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Divergent evolutionary pathways for aggression and territoriality in *Astyanax* cavefish

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Abstract

The surface morph of the Mexican tetra fish (*Astyanax mexicanus*) exhibits strong territoriality behavior and high levels of aggression. In contrast, the eyeless cave-adapted morph from Sierra de El Abra, México, rarely are aggressive and have totally lost the territorial behavior. These behaviors are part of what has been called the cavefish behavioral syndrome. Here, we report that several *Astyanax* cave populations of Sierra de Guatemala, unlike those reported for the Sierra de El Abra cave populations, display significant territoriality and aggression when confined into a reduced space. We discuss divergent evolutionary trajectories in terms of agonistic behavior for cavefish populations inhabiting different mountain ranges.

Keywords

Abra, behavior, stygobyte, troglobite

* These authors contributed equally to this work.

Introduction

Organisms in which variation in behavior can be linked to the environment and their evolutionary history are key to understanding how behavior evolve. The Mexican tetra *Astyanax mexicanus* has both an eyed surface morph and a blind cave-adapted morph. The cave-dwelling *Astyanax* are characterized by conspicuous morphological traits that have evolved in response to their subterranean environment. Among these traits, the most obvious are degenerate eyes and reduced pigmentation (Jeffery 2001; Krishnan et al. 2017). Behavioral differences are also found between the morphs, such as a reduction of intraspecific aggression in some cave-adapted *Astyanax* (Burchards et al. 1985; Parzefall 1985).

Astyanax genus has emerged as a powerful model system for genotype-phenotype analysis because surface fish and cavefish morphs are interfertile and high-quality genome information is available (Gross 2012; Warren et al. 2021). Furthermore, the molecular basis of repeated trait evolution can be examined in this species, as multiple cave populations have independently evolved the same traits (Jeffery 2001; Jeffery 2009). Thirty-four caves are currently known to be inhabited by *Astyanax* (Espinasa et al. 2020). They are found in three mountain ranges in Northern México (Sierra de Guatemala, Sierra de El Abra, and Micos), and one mountain range in Southern México (Guerrero; Granadas and La Joya). Multiple authors (e.g. Bradic et al. 2013; Gross 2012; Herman et al. 2018) have suggested that each mountain range has independent evolutionary histories, although colonization independence has been disputed for the Sierra de Guatemala and Sierra de El Abra by Espinasa et al. (2014). All cave localities (Molino, Caballo Moro, Jineo, Vásquez, and Escondido) involved in this study come from Sierra de Guatemala, with the exception of the Pachón and Tinaja cave populations, which are from Sierra de El Abra (Fig. 1).

Burchards et al. (1985) and Parzefall (1985) showed that epigeal specimens are intensely aggressive and develop territoriality in the laboratory. On the contrary, in the Sierra de El Abra cave populations, aggressive behavior is highly reduced, biting seldom occurs, and fish have lost territorial behavior (Langecker et al. 1995). Even the few attacks performed by cavefish during the behavioral tests have been linked to food-seeking behavior (Elipot et al. 2013). In the natural hybrid populations of Micos and Guerrero, aggression levels are also reduced (Espinasa et al. 2001). The virtual absence of aggressive behavior in these cavefishes has a genetic basis. F1 and F2 hybrids resulting from crosses between epigeal and Sierra de El Abra cave specimens exhibit a very high to intermediate level of aggression, respectively (Parzefall and Hausberg 2001; Elipot et al. 2013). In backcrosses with the cave morph, the aggressive behavior disappears (Wilkins 1988). Of note, F1 hybrids from crosses between Molino cavefish (Sierra de Guatemala) with Surface fish are exceptionally aggressive (Elipot et al. 2013).

Breder (1943) first described the aggressive behavior characteristic of surface Mexican tetra fish as “erratic viciousness”. Among the observable agonistic behavioral patterns are “fin spreading”, “snake-swimming”, and “tail striking”, with the most prominent being “ramming” and “biting” (Wilkins 1988). Subordinate individuals with no opportunity to hide or escape may eventually be killed. The intensity of the aggression depends

