

**CONFERENCE
SCIENTIFIC
PROGRAM**

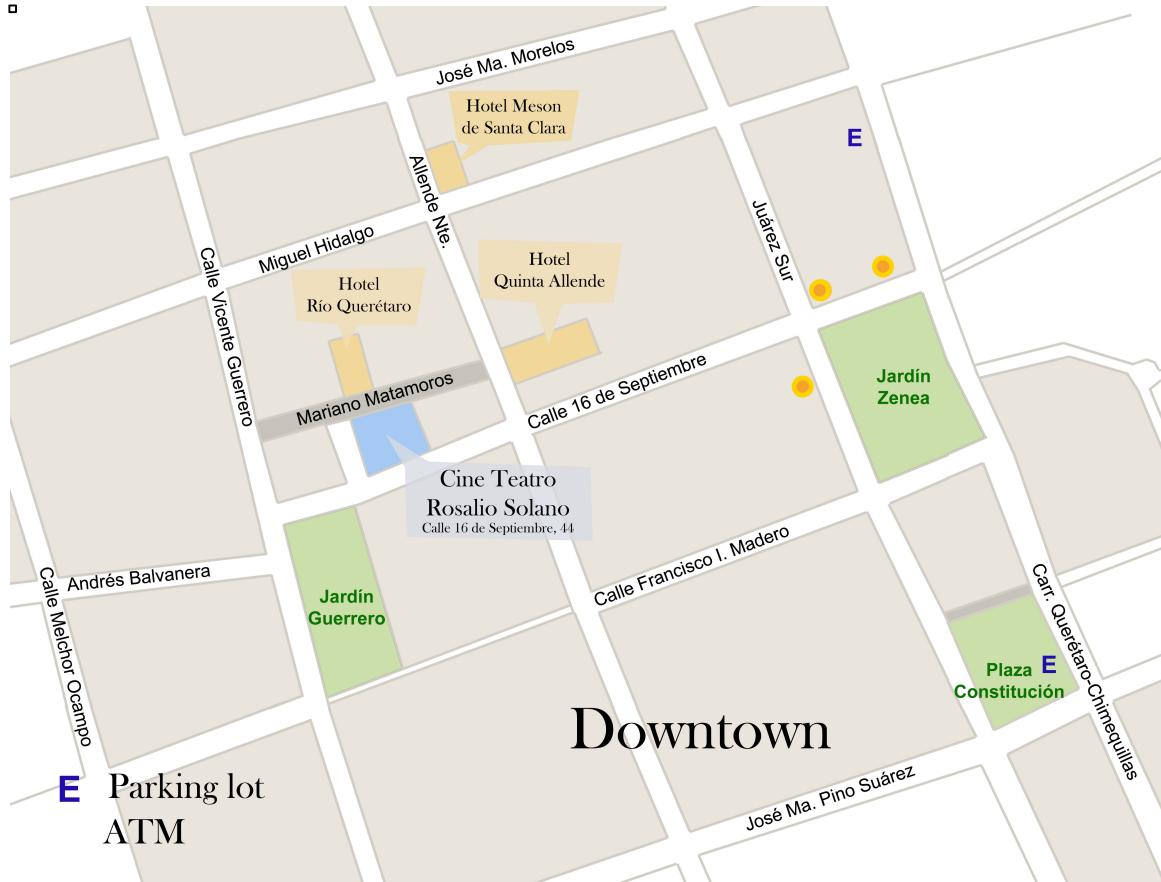


Fourth Astyanax International Meeting

October 26-28, 2015

Cine Teatro Rosalio Solano, Querétaro

MAP



Event	Places
Reception	Hotel Quinta Allende
Meeting	Cine Teatro Rosalio Solano
Meals	Hotel Río Querétaro
Banquet	Las Monjas

AIM2015
SCIENTIFIC PROGRAM

Monday, October 26 th Arrival of participants 6 pm – 9:00 pm	Welcome Reception at the Hotel Río Querétaro
Tuesday, October 27th	
7:00 – 8:30 am	<i>Breakfast</i> (Hotel Río Querétaro)
Morning session	
9:00 am	Meeting Welcome and Acknowledgements: Alex Keene, Suzanne McGaugh, Masato Yoshizawa, Patricia Ornelas
Genomics	Chair: McGaugh / Ornelas
9:00– 9:30 am	Martina Bradic “Caves with Eyed and Eyeless Populations of <i>Astyanax</i> ”
9:30 – 10:00 am	Richard Borowsky “Selection or Drift: Critical tests of hypotheses”
10:00 – 10:30 am	Suzanne McGaugh “Population genomics of cavefish”
10:30 – 10:45 am	<i>Coffee Break</i>
10:45 – 11:15 am	Julien Fumey “Evidence of Late Pleistocene origin of <i>Astyanax mexicanus</i> cavefish”
11:15 – 11:45 am	Bethany Stahl “High-resolution genomic mapping reveals genes contributing to complex melanophore variation in <i>Astyanax mexicanus</i> cavefish”
11:45 am - 12:15 pm	Patricia Ornelas – García “The <i>Astyanax</i> genus as model group in species delimitation”
12:30 pm – 2:00pm	<i>Lunch</i> (Hotel Río Querétaro)
Afternoon Session	
Development and Morphology	Chair: Yoshizawa
2:00 – 2:30 pm	Lucie Devos “Developmental evolution of the basal forebrain in cavefish”
2:30 – 3:00 pm	Joshua Gross “Craniofacial evolution in blind Mexican cavefish”
3:00 – 3:30 pm	William Jeffery “Molecular Analysis of an <i>Astyanax</i> Eye QTL Reveals a Potential Role of Cystathionine β -synthase A in Cavefish Eye Degeneration”
3:30 – 3:45pm	<i>Coffee Break</i>
3:45 – 4:15pm	Helena Balandzija

