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Defining the Unseen: Population-Specific Markers for *Astyanax mexicanus* Blind Cavefish

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Abstract

Astyanax mexicanus is an emerging model system used to study development, evolution, and behavior of multiple cavefish populations that have repeatedly evolved from conspecific surface fish. Although surface and cavefish live and breed in the laboratory, there are no rapid methods for distinguishing between different cavefish populations. We present 2 methods for genotyping fish for a total of 16 population-specific markers using methods that are easy and inexpensive to implement in a basic molecular biology laboratory. This resource will help researchers maintain independent stocks within the laboratory and distinguish between fish from different populations.

Keywords: *Astyanax mexicanus*, cavefish, husbandry, population-specific markers

Introduction

ASTYANAX MEXICANUS IS A SMALL tetra that exists in two forms, an eyed surface-dwelling fish and a blind cavefish that inhabits >30 caves across northern Mexico.[1,2] Multiple populations of cavefish display strikingly similar traits, including eye regression,[3] reduced melanin pigmentation,[4] and altered social behavior.[5] Given the phenotypic similarities between cavefish from different populations, it is crucial to have reliable methods for verifying fish stock ancestry among laboratory cavefish populations in this emerging model system.

We identified loci in *A. mexicanus* that have fixed variants in either restriction sites that prevent cutting in one of the four populations of interest or single nucleotide polymorphisms (SNPs) that are identifiable after PCR and Sanger sequencing. These provide efficient cost-effective approaches to verify the identity of individuals from different laboratory-bred stocks.

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Materials and Methods

All materials and methods are found in the Supplementary Data.

Results and Discussion

We utilized previously collected population genomic data to identify fixed differences in restriction enzyme cut sites, rendering cut sites nonfunctional in a population-specific manner. We identified 24 sites that are predicted to fail to cut in Pachón cavefish only, 277 sites in Molino, and 16 in Tinaja. We generated eight primer pairs that amplify a region containing an *Msp*I restriction enzyme site in wild-caught surface fish and two cavefish populations that are absent in the third cavefish population.

Next, we determined whether our laboratory populations follow the same pattern as wild populations. We isolated DNA from fin clips or pools of embryos from multiple individuals within our laboratory populations and genotyped fish with each primer pair (Supplementary Tables S1 and S2). At all Pachón population-specific marker locations, PCR products were cut by *Msp*I in all populations tested except for Pachón (Fig. 1A and Supplementary Fig. S1A, B). Similarly, PCR products amplified from Tinaja population-specific sites were uncut by *Msp*I in Tinaja, and PCR products from Molino population-specific sites were uncut by *Msp*I in Molino (Fig. 1B, C and