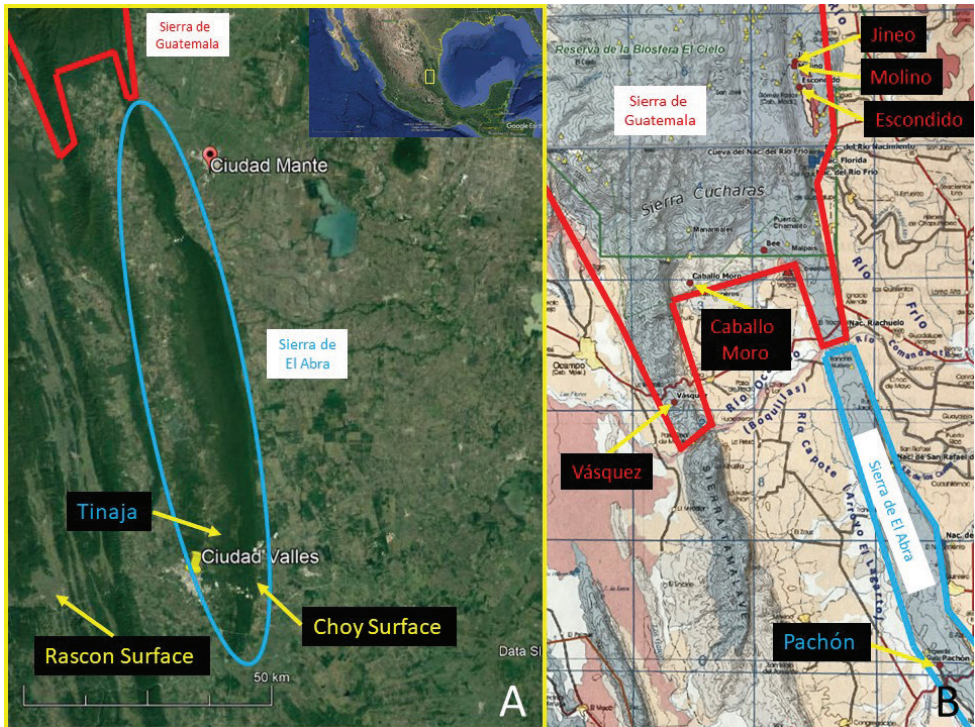


Figure 1. Localities of cave populations **A** satellite photo of Sierra de Guatemala (Red) and Sierra de El Abra (Blue). Inset shows the area of México. Map data 2021 (C) Google **B** topographic map modified from Elliot (2018). Sierra de Guatemala caves are shown in red while Sierra de El Abra caves in blue

on many factors, particularly an individual's size (John 1964), the amount of space available (John 1964), and the overall state of nutrition (Wilkins 1988; Elipot et al. 2013). Vision is not essential to trigger or release aggressive behavior (Espinasa et al. 2005). The agonistic behavior establishes a hierarchical order among the fish (Elipot et al. 2013).

Aggressive interactions and territoriality are correlated, with fish defending an individual space. *Astyanax* surface fish show territoriality that is dependent upon the aquarium space available. In small tanks less than 250 L, surface fish stop schooling and start defending territories (Burchards et al. 1985; Parzefall 2000). Hierarchy and territoriality are linked, with the dominant and most aggressive fish defending the largest area of the tank (Burchards et al. 1985). However, no territoriality behavior has been reported in the cave populations (Parzefall 1983).

The intense dominance-related aggressiveness in surface *Astyanax* is inversely correlated with the serotonin (5HT) levels in the hindbrain raphe nucleus (Elipot et al. 2013; Rétaux and Elipot 2013). A mutation has been identified in the monoamine oxidase (MAO) coding sequence of several cave populations from Sierra de El Abra (Elipot et al. 2014). The P106L MAO mutation would appear as an ideal candidate to serve as the genetic basis of reduced aggression. However, experiments using surface

fish and Pachón cavefish lines with or without the P106L *MAO* mutation have demonstrated that even though *MAO* P106L does increase 5-HT levels in fish, it does not affect aggression and cannot be considered as the genetic basis for the loss of aggressive behavior in cavefish (Pierre et al. 2020). The P106L mutation is present in Sierra de El Abra cavefish but absent in Sierra de Guatemala (Molino, Caballo Moro, Jineo, and Escondido cavefish) as well as in surface fish (Pierre et al. 2020).

Since populations in three mountain ranges (El Abra, Micos, and Guerrero) have independently converged in a reduction of aggression and territoriality, it has been suggested that the loss may be an adaptive change for cave *Astyanax* (Hinaux et al. 2015). The purpose of this study is to resolve whether troglomorphic *Astyanax* can retain aggression and territoriality by studying the Sierra de Guatemala cave populations.

Methods

Cavefish and surface fish localities used in this study can be seen in figure 1. Quantitative data was gathered from fish within four days after being collected from the field in 2016 (Pachón cave collected on August 6th, Caballo Moro cave on May 18th; and Rascón surface fish on August 5th). Data was also gathered from Molino, Vásquez, Caballo Moro, Jineo, and Escondido cavefish, kept in P. Ornelas' laboratory at IBUNAM, México, which had been collected on February 2016, four months prior to the study. Choy surface river and Tinaja cavefish originally bred in Borowsky's laboratory and now kept in Espinasa's laboratory were also analyzed. Fish kept in the laboratory have been under standard husbandry with a water filtration and aeration system. Fish have been fed about 3% of the fish's bodyweight daily. Fish used in experiments were not fed on the 24 hrs before the experiments. Since the Caballo Moro cave holds a mixed population of eyed and eyeless fish, only completely eyeless Caballo Moro specimens were used (Fig. 2). All other cave populations studied only have eyeless fish. Collecting permit # SGPA/DGVS/02438/16 from Secretaría del Medio Ambiente y Recursos Naturales, México, was issued to Patricia Ornelas García. To unify criteria of what constitutes an attack, one of us (LE) went to Rétaux (SR) laboratory to personally observe the pattern of behavior of the specimens described by Elipot et al. (2013) and kept at the French research facility. Afterwards both LE and SR observed the fish kept at Ornelas' Laboratory in México. All counts were performed by at least two observers at the same time to minimize bias. Counts were always very similar and were averaged to present the data.