	"The loss of body pigmentation has evolved independently in multiple animal taxa that have successfully colonized subterranean habitats"
4:15 – 4:45 pm	Nicolas Rohner "Hungry, Fat, and Healthy – Studying the physiological basis of cave adaptation"
4:45 – 5:15 pm	Amanda Powers "Investigating a potential relationship between constructive trait evolution and aberrant cranial phenotypes in <i>Astyanax</i> cavefish"
Dinner 7:30-9:00 PM	<i>Dinner (Hotel Río Querétaro)</i>
5:30 – 7:30 pm	Poster session and drinks
	Beatriz Robinson "The evolution of sleep loss in relation to metabolic processes in Mexican cavefish"
	Li Ma "Candidate genes analysis for pigment development in <i>Astyanax</i> cavefish"
	Ma. Pamela Bermúdez González "Morphological and ecological variation in contrasting environments in the Mexican sardine (<i>Astyanax mexicanus</i> de Filippi 1853)"
	Masato Yoshizawa "How to build a DIY water-flow system that successfully makes cavefish spawn"
	Andrea Herrera García "Phylogeography of <i>Astyanax aff. mexicanus</i> (De Fillipi, 1835) (Characidae) in surface and cave populations"
	David Iván Hernández Mena "Endohelminth parasites of the genus <i>Astyanax</i> (Characidae) through its geographical distribution in Mexico"
	Gabriel Isaias Cruz Ruiz "Analysis of Stomach content in population of <i>Astyanax aeneus</i> (Günther, 1860) from highlands on basin rio Papaloapan in oaxacan area of the Tehuacán-Cuicatlán biosphere reserve"
	Guillermo Abraham Peña Herrejón "Cavefish <i>Astyanax mexicanus</i> as a model to study genetic expression related to shoaling and aggressiveness"
	Julius Tabin "The evolution of temperature preference in <i>Astyanax</i> "
	James Jaggard

	"Distinct neural mechanisms underlie the convergent evolution of sleep loss in the Mexican cavefish" <u>Jamie Calderon</u> "Caves, infectious disease, and its reservoirs"
Wednesday, October 28th	
7:00 – 9:00 am	<i>Breakfast</i> (Hotel Río Querétaro)
Morning session	
Evolution, Behavior, Metabolism and circuits	Chair: Keene
9:00 – 9:30 am	<u>Sylvie Retaux</u> "Sensory evolution in blind cavefish is driven by early embryonic events during gastrulation and neurulation"
9:30 – 10:00 am	<u>Daphne Soares</u> "Functional imaging of circuits commonly associated with vision processing in the <i>Astyanax</i> blind cave fish"
10:00 – 10:30 am	<u>Misty Riddle</u> " <i>Astyanax mexicanus</i> as a model to study metabolism and the evolution of the digestive system"
10:30 – 10:45 am	<i>Coffee break</i>
10:45 – 11:15 am	<u>Oscar García</u> "Spatial Memory Experiments in <i>Astyanax mexicanus</i> "
11:15 – 11:45 am	<u>Ariel Aspiras</u> "Melanocortin 4 receptor mutations contribute to the adaptation of cavefish to nutrient-poor conditions"
11:45 – 12:15pm	<u>Alex Keene</u> "Convergent evolution of sleep loss"
1:00 – 3:00 pm	<i>Lunch</i> (Hotel Río Querétaro)
Afternoon Session	
3:00-4:00	<u>Keynote Address</u> <u>John Postlethwait</u> Spotted Gar Links Teleost Genomes to Human Biology
4:00 – 4:30 pm	<i>Coffee break/discussion</i>
6:30 pm - Late	<i>AIM2015 Banquet</i> (Restaurant Las Monjas)
Thursday, October 29th	
Jalpan Cave trip	Pick up at the Hotel Quinta Allende
6:00 am	All day trip

Trip to Jalpan Cave, October, 28th



The excursion will be all day long, please be aware in case that your flight is in the same day.

