Cavefish Evolution Development & Behavior

Astyanax International Meeting

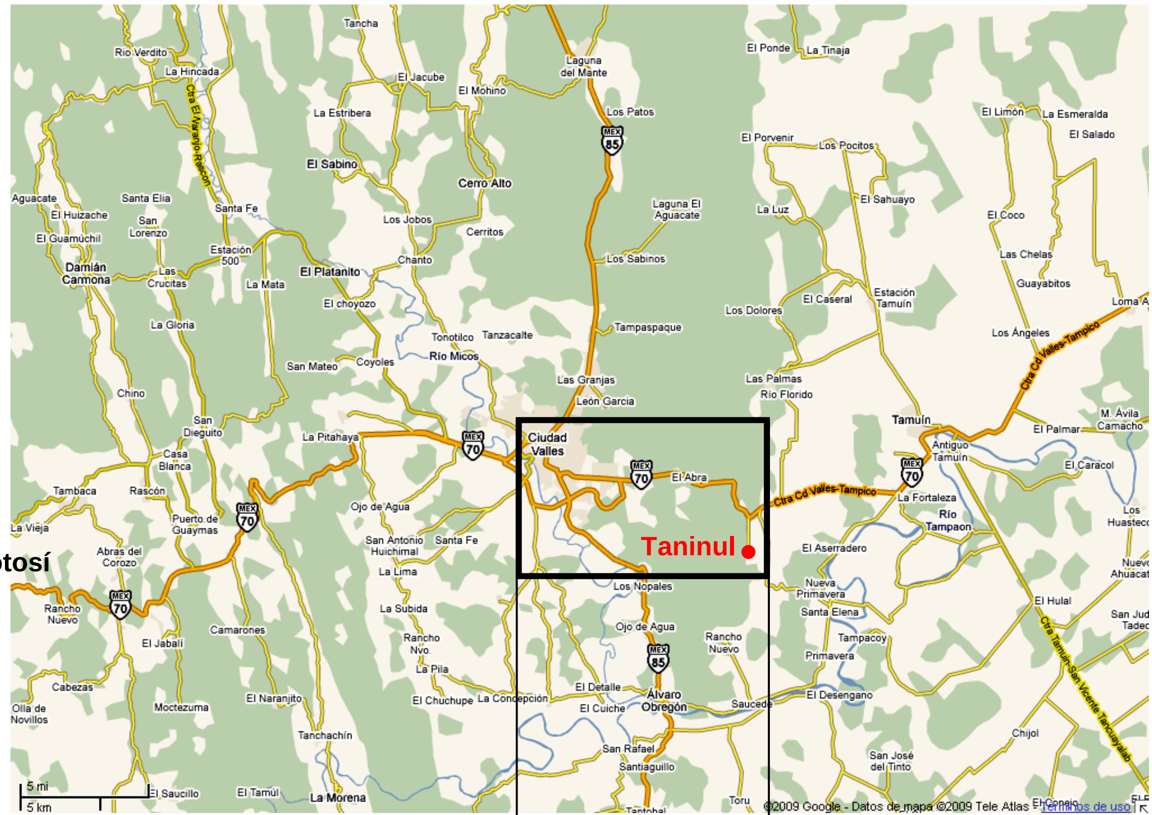
March 13-18, 2011

Ciudad Valles, Mexico

Meeting program, March 13th-18th, 2011

Welcome to AIM 2011 in Mexico

Meeting venue: Hotel Taninul in Ciudad Valles



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Donde Nace el Agua

Hotel Taninul and its Local Environment

Hotel Taninul is a former hacienda with long engaging corridors and sweeping balconies set in a lush oasis at the base of the eastern slope of the Sierra de El Abra, about 15 km east of Cuidad Valles. The oasis lies within a 2.8km² reserve dotted with springs, streams, gardens, meadows, and sugar cane fields. The main feature of the oasis is its springs, the reason for the hotel slogan “donde nace el agua” (where the water is born). There are two springs, a warm sulfurous spring (actually several individual springs) used for bathing, and a fresh water limestone spring, which used to be or might still be the home of an alligator, and definitely not for bathing. The springs were discovered by Europeans in 1808 but of course had already been in use for centuries by the Native Americans of La Huasteca. Soon people traveled here from all over Mexico to bath in the warm sulfurous water, which was believed to have healing properties.

In 1945 the Taninul hacienda was converted into a hotel, which received many famous visitors. The signatures of seven Mexican presidents, a soccer team, and Hollywood stars like Burt Lancaster can be seen in the guest book of the old hotel. In this era, the hotel had a museum of regional antiquities, an amphitheater, a small zoological garden, and a disco made from the mouth of a cavern (see below). Later, the hotel and its surroundings fell into disrepair and became disused. Now the hotel has new management and is being restored, the museum is usually open, although the disco is still closed. The disco cave is now rented out only for private parties (we are trying to get it), and at all other times may be freely visited by hotel guests. The hotel has a spa, a bar (the famous round bar), a restaurant, tennis courts, a gift shop, and bicycles are available. But there are no money distributing machines (the closest are in Cuidad Valles) and internet and telephone service is very limited. The most frequent activity is relaxing, swimming, and bathing in the warm sulfurous spring, especially in the early evening hours, when it has a dreamlike appearance.

Amusements

Hotel Gardens. The hotel and springs are surrounded by lush gardens featuring huge banyan trees. Rest for a while in hammock (provided) under a banyan tree or even sleep there one night for the experience of a tame jungle.

Swimming and Bathing. The warm sulfurous pool offers a tremendous opportunity for swimming, bathing, and general gathering site for our meeting; both during the day and in the evening (the springs are lighted). We are visiting during the dry season, so it is very unlikely to rain, and there will be pleasant bathing for certain. Soak in the slightly sulfurous waters, which are reputed to have healing properties. The sulfurous odor, which sometimes pervades the hotel, is mild and not particularly obnoxious. If you bathe in it, it will stay with you for days, even after showers, and help keep the insects away. You can take a warm water massage by sitting in a chair below the pool’s overflow, something that is sure to put you to sleep after a hard day of discussion with your colleagues.

Give yourself and a colleague a relaxing “mud” bath using the “ooze” from the pool, as many people you will see there are doing (to find out how to do it see the You Tube internet site http://au.youtube.com/watch?v=SXY8XmfouLY). The “ooze” is the greenish stuff usually floating along the edges. It is the sediment from the pool's bottom 10m below, uplifted by the pressure of the springs. The “ooze” is used for beautifying facial and body masks and you will probably see other guests covering themselves with it during your visit to the pool.

If you don’t choose to try out the water, then just sit at one of the tables under the thatched huts surrounding the pool, and let the attendant serve you a Mexican beer, or a “vampiro” (a special drink named after bats that are common in La Huasteca).