Aggression

Ramming and biting attempts were counted as attacks following Espinasa et al. (2005). Fish were in a 5-gallon fish tank under two 100-watt light bulbs placed 2 m above the center of the tank for illumination, as described in Espinasa et al. (2001). Fish had an

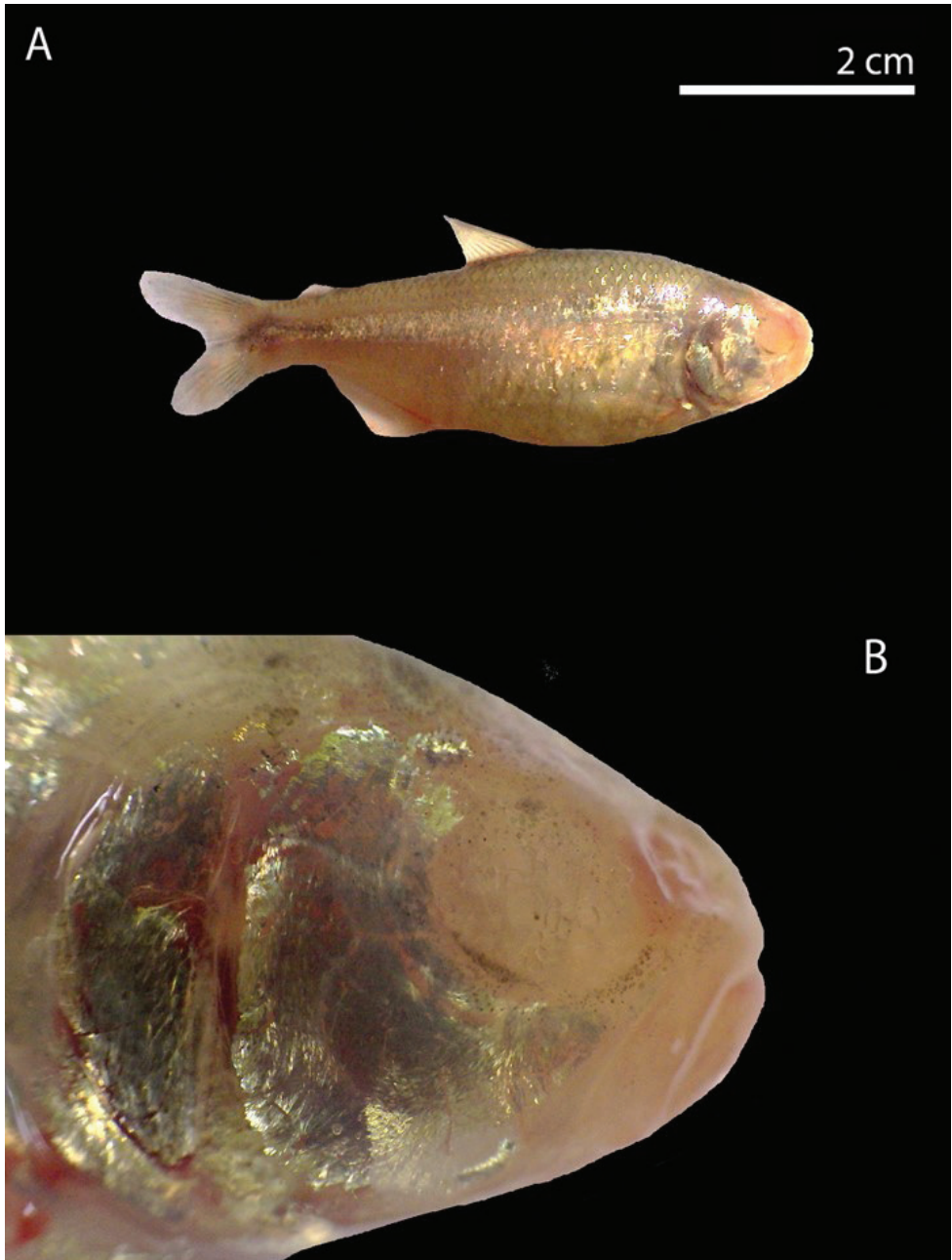


Figure 2. Eyeless Caballo Moro specimen used for aggression and territoriality studies **A** body **B** head. While the Caballo Moro cave is inhabited by both the eyed and the eyeless morphs, only eyeless specimens were used in this study. The other populations used in this study; Molino, Jineo, Vásquez, Escondido, Pachón and Tinaja caves are inhabited exclusively by eyeless fish.