General recommendations:

Equipment

- Two flashlights with extra batteries**
- Clothes easy to dry, like lycra or dry fit, try to avoid jeans.**
- Backpack to carry a change of clothes, once you get out of the cave you will need it!!!**
- Pair of gloves to protect your hands**
- Helmet (Please make every effort to bring your own)**
- Shoes**
- Food: water bottle, Snacks (seeds, energy bars, peanuts etc.).**
- small medical kit.**
- Hiking shoes or boots**

Caves with Eyed and Eyeless Populations of *Astyanax*

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To explore the genetic and ecological factors affecting relative condition factor (length corrected weight) in *Astyanax* cave fish, we studied the three most accessible caves containing mixed populations of eyed and eyeless fish: Rio Subterráneo (RS), El Sótano de Caballo Moro (CM) and Cueva Chica (CH). We characterized each of the populations in terms of condition factor, eye phenotype, proportion of cave genotype in each individual (PCave), and melanocortin 4 receptor (mc4r) genotypes. PCave was estimated from a STRUCTURE analysis of variation at 26 unlinked microsatellite markers reported earlier (Bradic *et al.* 2012). Mc4r mutations have been previously identified as major contributors to the adaptation of cave morphs to nutrient-poor conditions (Aspiras *et al.* 2015). Each of the cave populations is ecologically and genetically unique, varying in energy input and proportion of cave genome (including mc4r alleles). With respect to mc4r, the surface allele predominates in CM, the cave allele predominates in CH, and both are at intermediate levels in RS. In all three populations eye size was negatively correlated with PCave. All four variables are highly correlated in the RS population, reflecting recent migration from the surface and largely representing sorting of the phenotype and genotype into the two main categories, surface and cave. Correlations, while still strong, are significantly reduced in the other populations, suggesting sustained hybridization. Surprisingly, average condition factors of fish from CH, a cave with a high food input, and RS, a cave with a low food input, were identical. While this might reflect atypical conditions the year of the collections, we suggest an alternative ecological possibility: food availability may more affect population size than condition factor because of density dependent regulation through intraspecific competition. That is, more food = more (but not fatter) fish. Counterintuitively, fish from CM are in much better condition than those from RS or CH, in spite of the virtual absence of the mc4r cave allele in CM. We suggest that CM, which has input of light, may have a more extensive ecosystem resulting in the *Astyanax* population being regulated, in part, by predation rather than direct competition for food.

Selection or Drift: Critical tests of hypotheses

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Loss of eyes and pigmentation are common in cavefishes, but whether their evolution is driven by natural selection or genetic drift remains in dispute. These same convergences, however, present a remarkable opportunity to parse out these disparate evolutionary forces. Each cavefish species is a replicate of an experiment revealing an evolutionary response to an ecological shift to permanent darkness and increased competition for food. Thus, hypotheses based upon observations of one species may be tested by their predictions in other species. Application of this approach to independently evolved populations of *Astyanax* cavefishes and balitorid species from SE Asia provides strong evidence that the principal driver of eye loss is natural selection acting directly upon eye phenotype, and not drift or indirect selection through pleiotropy. The evidence for direct selection against eyes comes from: 1) studies of QTL polarities, 2) the relative rates of eye and pigmentation regression, and 3) analyses of genomic correlations of QTL and regions with hallmarks of selection. In contrast to eye regression, the likely principal driver of loss of melanophores is genetic drift. The metabolic demand of eye maintenance is a significant portion of the energy budget in these fish and is the likely target for direct selection (Moran *et al.*, 2015).

Population genomics of cavefish

Suzanne McGaugh*, Jimmy Weagley, Bill Jeffery, Masato Yoshizawa, Kelly O'Quin,
Luis Espinasa, Nicholas Rohner, Richard Borowsky, Wes Warren

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Here we accelerate the understanding of the genetic underpinnings of convergent evolution by conducting genome scans for divergent genomic regions between two surface fish populations and three cave fish populations, at least two of which are independently derived. We examined the data for 1) areas of exceptionally high genetic divergence between cave and surface fish across their genomes, and 2) regions that are different between the two independent evolutionary origins of cave fish. Since these divergent genomic regions are often the smoking guns of adaptation, we catalogued which genes are embedded in them and estimated the role of natural selection in creating these divergent regions.

Evidence of Late Pleistocene origin of *Astyanax mexicanus* cavefish

Julien Fumey*, Hélène Hinaux, Céline Noirot, Sylvie Rétaux and Didier Casane

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Populations of blind and depigmented cavefish belonging to the Mexican tetra species *Astyanax mexicanus* are outstanding models to study the tempo and mode of adaptation to cave environment because the phenotypic convergence of these independent populations allows us to examine whether the evolution of similar phenotypes involved the fixation of standing genetic variation and/or de novo mutations. However, we do not have yet accurate estimations of the time frames of evolution of these populations that are so important for such studies. We developed a cheap, convenient and robust method to detect a recently isolated small population and estimate its age. Our approach is based on the fact that there is a transient acceleration of the neutral substitution rate in such a population. We applied this method to estimate the age of Pachón cave population which is considered the “oldest” and most isolated cavefish population. Despite recurrent claims that the isolation of this population is ancient, within the range of the late Miocene to the middle Pleistocene, we obtained evidence that it has been isolated during less than 30,000 years, that is during the Late Pleistocene. This new time frame together with other recent evidence indicate that the many phenotypic changes observed in these cavefish mainly involved the fixation of cryptic genetic variants present in surface fish populations and within a short period of time.