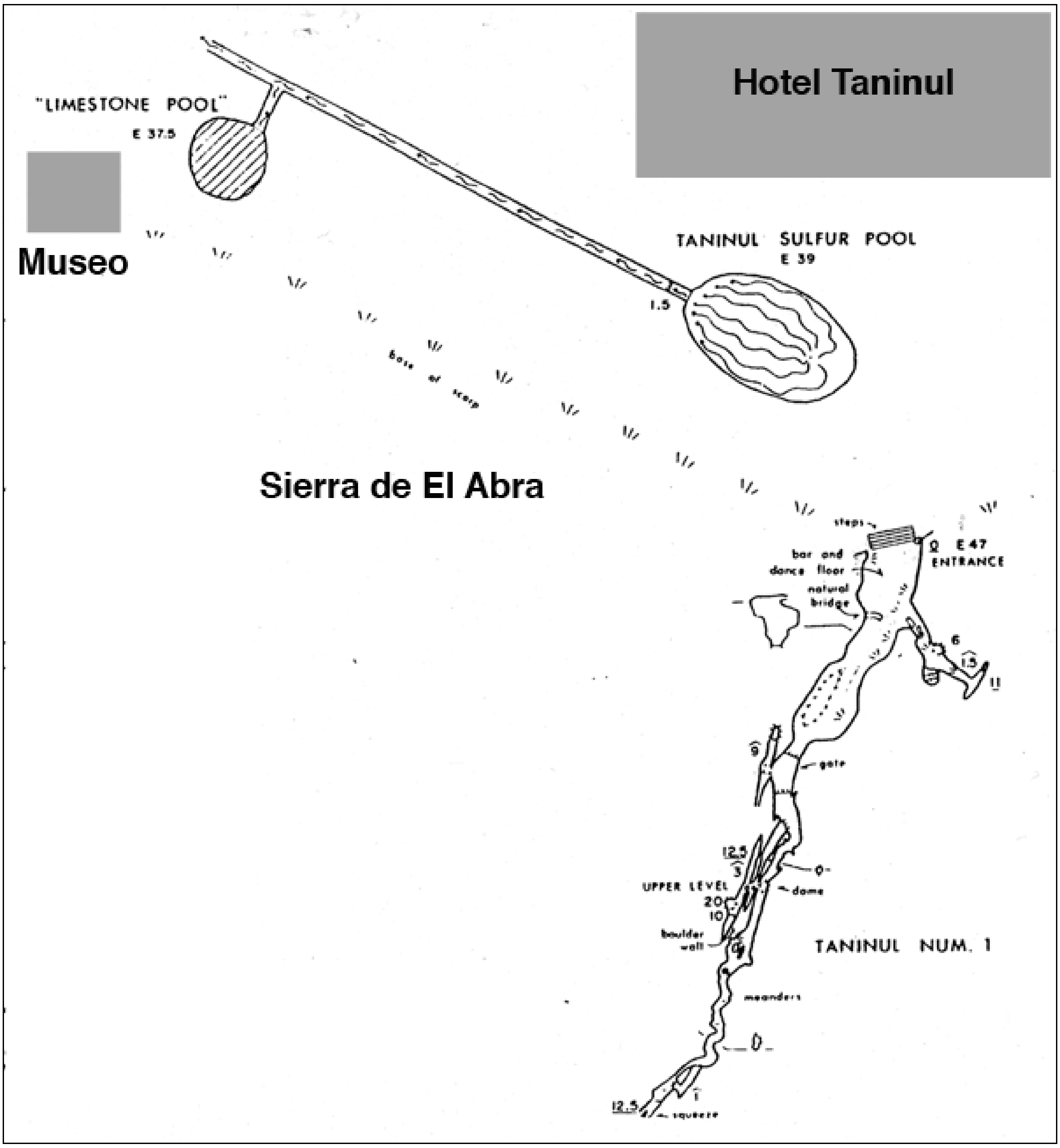
Go Birding. The grounds of the hotel are excellent for birding and wildlife observations. Snuggled up against the base of a mountain ridge that is part of the first range of foothills to the rugged Sierra Madre Oriental, Hotel Taninul offers a good introduction to the tropical birds of La Huasteca. Some of the birding highlights are nesting elegant trogons, which sit for hours in the lush vegetation near the thermal springs, tawny-collared nightjars coursing through the skies at dusk, rufous-capped brush-finches in the thickets on the hillsides, ferruginous pygmy owls, and the plaintive serenade of thicket tinamous in the cooler hours of the day. And of course, parakeets and parrots will be screeching overhead through the palms.

Follow the Stream. The sulfur spring at Hotel Taninul is very active, upwelling a large volume of warm water per hour. It exists into a sulfur stream, which is eventually joined by a tributary emanating from the cold fresh water limestone spring. After the confluence, the stream moves rapidly through the jungle into the meadows, becoming cooler and cooler, and eventually harboring some nice aquatic animals, including large turtles. Pathways border both sides of the stream. This is a nice little hike through the jungle and meadows.

Take a Little Cave Trip. Bring a light and follow the stone staircase at the back of the pool up to the Disco Cave (see above), officially known as La Cueva de las Quilas (The Cave of the Parrots) or (to local cavers) La Cueva de Taninul Numero 1. Here you will find a cavern with a natural bridge, a converted dance floor, and a built-in stone bar. There is a wild cave past the barred gate in the back, but please talk to some of the experienced cavers at the meeting before you try it (and take a colleague with you). If you are thinking that since there is a Numero 1 there must be a Numero 2, you are correct. La Cueva de Taninul Numero 2 is a larger cave than Numero 1 that requires a scramble up the steep rocky side of the sierra to get to its main entrance above the sulfur spring. Please let us know if you want to go there and take one or more of our experienced cavers with you.

Other Activities. There are numerous sights located farther away from Hotel Taninul and requiring a vehicle (and sometimes a hike) to explore. Nacimiento del Río Choy, a large and beautiful resurgence of water collected from the many blind cavefish caves located to the west has a large population of *Astyanax* surface fish. Please speak to one of the meeting organizers for instructions and directions to this location. The ruins of the ancient city of Tamtoc with sculptures, reliefs and an observatory are located south of the village of Tamuín, about 15 km east of Hotel Taninul. The waterfalls of the Río Tampaón and the Río Naranjo are located to the west of Cuidad Valles. Descriptions of the latter places can be found in brochures available in the hotel gift shop. Several tour guide companies offer day tours to these and other locations, and the hotel is a perfect base for climbers, kayakers, cavers, and regular tourists.

Hotel and cave map for exploration at Taninul:



Scientific conference program

Cavefish
Evolution
Development
&
Behavior

Monday 14th March (Chair: Bill Jeffery)

Introduction to the meeting

9h30: Sylvie Rétaux (Gif, France) “**AIM 2011 presentation**”

Plenary lecture

10h: John Avise (Irvine, USA) “**What's So Special About Cave Fish? (A Personal Reflection)**”

11h-11h30: coffee break

Morning session: population biology

11h30: Erik Garcia Machado (La Habana, Cuba) **“Phylogenetic relationships among Cuban and Bahamian species of the blind cave-fishes genus *Lucifuga*: A shared lineage among two isolated archipelagoes**”

12h: Martina Bradic (New York, USA) ”**Population genetic evidence for convergence and parallelism in the Mexican Blind Cavefish (*Astyanax mexicanus*)»**

Lunch at 12h30

Afternoon session1: physiology

14h: Natalya Gallo (Maryland, USA) “**Variance of Space Dependent Growth in *Astyanax mexicanus”***

14h30: Karine Salin (Lyon, France) «**Cave colonization without fasting capacities: An example with the fish *Astyanax fasciatus mexicanus »***

Afternoon session2: evo-devo

15h30: Hélène Hinaux (Gif, France) **“Placode Development in *Astyanax mexicanus* blind Cavefish and sighted Surface Fish”**

16h: Sylvie Rétaux (Gif, France) **«The nature and function of the *Astyanax* casquette»**

Diner at 19h

Tuesday 15th March (Chair: Richard Borowsky)