average size of 52.1 mm (max = 62.5 mm, min 43.6 mm). Two assays were used to assess aggression:

1. Four specimens were deposited into a fish tank and left to acclimate for at least five minutes or until two ramming or biting attempts (attacks) were initiated. Afterwards, the number of attacks were counted over a five min period. Replicates with different combinations of individual fish were performed for Molino (n = 18 tests), Caballo Moro (n = 17), Vásquez (n = 9), Jineo (n = 1), Escondido (n = 7), Pachón (n = 6) and Tinaja (n = 6) caves and for Choy (n = 10) and Rascón (n = 6) surface fish. A non-parametric Mann-Whitney U test was used to determine statistical differences among populations.

2. The same procedure was done, but with only two individuals per tank and for the following number of replicates: Molino (n = 9 tests), Caballo Moro (n = 2), Escondido (n = 7), Pachón (n = 8) and Tinaja (n = 6) caves as well as Choy (n = 10) and Rascón (n = 5) surface fish. A non-parametric Mann-Whitney U test was used to determine statistical differences among populations.

Territoriality

To study territoriality, four fish were left in a tank to acclimate overnight. The next morning, they were filmed with a DCRSR42 Sony Digital camera from above for three minutes. Since fish tend to scatter beyond their territories for a few moments after an attack, disrupting territorial patterns, the one-minute section of the film with fewer attacks was selected and examined in the laboratory to track the paths of each individual. For quantification, in the video the tank was divided into four quadrants. The track generated by the fish was analyzed and the number of quadrants not occupied throughout one minute by the individual fish were counted. The assumption was that territorial fish would stay in their territory and some quadrants would not be included in their paths, while non-territorial fish would swim throughout the tank. Samples analyzed were from Sierra de El Abra (Pachón n = 8 tests) and Sierra de Guatemala (Caballo Morro n = 8 and Molino n = 4). A Mann-Whitney U test was done to establish if the populations differed in how many quadrants were not occupied. Fish from other caves (Vásquez, Jineo, Escondido and Tinaja) were also observed qualitatively for signs of territoriality while in their host tanks at Ornelas' laboratory, where they had been acclimatized for months.

Since territoriality in *Astyanax* cavefish has not been previously reported, most authors have never witnessed this behavior in action. For illustration purposes and to facilitate uniformity of criteria in future studies, a video was recorded of cavefish being aggressive and how they establish territories using the intruder essay. Two Caballo Moro specimens were placed into one tank and five into another tank to acclimate overnight. The next morning, they were filmed as described above. Afterwards, an “intruder” fish from the tank with five fish was transferred to the tank with the two fish and left for 20 min to acclimatize, after which, another three minutes were filmed. Again, the one-minute section of the film with fewer attacks was selected to track and mark the preferred territories of each fish. Afterwards, another intruder was added to obtain the swimming paths of four fish.

Results

Aggression

In the four fish assay (Fig. 3A), Sierra de El Abra (Pachón \bar{x} = 0.8 attacks \pm 0.9 SD n = 6 tests with different combinations of fish; Tinaja \bar{x} = 0.5 attacks \pm 0.5 SD n = 6) and Escondido (\bar{x} = 0.5 attacks \pm 0.7 SD n = 7) caves showed significantly lower levels of aggression (P = 0.005–0.0007 Mann-Whitney U test) than the surface populations of Choy (\bar{x} = 13.5 attacks \pm 11.8 SD n = 10) and Rascón (\bar{x} = 52.1 \pm 34.8, n = 6), in agreement with previous studies.

On the contrary, Sierra de Guatemala cavefish populations (Molino cave fish \bar{x} = 16.0 \pm 11.0 SD, n = 18; Caballo Moro \bar{x} = 25.7 \pm 21.0, n = 17; Vásquez \bar{x} = 9.7 \pm 7.3, n = 9; and Jineo \bar{x} = 30, n = 1) had aggression levels not significantly different from surface Choy population (P = 0.68–0.15), and even Caballo Moro cave population was not significantly different (P = 0.06) from the highly aggressive Rascón surface population. All these Sierra de Guatemala cavefish populations were significantly more aggressive (P = 0.002–0.001) than the Sierra de El Abra and Escondido cavefish.

