Molecular Phylogenetics and Evolution 62 (2012) 62–70

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Parallel speciation in *Astyanax* cave fish (Teleostei) in Northern Mexico

Ulrike Strecker *, Bernhard Hausdorf, Horst Wilkens

Zoological Museum, University of Hamburg, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany

ARTICLE INFO

Article history:
Received 23 April 2011
Revised 22 August 2011
Accepted 9 September 2011
Available online 22 September 2011

Keywords:
Introgression
Hybridization
Sympatric speciation
Convergent evolution
Trans-Mexican Volcanic Belt
Astyanax jordani

ABSTRACT

We investigated differentiation processes in the Neotropical fish *Astyanax* that represents a model system for examining adaptation to caves, including regressive evolution. In particular, we analyzed microsatellite and mitochondrial data of seven cave and seven surface populations from Mexico to test whether the evolution of the cave fish represents a case of parallel evolution. Our data revealed that *Astyanax* invaded northern Mexico across the Trans-Mexican Volcanic Belt at least three times and that populations of all three invasions adapted to subterranean habitats. Significant differentiation was found between the cave and surface populations. We did not observe gene flow between the strongly eye and pigment reduced old cave populations (Sabinos, Tinaja, Pachon) and the surface fish, even when syntopically occurring like in Yerbaniz cave. Little gene flow, if any, was found between cave populations, which are variable in eye and pigmentation (Micos, Chica, Caballo Moro caves), and surface fish. This suggests that the variability is due to their more recent origin rather than to hybridization. Finally, admixture of the young Chica cave fish population with nuclear markers from older cave fish demonstrates that gene flow between populations that independently colonized caves occurs. Thus, all criteria of parallel speciation are fulfilled. Moreover, the microsatellite data provide evidence that two co-occurring groups with small sunken eyes and externally visible eyes, respectively, differentiated within the partly lightened Caballo Moro karst window cave and might represent an example for incipient sympatric speciation.

1. Introduction

Adaptation to different environmental conditions results in differentiation and may cause speciation (Schluter, 2000; Schliewen et al., 2001; Richmond and Reeder, 2002; Coyne and Orr, 2004; Strecker, 2006). The repeated evolution of traits contributing to reproductive isolation in separate populations as a by-product of adaptation to different environments may result in parallel speciation (Schluter and Nagel, 1995; Rundle et al., 2000). Schluter and Nagel (1995) specified three criteria that must be satisfied to demonstrate parallel speciation. First, separate populations in similar environments must be phylogenetically independent, such that shared traits responsible for reproductive isolation evolved separately. Second, ancestral and descendant populations must be reproductively isolated. Third, separate descendant populations inhabiting similar environments must not be reproductively isolated from one another.

The transition from living in habitats on the surface of the earth to subterranean life is especially suitable for studies of adaptation, differentiation, and speciation, because (1) the selection pressures in these alternative habitats are clearly defined and strikingly different, (2) the different habitats can be found in close proximity so that the analyses are not complicated by additional factors like different biogeographical influences, and (3) the many caves of different age in karst areas often represent replicated natural experiments (Barr and Holsinger, 1985; Holsinger, 2000; Sbordoni et al., 2000; Wilkens, 2001; Cooper et al., 2002; Schilthuizen et al., 2005; Niemiller et al., 2008; Juan et al., 2010).

We studied surface and cave fish populations of *Astyanax* (Characidae, Teleostei) in Mexico. *Astyanax* is an important model organism for examining adaptations to caves, including regressive evolution (Wilkens, 1988, 2010; Protas et al., 2007; Jeffery, 2008, 2009). Like other Neotropical primary freshwater fish *Astyanax* invaded Central and North America after the closure of the Middle American landbridge at the end of the Pliocene (Bussing, 1985; Bermingham and Martin, 1998; Strecker et al., 2004). It is widely distributed in this region, ranging as far north as the Rio Grande system between Mexico and the United States. Within a restricted karst area in northeastern Mexico a series of eye- and pigment-reduced cave populations has developed from the well-eyed and fully pigmented surface fish. At least 25 different cave populations are known (Mitchell et al., 1977).