Morning session: pigmentation

10h: Josh Gross (Cincinnati, USA) "**Towards an understanding of pigmentation reduction in cave forms of *Astyanax mexicanus*"**

11h-11h30: coffee break

11h30: William Jeffery (Maryland, USA) "**Convergent Evolution of Albinism in Cave Animals: A Defect in the First Step of Melanin Biosynthesis in Troglomorphic Fishes and Insects"**

Lunch at 12h30

Afternoon session: behavior

14h: Masato Yoshizawa (Maryland, USA) "**Evolution of a behavior mediated by the lateral line system adapts *Astyanax* to life in darkness"**

14h30: Yannick Elipot (Gif, France) "**Linking evolution of the aggressive behavior and the serotonergic system in *Astyanax mexicanus* surface fish and blind cavefish populations**"

15h: Nicolas Rohner (Harvard, USA) "**Cave Fish as a Model to Elucidate the Genetic Basis of the Evolution of Behavior"**

15h30: Erik Duboué (New York, USA) "**Evolutionary convergence on sleep loss in cavefish populations**"

Diner at 19h

Wednesday 16th March Cave trip

In past years, trips have been organized to the classic caves of Pachón and Chica, which are easy caves that almost anyone can enter. This year, town political difficulties for the first cave and fear of histoplasmosis for the second cave have made us decide to visit a different cave. While it is exciting to visit a new cave, especially if you have previously explored either of the aforementioned caves, it means that the trip involves a higher outdoor challenge: either a short pit at the end of a cave or a considerably longer hike in.

The exact cave we will visit has yet to be decided and depends on the field conditions at the time of the meeting. The Los Sabinos area is a good candidate to target. The village is now "showing" the Los Sabinos and Sotano Arroyo Caves. We may need to climb down to the cavefish areas with the help of cable ladders and ropes, unless they have constructed a staircase. The other alternative is Tinaja Cave. This is a “wild” cave that requires about a 2 hr hike in and a 2 hr hike out. A large section of the hike is through a steep tailless canyon that will require some non-technical climbing and boulder hopping. Clothing and especially shoes should be adequate for outdoor activities.

PRELIMINARY ITINERARY: The excursion will leave at about 9 AM on March 16 from Hotel Taninul. In the meantime those not visiting the cave could take an unguided hike to Naciemiento de Rio Choy. We will travel to the village nearest to the cave, which is approximately 2 hrs North of Hotel Taninul. There may be a Mexican police checkpoint along the way, so you are REQUIRED to bring your passport on the trip. At the village, we may divide into two groups. The cave trip will take about an hour, if we go to Sabinos, or five, if we go to Tinaja. In the afternoon, time permitting, we may visit the pleasant village of Micos to view *Astyanax* surface fish. Including this stop, the return will take 3-4 hrs. We should be back at Hotel Taninul by about 7 pm.

WHAT TO EXPECT: The hike to Sabinos Cave from the village is short and mostly over level ground (about 15 min). The path is slightly strenuous, although brief, and should be manageable for anyone in reasonable health. There may be need for hands and knees crawl in the cave and, depending on if they have constructed a staircase or not, we will need to use cable ladders and ropes to descend the short pit to the cave fish level. The passage may be muddy and slippery in spots, particularly at the end near the cavefish lake. We will rest at the underground lake for a while, view the cavefish, and then return to the surface in the opposite direction. The temperature in the cave will be warm (the mean annual temperature of the region, about 80^{o}F or 27^{o}C), a little cooler than outside but not uncomfortable, although the humidity will be high (your glasses might fog).

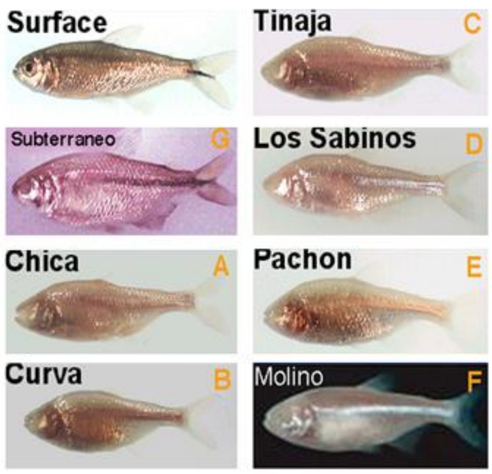
The hike to Tinaja from the village is about 2 hours. Certain sections are through a beautiful canyon without a trail. You will need to do some boulder hopping and some minor climbing. People participating will need to be in good physical condition and be accustomed to walk in the outdoors. Once inside the cave the passages are wide and the floor is mostly flat although it may be muddy and slippery in spots, particularly at the end near the cavefish lake. Return will be by the same route. The expected time from the car and back is about 5 hrs, but it can vary greatly depending on local conditions and the speed of the group. The cave and entry canyon also flood during wet weather, and thus selection of this cave will be weather dependent.

NOTICE: In Mexican caves, especially those harboring bats, there is risk of infection with histoplasmosis (http://www.cdc.gov/niosh/docs/2005-109/default.html). The caves we will visit are not particularly risky caves in this regard, as they do not contain a large bat population and our visit will be relatively short. However, to be cautious, each participant will be issued a respirator at the meeting and asked to wear it on their face throughout their visit to the cave. The N95 air-purifying respirator (NIOSH 42 CFR 84 standard) offers 95% protection from solid particles larger than 3 microns, including fungal spores (http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=INTERPRETATIONS&p_id=22251).

CLOTHING AND EQUIPMENT:
….Your respirator (you will be issued one at the meeting)
….Sunglasses and hat with a wide brim (for the hike to the cave)
….Flashlight (torch) with extra batteries and/or a light attached to your helmet (see below)
…. A SMALL backpack, but not heavily packed or otherwise cumbersome, to carry extra batteries, camera, water, and personal items, etc
….Pair of cotton or leather gardening gloves to protect your hands from thorns while hiking and damp mud in the cave
….Helmet (we will have some few extras, but certainly not enough for everyone. Please make every effort to bring your own)
….Jeans or other sturdy trousers
….T shirt
….Hiking shoes or boots with corrugated soles and cotton stockings
….Change of clothes if you don’t feel like returning in the same (possibly) muddy trousers

Note 1: There will be several experienced and superbly equipped cave explorers to guide you through the cave.
Note 2: You will be asked to sign a release of responsibility form to take the trip and enter the cave.
Note 3:There is absolutely no collecting of any kind allowed in the cave, although photography is permitted.

DONDE NACEN LOS PECES CIEGOS
Luis Espinasa and Bill Jeffery, Excursion Organizers



Thursday 17th March (Chair: Sylvie Rétaux)

Morning session: Genetics

10h: Richard Borowsky (New York, USA) “**Selection in *Astyanax* Cavefish**”

11h-11h30: coffee break and **group picture**

11h30: Patricia Ornelas-García (Madrid, Spain) “**Parallel adaptive divergence in a characid fish genus *Astyanax* Baird & Girard (1854) from Catemaco Lake (Mexico)**”.