High-resolution genomic mapping reveals genes contributing to complex melanophore variation in *Astyanax mexicanus* cavefish

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Loss of phenotypic characters is a widespread phenomenon that affects nearly every living organism, yet the genetic mechanisms underlying loss remain largely unknown. Cave animals represent excellent models to examine regressive evolution, owing to the common loss of eyes and pigmentation among lineages evolving in the subterranean habitat. The blind Mexican cavefish, *Astyanax mexicanus*, is a particularly powerful system because “ancestral” surface-dwelling forms are available, allowing for direct comparisons with cave-dwelling morphs. To date, only two genes (*Oca2*-albinism and *McIr-brown*) have been linked to specific pigmentation phenotypes in cavefish. However, pigment cell (melanophore) number is a complex trait governed by multiple genes, and the identities of these loci remain unidentified. To uncover these genes, we assembled a high-resolution, second-generation linkage map. We adapted an automated phenotypic scoring strategy, in which melanophore number is quantified across seven body regions in a surface x Pachón cave F₂ pedigree. QTL mapping analyses yielded several markers strongly associated with melanophore number variation in the dorsal mid-lateral stripe area and in the superior head region, which anchor to specific regions of the *Astyanax* draft genome and the zebrafish genome. Within these syntenic regions we identified two candidate genes, *Tyrp1b* and *Pmela*, with known roles in pigmentation-related processes based on Gene Ontology annotation. Mutant forms of these candidate genes in other systems cause global and regional pigmentation losses, respectively. In-depth analyses of these genes reveal coding sequence alterations and reduced expression in Pachón cavefish compared to surface morphs. This work uniquely identifies *Tyrp1b* and *Pmela* as genes involved with complex aspects of *Astyanax* pigmentation, and provides insight to genetic mechanisms governing regressive phenotypic change.

The *Astyanax* genus as model group in specie's delimitation

Claudia Patricia Ornelas García*, Carlos Pedraza Lara, David Hernández, y Gerardo Pérez Ponce de León

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The species is the fundamental unit in evolutionary biology, however, criteria used to delimit species are frequently not the same or are even contradictory between different groups (e.g., ecological and biological concepts), resulting in taxonomical discrepancies. In order to solve these drawbacks, it has been suggested the implementation of an integrative taxonomy approach, based on the incorporation and accommodation of multiple evidences for species delimitation. The main goal of this study was to evaluate the taxonomical status of *Astyanax aeneus*, sister species of *A. mexicanus*, under an integrative taxonomy framework. Among the data included for species delimitation was molecular (mtDNA and nucDNA), morphological (geometric morphometrics) and ecological (parasites loads) information. We sampled 36 populations within *Astyanax* distribution in southern Mexico. Considering the four mt-lineages previously described for the species, *cytb* and 12 loci microsatellites were sequenced and genotyped in order to evaluate the genetic structure of the species. In addition, description of shape variation was accounted by analysis of 27 body landmarks. Finally, the parasitic loads were characterized for the 36 populations within the species. We recovered higher taxonomical diversity than the previously recognized, and suggest a taxonomic proposal to integrate *Astyanax* variation under a timing and evolutionary context.

Developmental evolution of the basal forebrain in cavefish.

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Adaptation to the cave environment involves many modifications at morphological and behavioural levels. In the vertebrate forebrain, the preoptic region and the hypothalamus contain neuro-endocrine cell types that control complex motivated behaviours as well as body energy homeostasis. Previous work in the group showed that the hypothalamus of the cavefish is larger than that of the surface fish, that both the hypothalamus and preoptic area of the cavefish proliferate more than those of the surface fish during embryonic and larval development (Menuet *et al.*, 2007), and that specific neuronal groups such as the serotonin paraventricular nucleus is enlarged in cavefish (Eliot *et al.*, 2013). Moreover, we proposed a potential developmental trade-off between the neural plate regions fated to become the ventral part of the retina –which is lacking or reduced in the cavefish- and those fated to give rise to the hypothalamus/preoptic area, through a modification in Fgf8/Shh signalling (Pottin *et al.*, 2011). Here, we have therefore examined in details the development, morphogenesis and neural fates in the hypothalamus and preoptic region in a comparative manner in cavefish and surface fish. We generated a map of neuropeptide-expressing neurons and more particularly appetite-regulating peptidergic neurons (including orexigenic NPY and Hypocretin, and anorexigenic POMCa and POMCb). We found an increase in the numbers of orexigenic cells and a decrease in the numbers of anorexigenic cells in specific cell groups in cavefish. Further asking the origin of these modifications, we examined expression patterns of neuronal specifying transcription factors of the LIM-homeodomain family (including Lhx9 and Lhx7) and found some local modifications of their expression, generally just prior the first appearance of neuropeptide expression. Finally, we manipulated Fgf8 signalling through pharmacological treatments at neural plate stage, and found correlated changes in the morphology of the preoptic region and the size of neuropeptidergic groups. In summary, we will show that the increase in size of the hypothalamus and preoptic area is not the result of a global increase in all neuronal types, but rather of a complex modification of cell fates.