Studies of mtDNA sequences demonstrate that *Astyanax* invaded northeastern Mexico and the caves at least twice (Dowling et al., 2002; Strecker et al., 2003, 2004; Ornelas-García et al., 2008; Hausdorf et al., 2011). There was an older invasion by individuals carrying a basal mtDNA clade (clade G; Strecker et al., 2004) that is now mainly restricted to cave populations. A second

---

* Corresponding author. Fax: +49 40 428383937.
E-mail address: strecker@uni-hamburg.de (U. Strecker).

1055-7903/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2011.09.005

invasion occurred by individuals carrying haplotypes belonging to a clade (clade A) that is more closely related to southern mtDNA clades than to clade G. Today, clade A haplotypes predominate in the surface populations in northeastern Mexico, but also occur in some cave populations (Strecker et al., 2003, 2004; Ornelas-García et al., 2008; Hausdorf et al., 2011).

Based on microsatellite DNA data, two nuclear genotypic clusters could be delimited in northeastern Mexico that correspond to these two invasions (Strecker et al., 2003; Hausdorf et al., 2011). One of the clusters is restricted to cave populations and was mainly found in old cave populations of fish which are characterized by strongly reduced eyes and pigmentation (Wilkens, 2010), namely in Pachón, Sabinos and Tinaja. The population from Pachón cave is special, because it shares the strongly reduced eyes and pigmentation with the old populations from the Sabinos and Tinaja caves and belongs to the same nuclear genotypic cluster. However, it shares mitochondrial clade A haplotypes with the neighboring surface populations and the population from Chica cave. This has been explained by mitochondrial capture of the Pachón cave population (Strecker et al., 2003; Hausdorf et al., 2011). In contrast, microsatellite data revealed that the population from Chica cave is a younger one that descended from a more recent invasion, but is partly admixed with nuclear markers from an older invasion (Strecker et al., 2003; Hausdorf et al., 2011).

In some of the caves, e.g. Yerbaniz cave, subterranean forms with strongly reduced eyes and pigmentation and eyed surface fish co-occur without intermediates (Mitchell et al., 1977). In other caves, e.g. Micos and Chica caves, the fish are variable with respect to eye and pigment reduction. Some authors hypothesized that the variable populations reflect the high variability of eyes and pigmentation in early stages of evolution in caves as has been observed in other cave fish and many cave invertebrates (Kosswig and Kosswig, 1940; Kosswig, 1960; Peters et al., 1975; Meyer-Rochow and Juberthie-Jupeau, 1987; Wilkens, 2011). In contrast, other authors (Breder, 1942; Mitchell et al., 1977) ascribed this variability to gene exchange between cave and surface forms. Laboratory experiments reveal that surface and cave *Astyanax* readily interbreed (Sadoglu, 1958). Mitchell et al. (1977) assumed that the intermediate specimens in caves with "mixed fish populations" were hybrids and that the extent of hybridization depends on the food supply. The high amount of presumed hybridization in Chica cave was explained by the survival of surface fish owing to adequate food resulting from guano provided by large bat roosts. In contrast, Yerbaniz cave has little food and it was suggested that fish from the surface populations starve and serve as food rather than as mates for the cave forms (Mitchell et al., 1977).

A specific situation is observed in the Caballo Moro cave where daylight has access through a karst window to a part of a 90 m long stream pool. It contains a fish population consisting of specimens with small sunken eyes and specimens with externally visible eyes (Mitchell et al., 1977). The visible-eyed specimens favor the lighted area of the karst window whereas the sunken-eyed specimens concentrate in the dark part (Espinasa and Borowsky, 2000).