Lunch at 12h30

Afternoon session1: *Astyanax* for education

14h: Luis Espinasa (Poughkeepsie, USA) «**Promoting undergraduate research in large classrooms: Guerrero cave *Astyanax*- Old or young colonization?”**

14h30: Sylvie Rétaux (Gif, France) « **Projection of a movie promoting *Astyanax* as a model system**»

Afternoon session 2: poster presentations

Evo-devo:
Li Ma (Maryland, USA) «**The Function of αA-Crystallin in Lens Degeneration in *Astyanax mexicanus* Cavefish”**

Ernesto Maldonado (Mexico City, Mexico) “**Cloning Ear Development Related Genes in *Astyanax mexicanus*”**

Ressources and tools:
Hélène Hinaux (Gif, France) “***Astyanax mexicanus* cavefish and surface fish cDNA libraries”**

Laurent Legendre (Gif, France) «**I-SceI mediated transgenesis in *Astyanax* model”**

Karen Pottin (Gif, France) “**A developmental staging table for *Astyanax mexicanus”***

Diner at 19h

Friday 18th March

Morning session: Round table

10h: **community issues, prospective and the future of *Astyanax***

Lunch at 12h30

Free afternoon

Suggestions: resting by the pool, informal discussions, hiking to El Nacimiento Del Rio Choy…

AIM2011 closing banquet at 19h

Abstracts

Cavefish
Evolution
Development
&
Behavior

What's So Special About Cave Fish?

(A Personal Reflection)

John C. Avise,

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I will begin my talk with a brief stroll down memory lane by recounting my early (1970) protein-electrophoretic research on *Astyanax* cavefish. I will then use this personal experience as historical backdrop for describing some of my lab's more recent work on genetic mating systems and reproductive behaviors in a wide array of other equally fascinating fish species ranging from catadromous eels to cuckolding sunfish to male-pregnant pipefishes. To pick just one other such example, I will include a short discussion of our recent work on the mangrove killifish, which is unique among vertebrate species in displaying the following: simultaneous hermaphroditism with preponderant self-fertilization; androdiocy, with occasional male-mediated outcrossing; a mixed-mating system like some plants and invertebrate animals; and a population genetic pattern quite unlike that of any other known vertebrate animal. Perhaps the take-home message from my lecture is that fishes have fabulously diverse genetic and natural history profiles that make them valuable creatures for addressing many broader phenomena in ecology and evolutionary biology.

Phylogenetic relationships among Cuban and Bahamian species of the blind cave-fishes genus *Lucifuga*: A shared lineage among two isolated archipelagoes.

Erik García-Machado[1], Damir Hernández[1], Peter Rask Møller[2] Louis Bernatchez[3], Didier Casane[4]

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2 Zoological Museum, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen Ø, Denmark
3 Institut de Biologie Intégrative et des Systèmes (IBIS), Pavillon Charles-Eugène Marchand, Université Laval, Quebec QC, G1V 0A6, Canada.
4 Laboratoire Evolution Génomes et Spéciation (UPR9034), CNRS - 91198 Gif-sur-Yvette cedex – France.
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The fragmented geographic distribution of the *Lucifuga*, a stygobitic fish genus endemic from Cuban and Bahamian islands, is enigmatic. The geological evidences suggest that if both territories were ever in contact, this event have occurred during Upper Jurassic. However, it looks unlikely that this episode could have facilitated the interchange of shallow water biota between these territories since it took place at deep see waters. To investigate the relationships among Cuban and Bahamian species we carried out a phylogenetic analysis using sequences from mitochondrial and nuclear DNA gene regions. The mitochondrial phylogeny showed three deeply divergent clades that were also supported by nuclear and morphological characters. Two of the clades group species that are restricted to western Cuba while the third one includes species/lineages from both archipelagos. Within the two first main clades we identified five lineages, two of them representing putative new species. Interestingly, the Cuban-Bahamian clade shows no reciprocal monophyly among territories but is morphologically supported by two main characters: presence of highly pigmented eyes and ten caudal find rays. As most of the species/lineages shows restricted distributions we analysed the genetic variation of *L. dentata*, more widely distributed in Cuba, to figure out the possible scenarios leading to the present time distribution of the genetic variation. We used a fragment of the *cyt*b gene and the non-coding region. We found a strong geographical organization of the polymorphism at different geographic scales that can be explained by periods of population expansion and dispersion events followed by population fragmentation and restricted gene flow. At a larger temporal scale, these processes could also explain the diversification and the distribution of these subterranean fishes.

AIM 2011

Population genetic evidence for convergence and parallelism in the Mexican Blind Cavefish (*Astyanax mexicanus*)

Martina Bradic[1, 2], Peter Beerli[2], Richard L. Borowsky[1]

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The Mexican blind cavefish, *Astyanax mexicanus,* inhabits a food and light restricted cave environment and shows numerous morphological changes. This suite of changes has evolved in multiple cave populations, making this species an attractive model to understand the population relationships in the context of convergent and parallel evolution. We have collected molecular data from 568 fish from 21 populations:10 cave and 11 surface localities. These localities represent three distinct geographical areas of caves and their associated surface populations. Using 26 unlinked microsatellite loci to account for neutral demographic influences, we assessed genetic structure within populations and levels of genetic differentiation among populations. These data reveal that the widespread surface localities comprise a single panmictic population, in contrast to the cave populations which are differentiated and have at least four distinct origins in the three main regions of North-East Mexico. Measures of genetic diversity showed significantly lower values in hypogean than in epigean populations. All the cave populations showed increased numbers of loci out of Hardy-Weinberg equilibrium, typically with heterozygote deficiencies. This effect is greatest in those cave populations having migrants from the surface and may be due to Wahlund effects caused by geographic barriers to gene flow followed by genetic drift in the subpopulations. Estimation of migration rates support migration from the surface to the cave populations and among some caves, though the actual number of individuals moving per generation is relatively low; the exception was among those populations which are geographically very close. The study provides the clear evidence of the convergent and parallel evolution that are driven by the same environmental cue.

Variance of Space Dependent Growth in *Astyanax mexicanus*

Natalya D. Gallo and William R. Jeffery

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The role of environmental size constraints on growth regulation has received little previous attention in teleost fish. While it is common knowledge to most aquarium hobbyists and aquaculture workers that stocking density has a negative relationship with fish growth, the mechanisms of this relationship have not been fully explored. Decreased or stunted growth in small aquaria or under high stocking conditions is frequently attributed to external factors such as decreased water quality, oxygen and food availability, and limited exercise opportunity. Most previous studies failed to address the underlying mechanisms because they examined the average growth rate of a fish population, instead of examining the effects on individuals. Here we provide evidence for an alternative hypothesis: that fish have evolved the physiological ability to determine the size of their environment and regulate their growth as an adaptive trait that might be subject to natural selection in different environments. Multiple controlled growth experiments with *Astyanax mexicanus* surface fish and Pachón and Tinaja cavefish individuals have shown that fish possess this ability, but it is variable in strength between different *Astyanax* populations. Growth experiments conducted on individual fish raised in 10mL, 25mL, and 40mL containers indicate that surface fish show significant space dependent growth (SDG): their growth rate is significantly slowed in a constrained environment. Furthermore, surface fish have the ability to make up for arrested growth by growing at a faster rate when moved to a larger container, than they would have if not previously exposed to constrained conditions. This phenomenon has been referred to as compensatory growth in fasting and feeding experiments on fish growth, and is similarly exhibited when environmental size is the active stressor. While surface fish show significant SDG, there is variation in SDG between different troglomorphic cavefish populations. Similar growth experiments with Tinaja cavefish show significant SDG ability, while Pachón cavefish show very limited or no detectible SDG and exhibit the same growth rate irrespective of environmental size. Furthermore, Pachón cavefish show no compensatory growth, a characteristic of the surface fish phenotype, when moved from a constrained to an unconstrained environment. We are investigating this variation in SDG by exploring the differing intensities of the stress response exhibited by *Astyanax* surface and cavefish when exposed to space constraints while also looking at the underlying genetics of this trait by evaluating SDG in surface fish X Pachón F1 and F2 hybrids.