Craniofacial evolution in blind Mexican cavefish

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Despite the near absence of any nutrition or sunlight, many animals colonize caves to avoid predation, seek refuge during climatic cooling or expand an environmental niche. The cave-dwelling fish, *Astyanax mexicanus*, has a complex evolutionary origin, having repeatedly invaded 29 caves of the El Abra region of Mexico over the past several million years. Different populations demonstrate highly convergent characteristics, however the underlying mechanisms governing this convergence remain unclear. Certain traits like albinism and brown evolve through the same gene in geographically distinct populations. However, complementation studies revealed that eye loss evolves through different genes in different populations. To understand how gene expression patterns evolve following colonization of the cave, we performed mRNA-sequencing across critical developmental stages in two cave populations – Pachón and Tinaja – that demonstrate significant phenotypic convergence. We profiled genome-wide expression patterns and directly compared these changes to closely related surface-dwelling fish. We discovered many of the same gene functions (based on gene ontology terms) appear to be targeted during cave colonization, however each cave form demonstrated substantial GO term diversity unique to their own population. We also discovered several specific genes convergently over- or under-expressed in both cavefish populations compared to surface fish. Collectively, these results suggest that cave evolution is governed, in part, by a set of genes critically associated with adaptation to the subterranean environment. However, this genetic convergence is balanced by the expression of several novel genes, which may reflect the unique environmental pressures specific to each cave.

Molecular Analysis of an *A. mexicanus* Eye QTL Reveals a Potential Role of Cystathionine β -synthase A in Cavefish Eye Degeneration

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Genetic analysis indicates that multiple genes and quantitative trait loci (QTL) control eye degeneration in *Astyanax* cavefish. We are studying the eye QTL on chromosome 21 (C21 QTL), which contains 24 protein-coding genes located in scaffold KB871589.1 of the Pachón cavefish genome sequence. Semi-quantitative RT-PCR of 21 C21 QTL genes during the period of cavefish eye degeneration showed 15 genes with no changes in expression, 3 upregulated genes, and 3 downregulated genes in cavefish relative to surface fish embryos. The three-downregulated genes, cystathionine β - synthase a (cbsa), alpha A-crystallin (cryaa), and heat shock transcription factor 2 binding protein are contiguous in the center of the C21 QTL. In situ hybridization showed that only the cbsa and cryaa genes have eye specific expression in surface fish embryos. We previously reported that CRYAA serves as a survival factor in *Astyanax* eye development, but it is not detectably mutated in cavefish and probably under trans-acting regulation (Ma *et al.*, 2014, *EvoDevo* 5:28). The cbsa gene encodes an enzyme catalyzing the first step of the transsulfuration pathway, which is responsible for producing cysteine and the cysteine-containing tripeptide glutathione, a critical antioxidant in the eye and other tissues. In situ hybridization showed that cbsa expression is downregulated in the cavefish lens and retina, cbsa knockdown in surface fish embryos affects retina development, and the F1 hybrid test indicated that cbsa downregulation is likely caused by a cis-acting mutation. A nonsynonomous amino acid substitution (threonine > isoleucine) was also detected in the catalytic domain of cavefish CBSA, suggesting that mutations in both non-coding and coding regions may affect cbsa function. Similar coding region mutations in human CBS prevent proper enzyme folding and produce homocystinuria, a disease characterized by defective sulfur amino acid metabolism. One of the effects of this disease is on the eye. These results suggest that cavefish may suffer from a breakdown in the defense system protecting the eye against chemical and oxidative stress due to mutations in the cbsa gene and that this deficiency may be one of the causes of eye degeneration.