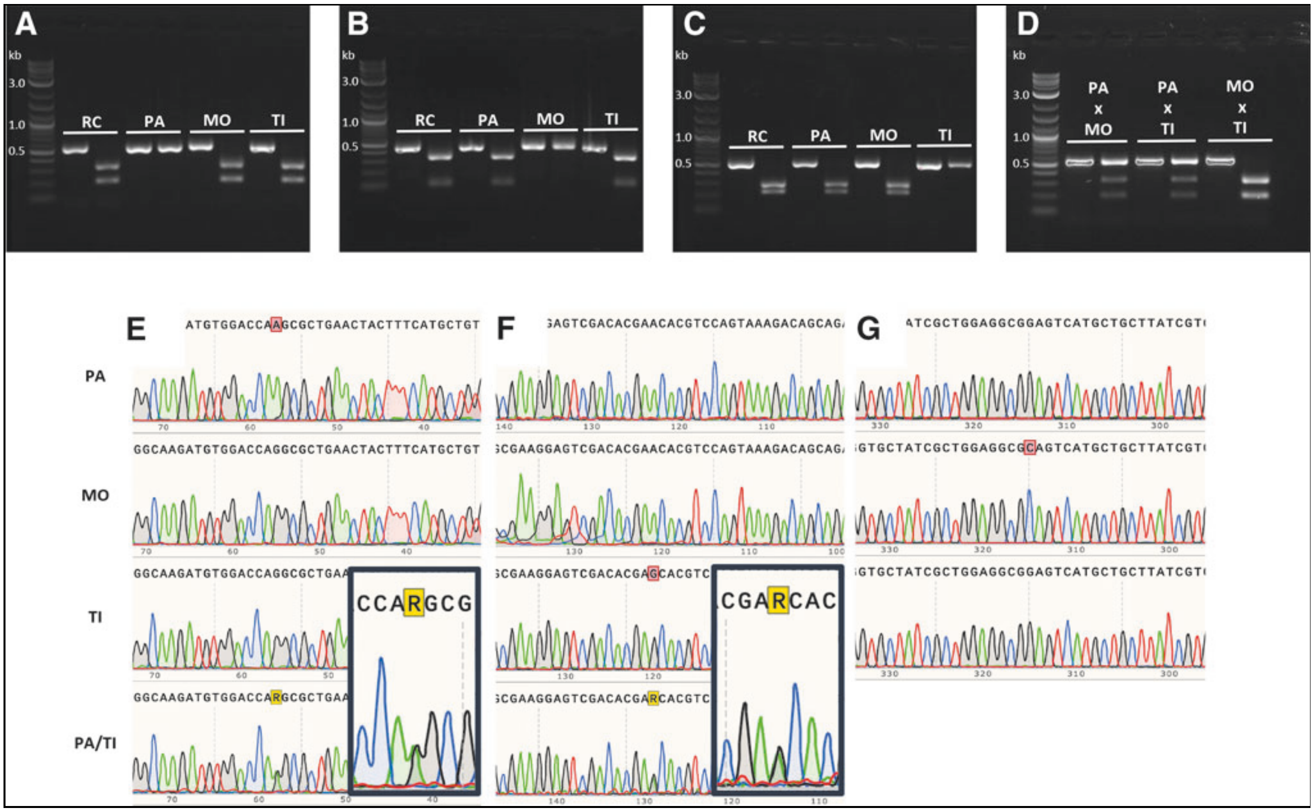

FIG. 1. Population-specific markers. **(A–C)** Amplicons undigested (left) and digested with *Msp*I (right) amplified from DNA from fin clips from surface (RC), Pachón (PA), Molino (MO), and Tinaja (TI) fish for population-specific population marker primer pairs **(A)** PA1, **(B)** MO1, and **(C)** TI1. **(D)** Amplicons undigested (left) and digested with *Msp*I (right) amplified from DNA from fin clips from Pachón x Molino, Pachón x Tinaja, and Molino x Tinaja hybrid fish using primer pair PA1. Note the presence of the *Msp*I restriction site, as indicated by the digest of the band, in all populations except for one cave population for each primer pair. **(E, F)** Sequence traces of regions amplified and Sanger sequenced from the genes *klhl15* **(E)** and *dyrk4* **(F)**, which distinguish Tinaja from Pachón fish. Insets show heterozygous peak in a lineage with a suspected history of hybridization between the two cave types. **(G)**. Sequence trace of *epha2a*, which distinguishes Molino fish from other morphotypes. MO, Molino; PA, Pachon; PA/TI, lineage with suspected hybridization between Pachon and Tinaja; TI, Tinaja.

Supplementary Fig. S1C–E). These results were consistent between adult fish and pooled larval fish. Thus, these fixed population-specific markers from wild-caught populations can be used to distinguish between different populations in the laboratory.

To determine whether two alleles are identifiable within one genomic sample, we extracted DNA from fin clips from cave-cave hybrid fish (e.g., Pachón x Tinaja). The hybrid fish were genotyped at population-specific loci. We found that restriction digest of PCR products amplified from parental population-specific sites in cave-cave hybrid fish produced three bands: a band corresponding to the uncut PCR product size and two bands at the cut PCR product sizes (Fig. 1D). Thus, hybrid fish can be distinguished from parental populations using this method. However, detection of an in-laboratory hybridization event that occurred multiple generations prior may need several markers to be identified due to recombination and independent assortment.

We also developed additional population-specific markers that are identifiable through Sanger sequencing. We first identified fixed SNPs between populations in sequence data from wild-caught individuals[6] and designed primers to amplify these loci (Supplementary Tables S3 and S4). We appended the commonly used M13-Reverse primer sequence to each reverse primer for seven sites that differed between cave populations. Amplicons sent for Sanger sequencing reliably identified laboratory fish from all three cave populations (Fig. 1E–G and Supplementary Fig. S2).

Notably, this method identified fish from a contaminated laboratory stock that had a suspected history of in-laboratory hybridization several generations prior. Although fish from the contaminated stock were not heterozygous for every marker, by testing five loci we were consistently able to identify individuals that were from a mixed lineage. Importantly, heterozygosity was only detected in the suspected contaminated lineage, suggesting that in-laboratory cave strains can be reliably differentiated using these markers (Fig. 1E–G and Supplementary Fig. S2).

In sum, we identified loci with fixed variants between populations that either result in the disruption of an *Msp*I restriction enzyme site in one cavefish population, or that contain SNPs fixed in specific cavefish populations that can be identified through Sanger sequencing. Of note, although these markers were identified in wild populations and distinguished between the populations in our laboratories, we cannot rule out that low frequency alleles are present in wild populations, which could in principle be in other or newly derived laboratory populations of cavefish.

Thus, these markers should be validated by individual laboratories before wide spread use for screening. By demonstrating that these sites are population specific in laboratory populations and outlining rapid and easy methods for genotyping fish at these loci, we describe a powerful tool to monitor cavefish populations in the laboratory. These resources will aid in maintaining laboratory stocks by quickly identifying contamination and increase the robustness of conclusions regarding repeated evolution from laboratory experiments.

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Authors' Contributions

Conceptualization of this study was carried out by A.E.M., H.G., S.M., and J.E.K.; methodology was taken care by A.E.M., H.G., R.M., R.R.-M., A.K.P., and S.M.; investigation was done by A.E.M. and H.G.; writing—original draft—was done by A.E.M., H.G., S.M., and J.E.K.; writing—reviewing and editing—was by A.E.M., H.G., R.M., R.R.-M., A.K.P., S.M., and J.E.K.; visualization was carried out by A.E.M. and H.G.; supervision was taken care by S.M. and J.E.K.; funding acquisition was taken care by H.G., S.M., and J.E.K.; and project administration was by J.E.K.

Authors Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Data

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