Cave colonization without fasting capacities: An example with the fish *Astyanax fasciatus mexicanus*

Karine Salin[a*], Yann Voituron[a], Jérôme Mourin[b], Frédéric Hervant[a]

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Subterranean animals from temperate regions have commonly evolved hypoactivity, hypometabolism and/or the sequential use of energetic reserves to tolerate long fasting periods imposed by the low food levels found in subterranean environments. However, some tropical caves are characterized by a potential high level of nutriments.

By using the tropical fish *Astyanax fasciatus* that presents both populations subterranean (*Astyanax fasciatus mexicanus*) and epigean (*Astyanax fasciatus fasciatus*) populations, we described behavioral, metabolic and biochemical responses during a long-term fasting period followed by a refeeding period.

The results demonstrated that fed hypogean fishes exhibited different energy stores together with a hypometabolism. But, despite drastic decreases in locomotory activity and oxygen consumption during fasting, hypogean fishes consumed significantly more glycogen, triglycerides and proteins during the starvation period than epigean fishes. This lower fasting capacity showed by hypogean fishes is confirmed by the higher activation of the compensatory metabolic pathways (ketogenesis and gluconeogenesis). After the refeeding period, cave fishes did not recover from the “food deprivation” stress, and resume fed levels in glycogen, triglyceride reserves and proteins, in contrast to epigean ones.

This study thus demonstrates that starvation adaptations are not necessary for cave life, but are rather correlated to the “energetic state” of each ecosystem, and that troglomorphism is not linked to starvation capacities and thus not to the impoverished food availability.

Placode Development in *Astyanax mexicanus* blind Cavefish and sighted Surface Fish

<u>Hélène Hinaux</u>, Sylvie Rétaux.

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In blind *Astyanax mexicanus* cavefish, the eyes first develop almost normally during embryogenesis. But 24 hours after fertilization, the lens enters apoptosis, which triggers the progressive degeneration of the entire eye.

The lens develops from a placode, a thickening of the ectoderm lying next to the anterior neural plate at the neurula stage. There are several placodes, giving rise to sense organs of the head, all originating from the "panplacodal" field. We hypothesize that the panplacodal field is modified in the cavefish as compared to the surface fish.

We tested this hypothesis by studying the regionalisation of the panplacodal field. We have thus established comparative maps of the panplacodal field in surface and cave tailbud embryos, by performing *in situ* hybridizations to detect the expression patterns of markers of different placodes, and by quantifying the size of their expression domains.

Thus, we found differences in the expression of Dlx3b, a marker of the anterior neural plate border, Pitx1, a marker of the anterior placodes, and Pax6, marker of the « eye field ». Pax6 expression is reduced in the cavefish placodal field, suggesting the presence of a smaller lens placode. These results point to a modification of the patterning of the panplacodal field in the cave embryos.

Moreover, we found that Dlx3b expression at 16hpf is expanded in the olfactory placode area in cavefish embryos, implying that olfactory placodes, giving rise to the olfactory epithelium, could be larger in cavefish.

Surprisingly, Dlx3b, a typical placodal gene, is also expressed in the casquette, a sensory mechanical structure located on the head of the *Astyanax* larvae and involved in the swimming behavior, raising the idea that the casquette could be of placodal origin.

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The nature and function of the *Astyanax* casquette

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The larvae of the fish *Astyanax mexicanus* develop transiently a flat and adhesive structure on the top of their heads, that we have called “the casquette “(meaning hat). We hypothesised that the casquette may be a teleostean homolog of the well studied *Xenopus* cement gland, albeit their different position and structure. We have found that the casquette has an ectodermal origin, secretes mucus, expresses *Dlx3b*, *Bmp4* and *Pitx1/2*, is innervated by the trigeminal ganglion and serotonergic raphe neurons, and has a role in the control and the development of the larval swimming behaviour. These developmental, connectivity, and behavioural functional data suggest that attachment organs can develop in varied positions on the head ectoderm by recruitment of a Bmp4-dependent developmental module. We have also found that the attachment organ of the cichlid *Tilapia mariae* larvae displays some of these features. We will discuss the idea that attachment glands are ancestral to chordates, and have been lost repetitively in many phyla.

AIM 2011

Towards an understanding of pigmentation reduction in cave forms of *Astyanax mexicanus*.

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In recent years, quantitative trait locus (QTL) analyses have been used successfully to identify the genetic basis for morphological traits in a number of natural animal systems. Using pedigrees of F_2 individuals derived from surface (epigean) x cave form crosses, similar studies have begun to reveal the identity of genes associated with cave colonization in *Astyanax mexicanus*. The evolution of two simple (monogenic) traits, albinism and *brown*, are governed by the genes *Oca2* and *Mc1r*, respectively. Interestingly, these genes also demonstrate functional variation in a number of other vertebrate species. Important similarities are evident in these two traits, possibly revealing a pattern to regressive phenotypic evolution. Phenotypic regression in both cases is accompanied by the presence of loss-of-function alleles in our mapping population (Pachón cavefish). Further, the phenotypic expression of both albinism and *brown* in other distinct cave populations can arise through a combination of both structural (coding) and inferred *cis*-regulatory alterations at both loci. Despite these advances, however, the precise evolutionary mechanisms leading to trait loss remains unclear. Is there a cryptic advantage to pigmentation loss in the microenvironment of the cave (e.g., reduction of an energetic cost)? Alternatively, does the evolutionarily labile nature of these genes, given how frequently they are mutated in other vertebrate taxa, indicate a particular vulnerability to mutation in the absence of any phenotypic benefit (i.e., in the darkness of a cave)? Is there an as-yet undiscovered pleiotropic effect of these genes that somehow increases fitness in the cave microenvironment? In the context of these unresolved questions, I present preliminary analyses seeking to clarify the role of different mechanisms in governing pigment regression in the blind Mexican cavefish. These approaches include studies of candidate genes demonstrating genomic and functional similarity to loci identified in prior studies. In addition, I discuss future QTL studies seeking to address the prospective role for pleiotropy through analyses of a more comprehensive set of phenotypes segregating within mapping populations.

Convergent Evolution of Albinism in Cave Animals: A Defect in the First Step of Melanin Biosynthesis in Troglomorphic Fishes and Insects

