation and ecological dynamics in newly occupied territories. Nevertheless, genetic studies of invasive species have generally focused on identifying source populations and the routes of spread and the role of genetic diversity, again without much integration with ecological studies. An exception is the study of rapid evolution and adaptation following introductions of fruit flies and salmonids (Huey et al., 2000, 2005). The paradigm of conservation biology is that populations have demographic and genetic thresholds below which non-adaptive, random forces (e.g. genetic drift) prevail over adaptive processes and extinction risk increases (Soulé, 1985). Invasive species seem to defeat this paradigm, given the often low number of individuals initially introduced and low genetic diversity (Lindholm et al., 2005; Poulet et al., 2009; Vidal et al., 2009). This apparent paradox is explained by an admixture of often unadvertised, multiple source populations and mechanisms that mitigate the impact of low genetic diversity (Roman & Darling, 2007).

The guppy *Poecilia reticulata* is a model system in the study of evolutionary ecology, sexual selection, and behaviour (Endler, 1995; Bronikowski et al., 2002; Reznick et al., 2004; Ghalambor et al., 2004; Magurran, 2009). Experimental introductions of guppy in Trinidad are a textbook example of rapid, predation-mediated life history evolution. The literature on this species is beyond the scope of our review but illustrates the wealth of information that can be gained with genetic tools, including, for instance, evidence of: i) genetic basis in mate-choice (Brooks & Endler, 2001) and shoaling (Huizinga et al., 2009) behaviours; ii) high levels of multiple mating in wild populations (Hain & Neff, 2007); and iii) high rates of natural selection and evolution in the wild (Reznick & Ghalambor, 2005).

### Conclusions

Genetics and ecology have a long history of little mutual appreciation and exchange (Berry & Bradshaw, 1992; Cain & Provine, 1992). Despite the prosperity of conservation genetics and phylogeography, particularly in large vertebrates, studies that combine both disciplines are still rare. We have tried to give an overview of the information available on the adaptive value of genetic variation in freshwater fish. Although this topic has been investigated for a few species, for most species we do not know how genetic variation affects survival and fitness in the wild. This knowledge is vital to understand the potential response of freshwater fish to global environmental change and to mitigate the impacts of the latter. For a number of reasons, however, we are optimistic about the future of ecological genetics of freshwater fish. The studies summarized reflect the importance and benefits of such an approach. Advances in the biology of fish are often delayed in comparison with those in birds or plants (e.g., García-Berthou, 2007), but with the development of new genetic resources and genomics tools this field is likely to expand greatly in years to come.

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