Similar results were obtained in the two fish assay (Fig. 3B). Pachón (\bar{x} = 1.3 \pm 0.9, n = 8), Tinaja (\bar{x} = 0.5 \pm 0.5, n = 6), and Escondido (\bar{x} = 0.7 \pm 0.7, n = 7) cavefish displayed significantly fewer attacks (P = 0.008–0.002), than Molino cavefish (\bar{x} = 9.3 \pm 7.5, n = 9), Caballo Moro cavefish (\bar{x} = 7.5 \pm 0.7, n = 2), Choy surface fish (\bar{x} = 26.4 \pm 21.4, n = 10), and Rascón surface fish (\bar{x} = 83.2 \pm 123.6, n = 5; P = 0). Molino cavefish were not significantly different (P = 0.08) from Choy surface fish. Aggression levels in our four- and two- fish assays over 5 minutes for Molino, Caballo Moro and Vásquez caves are within range of the number of attacks recorded by Espinasa et al. (2005) for three fish over 10 minutes for Molino cavefish (\bar{x} = 35 \pm 19, n = 6; i.e. about 17.7 in 5 min) and for a different surface fish locality, Río Boquillas (\bar{x} = 41 \pm 25, n = 6; i.e. about 20.5 in 5 min). Both Río Boquillas and Río Choy are surface streams neighboring the Sierra de El Abra and less than 10 km from a cave locality, while Rascón population is over 100 km away (Fig. 1). The Rascón population is isolated from the Choy and Boquillas rivers by the 100 m Tamul waterfall and belongs to a different *Astyanax* lineage, as evidenced by its mitochondrial DNA (Ornelas-García et al. 2008). In the four fish assay, the Rascón surface fish were significantly more aggressive (P = 0.01) than the Choy surface fish.

Examples of aggressive interactions of Sierra de Guatemala cavefish can be seen in <https://www.youtube.com/watch?v=8IW7hgZbWI>.

Territoriality

Sierra de El Abra cavefish (i.e. Pachón and Tinaja) show no tendency to establish territories. Pachón individuals swam throughout the tank, preferentially following the edges, crossing paths with other individuals constantly (Fig. 4A). All Pachón individuals shared and used the entirety of the tank. On the contrary, Caballo Moro (Fig. 4B), and Molino

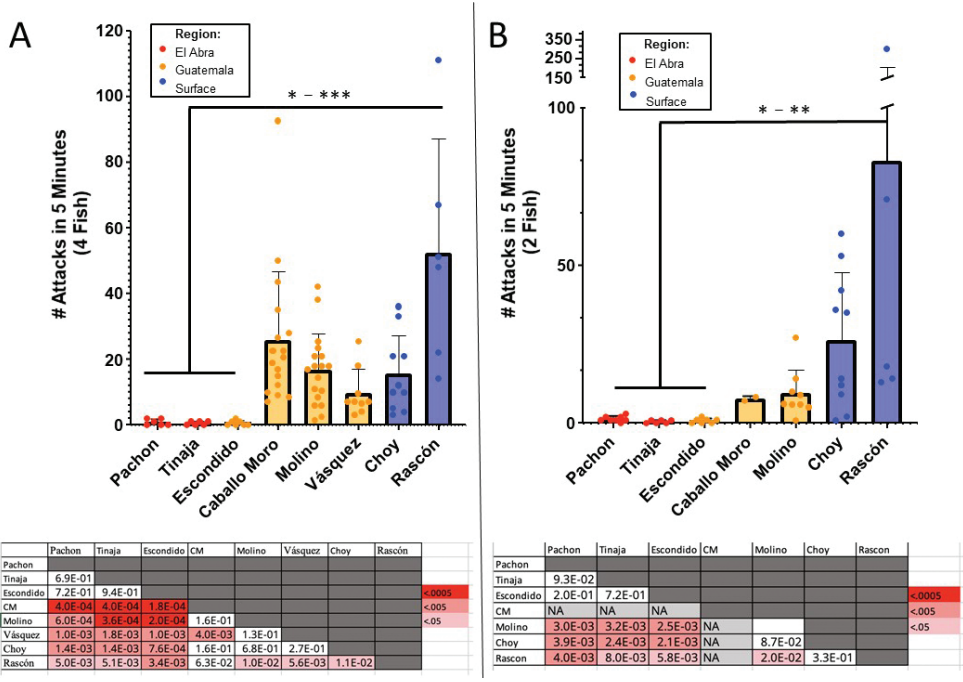


Figure 3. Number (with SD) of ramming and biting attempts in the four fish **A** and two fish **B** assays, for Sierra de El Abra (Pachón and Tinaja), Sierra de Guatemala (Escondido, Caballo Moro, Molino, and Vásquez) cave fish and surface fish (Choy and Rascón). With the exception of Escondido cave, Sierra de Guatemala cave fish were significantly more aggressive than Sierra de El Abra cave fish. In several comparison, Sierra de Guatemala cave populations were as aggressive and not significantly different than some of the surface fish. Tables below graphs show significance levels. Notice that Pachón, Tinaja and Escondido are significantly less aggressive than all of the rest.

fish developed territories where individuals kept to their own areas, with edges of the territories being the places of conflict where aggressive behavior and attacks occurred.