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Cave adapted animals are often characterized by the absence of melanin pigment, a condition known as albinism. Here we ask whether albinism has evolved by the same or different changes in the melanin biosynthesis pathway in two diverse lineages of cave adapted animals, fishes (teleosts) and insects (planthoppers). In *Astyanax mexicanus* cavefish, albinism is caused by mutations in the *oca2* gene, which acts during the initial step of melanin biosynthesis: the conversion of L-tyrosine to L-DOPA. We have developed a functional assay for detecting the position of changes in melanin biosynthesis by supplying exogenous substrates, such as L-tyrosine or L-DOPA, to lightly fixed specimens, and subsequently detecting melanin as deposits of black pigment. Because the melanin synthesis pathway is conserved across wide phylogenetic distances, this assay can assess whether the initial steps of melanin biosynthesis are functional in diverse cave animals. For example, black pigment can be rescued in independently evolved populations of *A. mexicanus* cavefish by supplying L-DOPA but not L-tyrosine substrate, demonstrating the conservation of functional tyrosinase in the pathway and a convergent defect at its first step. The rescue is blocked by prior treatment of the specimens with high temperature or co-assay with phenylthiourea, an inhibitor of tyrosinase, indicating that it is an enzyme-catalyzed reaction. Similar results were obtained with two other albino cavefish that evolved albinism separately from *A. mexicanus*, *Typhlichthys subterraneous* and *Amblyopsis spelaea*, but not in some surface-dwelling albino fishes, which may have changed a downstream step in the pathway. To investigate the phylogenetic scope of this convergence, we performed similar assays on independently evolved albino cixiid planthoppers from limestone caves in Mljet and Biokovo, Croatia (unidentified species) and lava tubes in Hawaii (*Oliarus polyphemus*). In each case, supplying exogenous L-DOPA or dopamine (another initial substrate in a parallel melanogenesis pathway in insects) but not L-tyrosine produced black pigment, implying a defect also occurs in the first step in the melanin biosynthesis pathway in these organisms. Therefore, albinism has evolved via convergent evolution by interfering with the same initial step of the melanin biosynthesis pathway in both cave-adapted fishes and insects. We are currently investigating the possibility that this convergence is even broader than described here by conducting the substrate assays in many different albino invertebrate species. In addition, we are developing testable hypotheses to explain the potential adaptive significance of repeatedly blocking melanin biosynthesis at its first step in cave-adapted animals.

Evolution of a behavior mediated by the lateral line system adapts *Astyanax* to life in darkness.

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The ancestor of cavefish adapted to a completely dark environment by shifting physiological functions. How did cavefish evolve these traits during the adaptation processes? Here we show the physiological and genetic basis of an adaptive behavior, vibration attraction behavior (VAB). VAB is the ability of fish to swim toward the source of a water disturbance in darkness. Quantitative laboratory assays indicate that VAB is common in cavefish but rarely observed and much less robust in surface fish. In competitive prey-capture experiments, surface fish with VAB predominated over those without VAB in darkness but not in light, showing that VAB is beneficial for feeding in the dark. VAB was evoked by vibration stimuli peaking at 35 Hz and was blocked by the lateral line inhibitors cobalt and gentamicin. The behavior appeared after a numerical increase in superficial neuromast (SN) during development, and was significantly reduced by bilateral SN ablation. The significant correlation between VAB and the number of SN supports the conclusion that enhanced SN mediated the evolution of VAB. Mating experiments between surface fish and cavefish suggested that VAB has a simple genetic basis involving paternal inheritance. Quantitative trait locus (QTL) mapping using 140 F2 offspring showed a single major QTL on the linkage group 17, which accounts for 12% of the phenotypic variance in VAB. The increase in SN number also had a simple genetic basis (3 QTLs) but none of the SN QTLs was the same as the VAB QTL. Considering the fact that surface fish individuals with VAB do not show an increase in SN or tuning at 35 Hz, we propose that the cavefish-type VAB evolved through sequential SN-independent and SN-dependent steps. As a first step in cavefish evolution, the surface-dwelling ancestor likely used the surface fish-type VAB for initial adaptation to cave environments. In the subsequent step, cavefish with enhanced SN and VAB tuned to 35 Hz were able to detect prey more efficiently in the dark. This scenario is perhaps one of the ways in which *Astyanax* became adapted to caves and eventually evolved into cavefish. Our results underscore the importance of behavioral diversity in adapting animals to new environmental challenges.

Linking evolution of the aggressive behavior and the serotonergic system in *Astyanax mexicanus* surface fish and blind cavefish populations.

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Most cavefish populations have lost the aggressive behavior that is a trademark of their surface fish counterparts. Here we investigated the possible developmental and neuro-anatomical origin of the loss of aggressive behavior in cavefish.

We first characterized aggressive behavior in Astyanax. Using an “intruder assay”, we found that surface fish (SF) not only attack 80% more times during a one hour period, but also show a significantly different pattern in the temporal distribution of their attacks: while 2 cavefishes (CF) attack mostly during the first minutes after they are put together, the surface fishes (SF) attack more and more frequently as time goes by during the test. As levels of aggressiveness and serotonin (5-HT) neurotransmitter levels are known to be inversely correlated in several vertebrate species including fish, we have then compared the brain serotoninergic system in SF and CF. The two populations show an identical organization of their serotonin networks in the hindbrain raphe nucleus and in several hypothalamic nuclei. One of these, the anterior paraventicular nucleus (PV) of the anterior hypothalamus, is significantly enlarged and contains more 5-HT neurons in CF from early larval stage to adulthood. This anatomical observation was further confirmed by HPLC dosage of 5HT, showing higher 5HT levels in the hypothalamus of cavefish, but identical 5HTlevels in the raphe nucleus of the two populations.

To establish a functional link between aggressiveness and serotonin, we performed pharmacological treatments modulating serotonin brain level (deprenyl, fluoxetine).These treatments (which both increase brain 5HT level by different mechanisms) decrease the number of attacks and abolish the typical SF pattern of aggressiveness. Thus, aggressiveness and serotonin levels are inversely correlated in SF.
To further investigate the developmental origin of this SF/CF difference, we compared the development of their serotonergic systems. We found a time-shift in the appearance of hypothalamic 5-HT neurons. In fact, the PV 5-HT neurons develop four to six hours earlier in CF while there was no difference in the development of the raphe neurons. We are currently testing the hypothesis that early modification in signaling systems (Shh, Fgf8, previously described by us and others) could be responsible for this specific difference in neurogenesis in the two populations.

Finally, we will discuss the adaptive nature of the loss of aggressiveness for living in darkness and cave conditions, and hypothesize a possible link with modifications for reproductive and/or feeding behaviors.

Cave Fish as a Model to Elucidate the Genetic Basis of the Evolution of Behavior

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A wealth of studies elucidating the genetic basis of morphological variation has been performed in the last decades. In comparison, there have been relatively few studies addressing the genetics underlying behavior, especially regarding differences in behavioral traits arising through evolution. The cave fish *Astyanax mexicanus* provides a beautiful system to study the genetic basis of the evolution of behavioral traits. These cave animals display a series of behavioral adaptations relative to their river cousins that are of importance for survival in caves.

One such behavior is the grouping behavior that is seen when fish swim in a school. Schooling conveys several critical advantages including predator avoidance and foraging. *Astyanax mexicanus* is an excellent model to study schooling behavior as the surface forms school, while cave forms have lost this behavior. Previous studies have shown that schooling behavior in *Astyanax* is a polygenic trait, but so far nothing is known about the particular genes or underlying neuronal signaling being involved in this behavior. We are using a model school to quantify schooling preference in the F2 population of a Tinaja-cave/surface intercross and QTL linkage mapping to decipher the genetic changes underlying this behavioral adaptation. As the ability to see might have a direct effect on schooling, we use a high throughput dark/light preference system to correct for differential visual ability in the hybrids. Our preliminary data implies a genetic basis for schooling as a polygenic trait which is partially independent of visual capacity, and potentially a behavioral adaptation of living in the cave.

Another interesting behavior is that cavefish “scan” the ground during feeding at a shallow angle compared to a very steep feeding posture of the surface form. This feeding posture is displayed in multiple independently derived cave populations indicating that it may be adaptive for finding food in the dark. A previous study suggests a main genetic locus underlying this behavioral change. We are measuring the feeding angle in the F2 population of our Tinaja-cave/surface intercross and are including this trait in the QTL analysis. Although sight does not seem to be correlated with this trait we are performing the experiments in complete darkness to avoid confounding effects with visual ability.