The Central American *Astyanax* is part of the Neotropical *Astyanax fasciatus* species group (Melo and Buckup, 2006), which is taxonomically difficult and not fully resolved (Lima et al., 2003). Based on mitochondrial DNA sequences Ornelas-García et al. (2008) would divide Central American *Astyanax* into more than 15 species. Populations from northeastern Mexico were assigned to two separate species, both of which would include both cave and surface forms. This classification ignores previously demonstrated mitochondrial capture in cave populations (Strecker et al., 2003, 2004; Hausdorf et al., 2011).

In this study we investigated differentiation processes in Mexican *Astyanax*. In particular, we used microsatellite DNA genotyping and mtDNA sequences to assess levels of gene flow between surface and subterranean populations as well as between populations of independent invasions of subterranean habitats to test whether the evolution of the cave fish represents a case of parallel speciation.

2. Materials and methods

2.1. Sampling

Astyanax individuals were collected in four caves in northeastern Mexico in 2000 and 2002 using handnets (Fig. 1a). Fin clips were immediately preserved in 95% ethanol.

2.2. Microsatellite analyses

We assayed 163 individuals from four Mexican caves at six microsatellite loci. DNA extraction, polymerase chain reaction conditions and scoring procedures were as described by Strecker (2003). Additionally, we included microsatellite data from 385 individuals from Strecker et al. (2003) and Hausdorf et al. (2011) in our analyses. The dataset includes fish from seven caves: Caballo Moro, Pachón, Yerbaniz, Sabinos, Tinaja, Micos, and Chica, as well as seven surface populations: Coahuila, Pachón, Yerbaniz (surface fish washed into the cave), Micos (surface fish from outside the cave and sampled in the cave), Rio Coy, Rio Sarabia at Malatengo (Rio Coatzacoalcos drainage), and Mahajual in eastern Yucatan. The microsatellite data are available in Supplementary material Table S1.

We used GENEPOP version 4.0.10 (GENEPOP on the Web at http://genepop.curtin.edu.au/; Raymond and Rousset, 1995) to obtain general statistics of microsatellite diversity and to test for deviations from Hardy–Weinberg equilibrium and linkage disequilibrium. Significance was estimated by the Markov chain method with 10,000 iterations (Guo and Thompson, 1992) and significance ($\alpha = 0.05$) was based on the sequential Bonferroni method for multiple comparisons (Rice, 1989). Pairwise F_{ST} values were computed with Arlequin version 3.01 (Schneider et al., 2000). D_A distances (Nei et al., 1983) between populations were calculated with POPULATIONS version 1.2.30 (Langella, 1999). Neighbor-joining trees (Saitou and Nei, 1987) were constructed with MEGA 4.1. using the D_A distances or F_{ST} values. The trees were rooted by the cave populations with strongly reduced eyes and pigmentation. The basal position of this group of populations is supported by the occurrence of clade G mitochondrial haplotypes in Sabinos and Tinaja cave. Clade G haplotypes represent a more basal branch in the mitochondrial gene tree than clade A, B and C haplotypes, found in the other populations studied (Strecker et al., 2004; Hausdorf et al., 2011). Confidence values were determined based on 1000 bootstrap resamplings. We calculated a phylogenetic network with the neighbor-net algorithm (Bryant and Moulton, 2004) implemented in Splits-Tree4 version 4.6 (Huson and Bryant, 2006) to visualize the relations between the individuals from Caballo Moro cave based on the microsatellite data using D_A distances.

We used STRUCTURE (Pritchard et al., 2000; Falush et al., 2007) to investigate the population genetic structure of Mexican *Astyanax* based on the microsatellite data without a priori grouping of individuals into populations. We explored the pattern of admixture by varying the number of groups (K) and assigning proportions of each individual to these groupings using STRUCTURE version 2.3.3 with the admixture model. Twenty runs with 80,000 iterations after a burn-in of 20,000 iterations were carried out for each K from 1 to 10. To estimate K, we used the mean estimates of the posterior probabilities ($L(K)$) of the data and the statistic $\Delta K = m(|L(K+1) - 2L(K) + L(K-1)|)/s[L(K)]$ proposed by Evanno et al. (2005). DISTRUCT version 1.1 (Rosenberg, 2004) was used