Pachón cavefish had fewer quadrants not traversed in their paths (0.0 +/- 0.0 SD, n = 8 individual fish) in one minute than Caballo Moro (2.5 +/- 0.7, n = 8; P=.00094 Mann-Whitney U test) and Molino (1.25 +/- 1.5, n = 4; sample size too small to test with U test). In all tests, Pachón cavefish shared all sectors of the tank and all individual's paths crossed all four quadrants of the tank. The average time spent in the single most used quadrant for Pachón cavefish was 47.9% (\bar{x} = 28.7 +/- 8.0 seconds out of 60), while Caballo Moro cavefish spent 90.8% (\bar{x} = 54.5 +/- 5.0 seconds out of 60) and Molino 75.4% (\bar{x} = 45.2 +/- 13.8 seconds out of 60) of the time in a single quadrant or corner of the tank and all fish failed to enter between one to three of the other quadrants. This suggests that Sierra de Guatemala cavefish remained within a constrained area or territory, unlike Pachón fish. Fish from other Sierra de Guatemala caves (Vásquez and Jineo) appeared to establish territories at a slower pace than Caballo Moro and Molino fish. Therefore, only a qualitative observation was done in their host

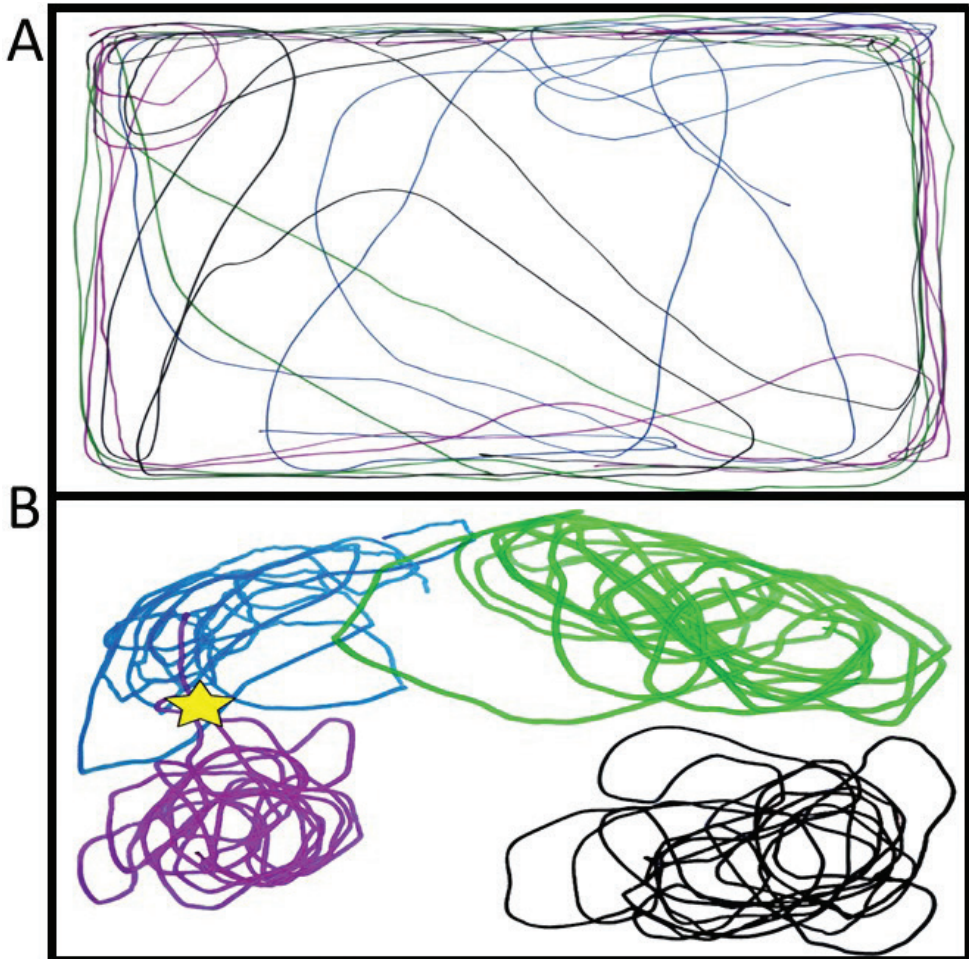


Figure 4. Territoriality from a top view of a tank. Black straight lines represent edges of the tanks (size 20cm X 40cm). Colored lines follow the swimming paths of four individuals over a 1 min period. A Thin lines follow four Pachón cavefish. B Thick lines follow four eyeless Caballo Moro cavefish. Yellow star signals an attack. Specimens used for both cavefish populations are eyeless. Notice that while all Pachón fish swim throughout the entirety of the tank and the paths of individual fish converge over the paths of other individuals, those of Caballo Moro are mostly restricted to a corner, and each individual holds a territory seldom crossed by another individual. However, when they do cross, it can result in an attack.

tanks at Ornelas' laboratory where they had been acclimatized for months for signs of territoriality. In those conditions, cavefish were also seen swimming in paths clearly restricted to only certain areas of the tanks. Qualitative observations of Pachón, Tinaja and Escondido cavefish in their host tanks where they have also been kept for months failed to show equivalent swimming paths restricted to territories.