Evolutionary convergence on sleep loss in cavefish populations

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The blind Mexican cavefish, Astyanax mexicanus, is a well established model system for studying the genetics of evolutionary changes in development and morphology. The species includes surface populations with eyed fish, and numerous blind cave populations. Cave and surface forms are interfertile, which facilitates genetic analysis of their differences, and the independent origins of many of the cave populations allow investigation of the genetic bases of convergent evolution. We report that this system is also uniquely valuable for the investigation of variability in patterns of sleep. Sleep is a fundamental behavior, perhaps universally exhibited throughout the animal kingdom, but the functional and evolutionary principles responsible for sleep diversity remain largely elusive. Here we show that a clearly defined change in ecological conditions, from surface to cave, is correlated with a dramatic reduction in sleep in three independently derived cave populations of A. mexicanus. Analyses of surface/cave hybrids show that the alleles for reduced sleep in the Pachón and Tinaja cave populations are dominant in effect to the surface alleles. Genetic analysis of hybrids between surface and Pachón cavefish suggests that only a small number of loci with major effects are involved. Our results demonstrate that sleep is an evolutionarily plastic phenotype that is responsive to changes in ecological conditions. To our knowledge, this is the first example of a single species with two radically different sleep phenotypes correlated with differences in ecological settings.

Selection in *Astyanax* Cavefish

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Most cavefish populations of the genus *Astyanax* have reduced eyes and pigmentation, as well as changes in other senses, in behavior and metabolism. We now add sleep reduction to the list of troglomorphic features. The forces driving the evolution of the different troglomorphies vary from one trait to the next. QTL analyses suggest that the reductions in eyes are driven by selection while reductions in pigmentation result from recurrent mutation and genetic drift. Quantitative genetic analysis suggests that the alleles for reduced sleep in cave populations are dominant in effect over those of the surface alleles. This pattern is markedly different from the dominance effects of QTL for eye and pigment reduction and suggests that sleep reduction is driven by strong selection. Our working hypothesis is that increased wakefulness in the cave is selected for because it increases the probability of obtaining food that is episodically and unpredictably present. Preliminary analyses of rest patterns in balitorid cave fish suggest that sleep reduction in cavefish populations may be as common as eye regression. The hypothesis that eyes are selected against in the cave environment but pigmentation regresses because of drift makes the prediction that eyes should be lost faster than pigmentation in the cave. This prediction is supported by analyses of eyes and pigmentation in unrelated balitorid cave fish. Finally, we have documented patterns of significant gene flow from surface populations into cave populations. The maintenance of troglomorphy in the face of persistent gene flow indicates that selection against surface genes in the cave populations is ongoing.

Parallel adaptive divergence in a characid fish genus *Astyanax* Baird & Girard (1854) from Catemaco Lake (Mexico).

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The fish genus *Astyanax* is characterized by a high phenotypic plasticity such as that seen in recurrent cave-adapted phenotypes. Another kind of adaptations are those related to trophic traits, evident from the characid system of *Astyanax* and *Bramocharax* from the Lake Catemaco in Mexico. This system represents a good model to study parallel evolution of trophic traits, usually assigned to a generalist form (*A. aeneus*) and a specialized one (*Bramocharax caballeroi*). Using geometric morphometrics, we examined variation in body size and shape of 210 characid individuals from Lake Catemaco. In order to evaluate the possible genetic correspondence with morphology, we also analyzed the mitochondrial structure using Cytochrome *b* (Cyt*b*) sequences and 12 microsatellite loci of 305 individuals. Characid morphotypes were significantly different with respect to the shape and orientation of the head and to body depth. In contrast, we did not find correspondence between phenotypic differentiation and molecular structuring, neither at mitochondrial nor nuclear evidence. We found two mitochondrial lineages showing less than 1% divergence, revealing a convergence of the specialized phenotype in both lineages. We suggest that differences observed in biotypes may have resulted in reduced resource competition through modification of trophic morphology, body shape, and feeding behavior.

AIM 2011

Promoting undergraduate research in large classrooms: Guerrero cave *Astyanax*- Old or young colonization?

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In recent years, the inclusion of research in undergraduate work has been deemed as one of the best practices in undergraduate education. While this is acknowledged by most, efforts are typically restricted to providing just a handful of outstanding students the opportunity of working on a research project. The great challenge is how to bring large classes into the laboratory and involve them in novel research. My talk will concentrate on presenting a successful model occurring at Marist College which involves up to 72 undergraduate students a year while fulfilling the standard academic goals of an upper level course Genetics. The key to the model program is that, instead of purchasing standard educational kits, all students get to use real reagents and samples, and they perform genuine research for publication. The research has been aimed at sequencing the DNA of cave organisms, such as *Astyanax*. There are so many unanswered and interesting questions regarding cave organisms that can be approached by sequencing DNA, a technique which is well within the capabilities of undergraduate students, that finding novel research projects every year is relatively simple. Results have been published in peer-reviewed journals and have included undergraduate students as authors. Students in large classrooms get to experience how scientific research is really conducted and, while they do so, they become proficient in techniques used in modern genetics such as gel electrophoresis, PCR, DNA sequencing, sequence analysis, and bioinformatics.

I will present the results obtained by my genetics students from the class of fall 2010. Students sequenced the 16S rRNA gene of *Astyanax* from a cave in Guerrero, Southern Mexico, to establish when the colonization of the cave environment by these fish occurred. Previous studies using mitochondrial DNA have shown that there have been at least two colonization events of the cave environment by *Astyanax fasciatus* in the caves of Sierra de El Abra in Northern Mexico. One is old (Piedras, Sabinos, Tinaja, and Curva populations) and most likely influenced by major climate changes during the Pleistocene. The other event is more recent. For this study we asked whether the Southern *Astyanax* blind population from Guerrero is also a relic population from an old colonization or if it is instead the product of a recent colonization. Mitochondrial DNA sequences from the Guerrero cave populations were identical to the surface sequences of fish from the Amacuzac River, supporting a recent colonization.

Projection of a movie promoting *Astyanax* as a model system

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A 4’30” movie on *Astyanax* cavefish and surface fish used as laboratory model animals will be presented.

The making of this vulgarization movie has been promoted and supported by EFOR, a French network of laboratories and platforms whose objective is to promote conventional and less classical model organisms for studies of gene function. EFOR helps and facilitates the use of varied species through the valorization of their dedicated infrastructures. The EFOR network is coordinated by Jean-Stéphane Joly (joly@inaf.cnrs-gif.fr) and Johanna Djian-Zaouche (djian@inaf.cnrs-gif.fr).

The movie has been realized and produced by Noé Sardet from Parafilms, a company based in Montréal, Canada (noe@parafilms.com).