**The loss of body pigmentation has evolved independently in multiple animal taxa
that have successfully colonized subterranean habitats.**

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The loss of body pigmentation has evolved independently in multiple animal taxa that have successfully colonized subterranean habitats. By using the melanogenic substrate assay, we have gained two insights into the mechanisms underlying albinism in a diverse variety of cave-dwelling animals. The first insight is that pigment cells capable of using L-DOPA to synthesize melanin are conserved in a variety of cave animals in spite of the absence of melanin synthesis, suggesting that these cells may have roles other than pigment formation. Studies of wound healing in arthropods have provided a clue for one of these roles: melanization of wound sites is a widespread feature that is conserved even in cave-dwelling arthropod species that have lost body melanin body pigmentation. Furthermore, we have found that melanophores or colorless cells capable of melanin synthesis congregate near wound sites in *Astyanax* embryos. These studies suggest that pigment cells may be important in innate immunity, which would explain their conservation during troglomorphic evolution. The second insight is that melanin synthesis is blocked at its first step in a wide variety of cave-dwelling animals. This first step could be a frequent target of disruption because: i) it is energetically most efficient to block melanin synthesis at its beginning, ii) the disruption of other steps in the pathway would generate reactive oxygen species and other toxic intermediaries, and/or iii) L-tyrosine, the initial substrate can be used to enhance other metabolic pathways, such as the catecholamine synthesis pathway. We are testing these possibilities and have demonstrated a link between the catecholamine and melanin synthesis pathways via oca2 loss of expression in *Astyanax*. Preliminary evidence also suggests that catecholamines are increased in albino cave invertebrates.

Hungry, Fat, and Healthy – Studying the physiological basis of cave adaptation

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With the prevalence of obesity rising to epidemic proportions, new approaches will be needed to address resulting diseases such as diabetes or non-alcoholic fatty liver syndrome. The cavefish *Astyanax mexicanus* is providing us with a unique angle into the emerging field of evolutionary medicine. As a consequence to nutrient limited environments, cavefish have acquired different strategies to survive long periods of starvation. For example, Tinaja cavefish can acquire substantially higher fat levels than surface fish and as a consequence display impressive starvation abilities. Despite such high fat levels and additionally very low insulin levels, cave fish seem healthy and do not display obvious signs of similar phenotypes seen in humans. I will describe some of these impressive physiological adaptations in detail and discuss future approaches to study its underlying genetic basis and how I plan to translate these molecular mechanisms into other species, including our own.

Investigating a potential relationship between constructive trait evolution and aberrant cranial phenotypes in *Astyanax* cavefish

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Cave animals have undergone remarkable adaptive changes in order to survive in their dark, nutrient poor environment. For instance, cavefish exhibit a dramatic increase in number of sensory neuromasts relative to closely related surface-dwelling fish. Neuromasts are specialized components of the lateral line system in aquatic species encompassing hair cells that are stimulated by movements in the surrounding water. Neuromast expansion is particularly apparent in the cranial region and has been linked to vibration attraction behavior (“VAB”) in cavefish, which is associated with enhanced foraging ability in the absence of light. Interestingly, ablation of superficial neuromasts proximal to the third suborbital (SO3) bone decreases VAB, suggesting this region plays a key role in cave-associated behavioral changes. Interestingly, several independent cavefish populations harbor defects in the suborbital bony series, including the fragmentation of the SO3 bone into multiple, distinct elements. The cellular basis for this fragmentation has long remained unknown. Here, we integrate quantitative and qualitative analyses to determine if there is a relationship between cranial neuromast positioning and density, and SO3 bone fragmentation. Approximately 86% of our lab-reared Pachón cavefish exhibited SO3 fragmentation compared to zero observations in surface fish. In addition, despite greater numbers of SO3 neuromasts in cavefish ($p<0.001$), surface fish SO3 bones are significantly larger ($p<0.001$). Using Delaunay triangulation to quantify density, we found surface fish have a significantly greater distance between neuromasts than cavefish in the SO3 region ($p<0.001$). Since higher neuromast density is associated with SO3 fragmentation, there may be an antagonistic relationship between neuromast expansion and cranial bone development, leading to the failure of SO3 bony elements to fuse together to form an intact bone. Thus, maladaptive cranial phenotypes in cavefish may evolve through an indirect relationship with the strong selection of constructive phenotypes.

Sensory evolution in blind cavefish is driven by early embryonic events during gastrulation and neurulation

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Natural variations in sensory systems constitute adaptive responses to the environment. Here, we have compared the development of the sensory placodes in the blind, cave-adapted morph and the eyed river-dwelling morph of the fish *Astyanax mexicanus*. Focusing on the lens and the olfactory placodes, we found a trade-off between these two sensory components in the two morphs: from neural plate stage onwards, cavefish have larger olfactory placodes and smaller lens placodes. Using lens placode laser ablation experiments, we ruled out the possibility of a defective community defect among cavefish lens cells that would explain their apoptotic death later in development. Searching for the developmental mechanisms underlying cavefish sensory evolution, we analyzed the roles of Shh hyper-signaling from the ventral midline, Fgf8 heterochronic signaling from the rostral forebrain, as well a newly discovered modification in Bmp4 expression dynamics in the prechordal plate of cavefish embryos. Using pharmacological treatments and injections to interfere with these three signaling systems at the end of gastrulation, we establish a regulatory network in which the subtle modifications of Shh, Fgf8 and Bmp4 signaling in space and time in cavefish embryos explain the observed changes and trade-off in lens versus olfactory placode fate, and account for the observed size of their sensory derivatives. We suggest that such evolutionary developmental mechanisms may be used throughout vertebrates to drive adaptive sensory specializations according to lifestyles and habitats.