Examples of territoriality of Sierra de Guatemala cavefish can be seen in <https://www.youtube.com/watch?v=8IW7hgZbWI>.

Discussion

While most authors agree that aggression in *Astyanax* cavefish populations from Sierra de El Abra, Micos, (reviewed in Hinaux et al. 2015) and Guerrero (Espinasa et al. 2001) is highly reduced when compared to surface fish, there have been conflicting accounts for the populations in Sierra de Guatemala. Elipot et al. (2013) reported that both Molino and Pachón fish showed ten times less aggression than surface fish during an hour-long resident-intruder assay. In contrast, an earlier study (Espinasa et al. 2005) reported that Molino cavefish had the “erratic viciousness” equal to surface fish and were clearly more aggressive than the Pachón cavefish.

In the present study, cavefish were directly observed by authors of both the Elipot et al. (2013) and Espinasa et al. (2005) studies to standardize criteria. Five populations were examined (Molino, Caballo Moro, Jineo, Vásquez, and Escondido). Assays used were for two to four individuals. Fish were observed with or without acclimation (like in resident-intruder assays), and with individuals that had just been collected from the field or that had been kept in the laboratory for extended times. In all these tests, 4 out of 5 Sierra de Guatemala cave populations (except Escondido cavefish) have displayed levels of aggression significantly higher than Sierra de El Abra cavefish. The Molino fish have similar histological characteristics of eye reduction to El Abra cavefish (Wilkins 1988). So, their higher levels of aggression are not due to having significantly better vision.

Espinasa et al. (2005) reported that in a “three fish assay”, Molino cavefish had aggression levels over a 10 min period equivalent to surface fish from Río Boquillas. Similar results were obtained by us when comparing against the surface population of Choy. Yet, in our study, surface fish from Rascón were 2 to 5 times more aggressive than both the surface Choy population and the aggressive Sierra de Guatemala cave populations. Two interpretations seem plausible. The first is an ongoing process to loss the aggression, in which most Guatemala fish display “intermediate” aggressiveness between surface Rascón and cave Sierra de El Abra fish. This corresponds to a plastic behavior whose environmental conditions, phylogenetic inertia, or genetic architecture have not promoted the genetic fixation in comparison with El Abra system, while in Escondido the lack of aggressiveness has been favored.

But there is a second alternative that focuses on the variability in aggression levels among surface populations. Sierra de Guatemala cavefish are as aggressive as surface fish from Río Boquillas and Choy, but less aggressive than those from Rascón. Qualitative and quantitative observations in the fish kept at the UNAM laboratory suggest that Rascón fish are more aggressive than other surface populations from streams closer to Sierra de El Abra. While few live specimens are available, tanks with Rascón fish typically need to have reduced number of individuals so as to prevent them from killing each other. Rascón population is isolated from the rest of the surface populations by the 100 m high Tamul waterfall and its known to be genetically distinct by harboring a different mitochondrial haplotype from other surface populations (Ornelas-García et al. 2008) as well as with nuclear data (Herman et al. 2018). So, it could be that Rascón is a particularly aggressive population of surface *Astyanax* and the Sierra de Guatemala cavefish are simply as aggressive as their neighboring surface populations of Choy and Boquillas.

Our study also confirmed that the absence of aggression in the three Molino individuals kept in France used in the Elipot et al. (2013) study was “real” and not an artifact of subjective evaluation or different data collection techniques. We propose that apart from variability among populations, there is variability among individuals within a population regarding their aggression levels. For stochastic reasons, the handful of specimens that were shipped to France displayed little aggression and represented a divergent subset of the natural population with regards to average aggression. In fact, while the norm is that levels of aggression in Sierra de Guatemala cavefish are higher than in Sierra de El Abra, the absence of aggression displayed in the Escondido cavefish housed in Ornelas’ laboratory in México may be the result of this same natural variability. Escondido cave is part of the same hydrologic system as Molino and Jineo Cave (Espinasa and Espinasa 2015). It remains to be seen if for stochastic reasons the pool from which Escondido cavefish were collected happened to have a high number of non-aggressive fish, or if it is the norm throughout that particular cave.