Poster 1

The Function of αA-Crystallin in Lens Degeneration in *Astyanax mexicanus* Cavefish

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The αA-crystallin gene encodes a potent cell survival factor that normally protects the developing lens from apoptotic cell death. The lens dies by apoptosis during cavefish development, and this phenotype is considered to be one of the causes of subsequent eye degeneration. Although cavefish eye regression is a multi-factorial trait controlled by many genes acting in concert to suppress optic development, recent QTL and candidate gene analyses suggest that mutations in the αA-crystallin gene may be partially responsible for this phenomenon. Our objective is to determine the molecular role of αA-crystallin in cavefish lens degeneration. There are four main goals: first, to compare and confirm the spatial and temporal expression patterns of the αA-crystallin genes during surface fish and cavefish development; second, to identify changes in the sequence of the αA-crystallin gene and surrounding regulatory regions that may be responsible for the differences in expression between surface fish and cavefish; third, to determine if identified sequence differences are the actual mutation(s) leading to gene dysfunction and lens apoptosis; and finally, to determine whether the same or different DNA sequence changes and putative mutations occur in cavefish populations that evolved eye degeneration independently. *In situ* hybridization has shown that αA-crystallin mRNA expression is lens specific in both surface fish and cavefish. Compared to surface fish, αA-crystallin mRNA is not detectible in Tinaja cavefish and very weakly expressed in Pachón cavefish (as previously demonstrated by others: Behrens et al., 1998, Gene 216: 319; Strickler et al., 2007, Dev. Genes Evol. 217: 771). The low levels of αA-crystallin expression are in striking contrast to several other crystallin genes, whose expression levels in the developing lens are not appreciably changed in cavefish (Strickler et al., 2007), suggesting specific downregulation of this gene during eye degeneration. Thus far, we have discovered several sequence differences between surface fish and Pachón cavefish within a 10 kb region in and around their αA-crystallin genes, including changes in the coding region, the second intron, the basal promoter, and an interesting region upstream of the gene that contains a putative enhancer. In cavefish, the latter region contains a 633 bp insert, which is absent from surface fish and a strong candidate for the critical mutation leading to αA-crystallin downregulation. The possible function of these sequence differences in transcriptional regulation of αA-crystallin are under further investigation.

Cloning Ear Development Related Genes in *Astyanax mexicanus*

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Several subterranean fishes have been found in Mexico; the phantom blindcat (*Prietella lundbergi*) the Mexican blindcat (*P. phreatophila*), the Olmec blindfish (*Rhamdia macuspanensis*), a blindwhiskered catfish (*R. reddelli*), the blind swamp eel (*Ophisternon infernale*). Among them a blind characid has been recognized as a subspecies of *Astyanax mexicanus* as chromosomal differences have not been detected and natural hybridization occurs between the eyed, surface ancestor and the eyeless, cave-dwelling populations. Interesting results has been published about an increased expression of some members of Hedgehog (Hh) signaling family, during embryonic development of a blind subpopulation of *Astyanax mexicanus*. In zebrafish (*Danio rerio*) it was found that Hedgehog (Hh) signaling family is necessary for ear patterning, concomitant in time and space with the increased Shh expression observed in *Astyanax* cavefish embryos. The effects in cavefish ear development of such natural Shh overexpression will be analyzed. Therefore we will study the expression pattern of genes involved in ear development, as *patch* (*ptc*), *Brn3* and *islet-3* (*isl-3*), which are expressed in the posterior otic placode, in ciliated cells and otic neurons, respectively. We are presenting our first attempt to clone these genes from *A. mexicanus,* using tail tissue total RNA and RT-PCR experiments.

Poster 3

Astyanax mexicanus cavefish and surface fish cDNA libraries

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The molecular studies of *Astyanax mexicanus* can be uneasy due to the lack of genomic data. Each gene has to be cloned individually through PCR using degenerate primers, a tedious technique which lengthens the duration of each experiment.

We decided to establish 8 cDNA libraries of *Astyanax mexicanus* cave and surface fish. We extracted total RNA of 8-18hpf embryos, 1 day-larvae, 2 day-larvae and 1-2 weeks larvae for each population. RNA was then reverse-transcribed and cDNA was cloned in a vector derived from pCMV-Sport6 by a German company called Agowa genomics, now LGC Genomics. The French sequencing center Génoscope transformed bacteria with these ligations and arrayed them as glycerol stocks in 384 wells plates. As of today, 80.000 resulting clones have been sequenced by the Sanger method. 120.000 more clones are currently under sequencing.

We now have 2 normalized libraries, including all stages of each population, and 6 non-normalized libraries, for the 3 younger stages of each population. Two copies of the libraries have been arrayed, one being held in the NeD laboratory in France, the other in Bill Jeffery's group in Maryland.

These libraries seem to be representative of the transcriptome, with a small content in ribosomal genes, and an interesting repartition of the cDNAs in various gene ontologies. The 2 normalized libraries saturate particularly slowly, containing thus a lot of various cDNA sequences.

This transcriptomic resource represents a major contribution to the *Astyanax* scientific community and will be available within a year or so.

Poster 4

I-SceI mediated transgenesis in *Astyanax* model

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Transgenesis techniques in *Astyanax* model (*A. mexicanus*) have been poorly used in the past years although this fish has become an increasingly popular microevolution model (cf Yamamoto et al. Dev Biol. 2009). It is however an interesting and fruitful way to analyze how genes work or to visualize gene expression in live animals when the transgene contains a fluorescent protein.

We used Involin, an I-SceI meganuclease-mediated method (meganucleases are restriction enzymes which cut 18bp recognition sites, typically found only once in genomes). We choose a vector routinely used and made by the AMAGEN platform (aquatic animals transgenesis platform, Gif sur Yvette, France). The construct contains the zebrafish β-crystallin promoter controlling CFP reporter protein expression. We co-injected this vector together with the meganuclase in embryos from one to four cells stages. The method has proved efficient at the AMAGEN platform in two other fish models, *Oryzias latipes* (Medaka) and *Danio rerio* (Zebrafish). The advantage of this technique is a high frequency of transgene insertion resulting most frequently in a single locus - low copy number insertion in the genome.

Three batches of surface fish embryos (total: 134) were successfully injected from which 41 larvae with CFP-expressing lenses were obtained. A good overall survival rate was observed and most of the fish are still alive at the time of writing (n=39, aged 2 to 5 months old). As expected, various types of expression were observed in the lenses, from monolateral to bilateral and from strong to low expression in a manner very reminiscent of Involin transgenesis in zebrafish. Statistics will be presented. Based on F0 mosaic animals observation and AMAGEN experience of other fish species transgenesis, we expect that one quarter to one third of the CFP-expressing animals will be founders. Similarly, we expect the rate of transgene transmission for each founder F0 animal to vary from 1 to 50% for one insertion, and sometimes more if there are several insertions. We have started the screen to establish β-crystallin-CFP lines through breeding of the positive FO surface fish we obtained.

All observations made so far, let us believe that surface Astyanax will be a species very amenable to transgenesis. To bring its full potential transgenesis must indeed be equally applied to cavefish; we are therefore currently trying to perform the same experiments in cavefish embryos.

AIM 2011

A developmental staging table for *Astyanax mexicanus*

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Each model species has its own developmental table. Studies on *Astyanax mexicanus* taking more and more importance, a developmental staging table becomes increasingly necessary, especially as comparative analysis of early developmental events are more widely used by researchers.

We collected freshly spawned embryos from surface fish or Pachón cavefish population and took pictures every 10-12 minutes in the first day of their development, followed by more spaced pictures in the following days. The results provide an illustrated comparison of different stages from one cell stage to hatching for the two populations and show a mainly very synchronous development of the two types of embryos regarding major events like epiboly, neurulation, somitogenesis, heart beating or hatching. We will also present data on particular morphological characters such as lens apoptosis during development, neuromast and lateral line aspect or fin development with high magnification pictures taken with an Apotome microscope (Zeiss).

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Cavefish
Evolution
Development
&
Behavior

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Meeting notes

Cavefish
Evolution
Development
&
Behavior