**Functional imaging of circuits commonly associated with vision processing in the
Astyanax blind cave fish**

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To study the adapting roles of the neural circuits associated with vision in *Astyanax mexicanus* we have performed two-photon calcium imaging of the retina and the optic tectum in intact, non-anesthetized larvae of both epigean and hypogean forms. We found that both the retina and the optic tectum of cavefish show extensive ongoing spontaneous activities. Yet, activity induced by visual stimuli were never observed. This lack of visually-induced responses comes in sharp contrast to surface fish which show large visually-induced tectal responses. Furthermore, the cavefish optic tectum did not respond to either vibrational or acoustic stimuli which trigger specific behavioral responses. Anatomically, both 5-14 days post fertilization cave and surface larvae displayed similar size optic tecta, with retinal ganglion cells projecting to the optic tectum at comparable extents. Our results also show that while deprived from sensory input, the visual network remains highly active, showing extensive ongoing spontaneous activity. The functional role of the retino-tectal circuit in a developing blind fish is still uncertain. One explanation rests upon the developmental role of the tectum which can be fundamental to patterning the CNS, or maintain the proper connectivity between different brain regions (e.g acting as a relay rather than being a processing brain area). It is worth noticing that cave *A. mexicanus* could have diverged as early as 10,000 years ago (Avise and Selander, 1972; Chakraborty and Nei, 1974; Mitchell *et al.*, 1977), therefore providing us with only an early snapshot of a continuously adapting brain. Spontaneous activity in the visual pathway of cavefish could therefore be present as a vestigial surface fish code for processing visual information.

***Astyanax mexicanus* as a model to study metabolism and the evolution of the digestive system**

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To survive and thrive in unique environments animals have evolved a host of mechanisms to maximize the intake, storage, and use of energy. *Astyanax mexicanus* is a proven model for understanding the genetic basis of adaptation and represents a particularly strong system to investigate evolutionary changes in metabolism. To exploit the prevailing food source in an environment an animal can increase the amount eaten during rare times of abundance, alter digestive transit time to extract more from the food ingested, and/or alter the storage and processing of fats. There is evidence that these strategies have been adopted in the cave morphs of *A. mexicanus*; recently published data indicate that genes known to regulate human appetite (MC4R) are altered in the cavefish leading to binge eating. We also found that the Tinaja digestive tract is longer than the surface digestive tract possibly representing increased digestive absorption capacity. In addition, we observed that the color of Tinaja visceral adipose tissue is strikingly different from surface possibly representing altered carotenoid absorption or metabolism; Tinaja visceral fat is yellow, while surface fat is white. Interestingly, while Pachon fat is similar to Tinaja, Molino fat is white like surface fat. These findings are leading us toward a better understanding of the adaptations used to survive in an environment with infrequent and minimal food.

Spatial Memory Experiments in *Astyanax mexicanus*

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In evolution, behavior is one of the most moldable features that organisms have at hand, in order to adapt to new habitats. It is through an array of different sensory systems that they can gather information about their surroundings, colonizing even extreme environments. This is the case of the Characid fish *Astyanax mexicanus* with two morphotypes: Surfacefish (SF) with normal vision that inhabits rivers and blind Cavefish (CF) living in cave ponds. CF evolved in perpetual darkness and scarce food through morphological changes such as eye loss and acquisition of a greater number of larger neuromasts. It also experienced behavioral shifts as; gain of vibration attraction, and loss of traits as aggression and schooling. CF originated from SF trapped in caves. Our main question is: How *Astyanax* SF managed to survive in the caves? First we collected SF and CF in the wild and performed a maze experiment in complete darkness while filming with IR cameras. Spatial learning in SF has a slower learning curve than CF, however soon enough SF was completely able to form a spatial memory solving the maze in the complete absence of light.

Melanocortin 4 receptor mutations contribute to the adaptation of cavefish to nutrient-poor conditions.

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Adaptation to new environment necessitates changes in morphology, behavior, and physiology. While we now have a better understanding of morphological and behavioral evolution, how physiology evolves is still mysterious. Here we use independently derived populations of Mexican cavefish, *Astyanax mexicanus*, to elucidate the genetic basis of population level metabolic changes associated with the colonization of nutrient poor environment. Many cavefish populations rely on sporadic flooding for nourishment as caves lack primary producers. Thus to survive, cavefish evolved a range of adaptations including starvation resistance and binge eating when food becomes available. We found that the use of these adaptive strategies differs among independently derived populations. While all cavefish populations tested lose weight more slowly than their surface counterparts, only a subset of cavefish populations exhibit the hyperphagia phenotype. A candidate gene approach identified coding mutation in conserved residues of the melanocortin 4 receptor (MC4R) gene, contributing to the insatiable appetite found in some populations of cavefish. Intriguingly, one of the mutated residues has been shown to be linked to obesity in humans. We demonstrate that the allele results in both reduced maximal response and reduced basal activity in vitro. We further validate in vivo that the mutated allele contributes to elevated appetite, growth and starvation resistance. The allele appears to be fixed in cave populations in which the overeating phenotype is present. The presence of the same allele in multiple caves appears to be due to selection from standing genetic variation present in surface populations.