It has been suggested that agonistic behaviors in surface *Astyanax* rely on visual cues and that aggressiveness is no longer performed in darkness at all (Burchards et al. 1985; Parzefall and Hausberg 2001; Langecker et al. 1995). In contradiction, Elipot et al. (2013) reported that surface fish remain aggressive in the dark. These differing findings come about by the fact that Elipot et al. (2013) observed the fish in darkness by using infrared lamps. This method does not consider that many fish (including characids) are able to perceive near-infrared (Meuthen et al. 2012). It is interesting to note that surface fish that have been blinded by surgical lens removal at larval stages are aggressive (Espinasa et al. 2005).

Our analyses of the swimming paths indicate that unlike Sierra de El Abra cavefish, most Sierra de Guatemala cavefish develop territories. Individuals appear to try to minimize contact with each other and the available space is subdivided. The borders of these territories are where most attacks are concentrated. Again, it is worth noting that the cave specimens in these assays were completely eyeless individuals and thus they could not use visual cues to generate a spatial mapping of the tank and their territories. Nonetheless, individuals adjusted their swimming paths to form new and stable territories without the help of visual cues.

Swimming paths of most Sierra de Guatemala cavefish kept inside the laboratory are drastically different from Sierra de El Abra cavefish. Pachón and Tinaja fish typically swim near the edges of the tank, following the entirety of the side and turning at the corners, thus going in circles around the whole tank. Aggressive Sierra de Guatemala fish that hold territories have swimming paths that do not follow the entirety of the side of the tank. Instead, they swim a few strokes and turn around before encountering any physical obstruction. Non-visual spatial mapping must allow them to turn at the frontier of their territories, even when the neighboring individual is distant. At high densities, swimming paths can even be restricted to reduced circling around in areas only slightly larger than the fish itself. Teyke (1985) has shown that active flow sensing, using locomotion and the lateral line are utilized during cavefish navigation. Blind cavefish use a higher frequency of swim cycles than eyed surface fish, consisting of burst and glide in straight trajectories. Santacruz et al. (2015) have suggested that three-dimensional space is learned and remembered differently in *Astyanax* surface fish and cavefish. It is likely

that the modifications to the sensory, processing, and motor neural system of the Sierra de Guatemala cavefish must have occurred such that they are equipped for navigation in perpetual darkness to generate territoriality without the need of visual triggering stimuli.

Based on studies of the Pachón and Micos cave, the surface fish, and F1-hybrids between surface and Pachón fish, Langecker et al. (1995) revealed two releasers for aggressive behavior, ‘natural shape’ and ‘locomotion’. Both would differentially evolve in *A. mexicanus* during the transition from a surface to a subterranean life style. The releaser ‘natural shape’, on the one hand, cannot be manifested in blind cave specimens and became a subject to regressive evolution because of the lack of stabilizing selection. The releaser ‘locomotion’, on the other hand, may be progressively selected because it can still be stimulated by the lateral line system. It is likely to play a role in the new aggressive behavioral patterns of cave fishes (Langecker et al. 1995).

Elipot et al. (2013 and 2014) proposed that the loss of aggressiveness in the Sierra de El Abra cavefish is a “side effect” and not something to be directly selected by living in caves. Raphe serotonergic neurons control aggressive/dominance behavior while at the same time, hypothalamic serotonergic paraventricular neurons regulate food-seeking behavior. They suggested that evolution of the Sierra de El Abra cavefish, in seeking to enhance feeding, lost aggression as a pleiotropic side effect due to modifications of their shared serotonergic neuronal network. We propose that the Sierra de Guatemala cavefish may have achieved the regulation of adaptive feeding behaviors or the control of serotonergic networks through different evolutionary, genetic, and physiologic pathways (e.g., mc4R mutation in Molino cavefish; Aspiras et al. 2015). Thus, the pleiotropic and side effects are different such that aggression and territoriality were maintained.

Espinasa et al. (2021) have found that variability of food resources among different pools within a single cave can be higher than between different caves. Thus, we can assume that it is unlikely that caves in Sierra de Guatemala are environmentally significantly different from those in Sierra de El Abra with regards to selective conditions for loss of aggression and territoriality. We suggest that the behavioral differences are rather due to their independent evolutionary histories, where adaptation occurred through modification(s) of different genes with different pleiotropic effects. Future studies may resolve if aggression and territoriality, or their absence, evolves differently among karstic zones.

Conclusion

Several *Astyanax* cave populations of Sierra de Guatemala, unlike those reported for the Sierra de El Abra cave populations, display significant territoriality and aggression when confined into a reduced space. The Sierra de Guatemala cavefish may have achieved the regulation of adaptive feeding behaviors or the control of serotonergic networks through different evolutionary, genetic, and physiologic pathways than at Sierra de El Abra populations. Thus, the pleiotropic and side effects may be different such that aggression and territoriality were maintained.

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