Convergent evolution of sleep loss

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Sleep is characterized by extended periods of quiescence and reduced responsiveness to sensory stimuli. Animals ranging from insects to mammals adapt to environments with limited food by suppressing sleep and enhancing their response to food cues, yet little is known about the genetic and evolutionary relationship between these processes. The blind Mexican cavefish, *Astyanax mexicanus* provides a powerful model for elucidating the genetic mechanisms underlying behavioral evolution. Evolutionary convergence on sleep loss and vibration attraction behavior, which is involved in prey seeking, have been documented in cavefish raising the possibility that enhanced sensory responsiveness underlies changes in sleep. We established a system to study sleep and vibration attraction behavior in adult *A. mexicanus* and used high coverage quantitative trait loci (QTL) mapping to investigate the functional and evolutionary relationship between these traits. Analysis of surface-cave F2 hybrid fish and an outbred cave population indicates that independent genetic factors underlie changes in sleep/locomotor activity and vibration attraction behavior. While the genetic and neural mechanisms underlying sleep loss are distinct from those regulating VAB, ablation of the entire lateral line with gentamicin, or localized ablation of superficial neuromasts in the head or trunk regions restore surface fish levels of sleep in Pachón cavefish. Strikingly, ablation of the lateral line has no effect on sleep in surface or other cave populations suggesting the evolution of a wake-promoting role for the lateral line in Pachón cavefish. Analysis of surface-Pachón-cave F2 hybrid fish reveal sleep is inversely related to the number of superficial neuromasts in the head or trunk, fortifying the notion that sleep loss in Pachón is linked to increased sensitivity of the lateral line. Taken together, these findings present Mexican cavefish as a model for understanding the evolutionary changes in sleep and provide insight into the relationship between sleep and the gating of sensory information. Current research is seeking to identify the genetic basis for lateral line-dependent sleep loss in Pachón cavefish

Posters session

The evolution of sleep loss in relation to metabolic processes in Mexican cavefish

Beatriz G. Robinson, James B. Jaggard, Masato Yoshizawa, Alex C. Keene

Sleep is an essential behavior that is universal throughout the animal kingdom, yet it is highly variable in timing and duration between species. Food supply is one of the multiple factors underlying evolutionary changes in sleep. Changes in sleep behavior have been linked to energy stores; however, the relationship between sleep modulation and metabolic processes remains poorly understood. The Mexican cavefish, *Astyanax mexicanus*, is a model system for investigating the evolution of sleep in response to new environmental pressures. *A. mexicanus* consists of cave-dwelling cavefish, that have been isolated in cave environments and its ancestral-type river-dwelling surface fish. Here, we are using *A. mexicanus* to investigate the relationship between diet and sleep. RNAseq in Pachón cavefish revealed modification of genes related to metabolic function and activity in cave populations, suggesting a relationship between metabolism and sleep. To address this hypothesis, we have systematically investigated the effect of diet on energy stores and sleep of several cave populations of *A. mexicanus*. Unlike surface-fish and several other cave populations, the Molino population exhibits a robust increase in energy stores and sleep while on a high fat diet. Current work is examining the relationship between basal triglyceride levels and sleep in surface-cave hybrid fish. Our findings provide evidence for functional differences in the evolution of metabolic changes between Molino and other *A. mexicanus* populations.

Candidate genes analysis for pigment development in *Astyanax* cavefish

L. Ma, B. Carlson, B. Stahl, M. Powers and J. B. Gross

Depigmentation is one of the most common phenotypes evolving in *Astyanax* cavefish. The identification and characterization of genes controlling coloration will enable us to better understand the genetic and evolutionary mechanisms underlying cavefish depigmentation. To understand the genetic differences of pigment cell development between surface fish and cavefish, we identified 165 pigment-related transcripts from prior QTL studies and the literature. We analyzed 158 genes from the current draft of the *Astyanax* genome, distributed across 133 scaffolds, and found 95 genes harbored SNP changes, and 75 genes harbored other coding sequence changes. We quantified mRNA expression level differences in these 75 genes in surface fish and cavefish across early development and identified 50 genes with expression level fold-change differences between surface and Pachón cavefish >1.5 . We further examined whether the expression levels change appeared in two geographically-distinct populations from the Sierra de El Abra – Pachón and Tinaja. Our results identified 17 genes that are down-regulated in both cave populations and 7 genes which are up-regulated in both cave populations. When we performed sequence alignments of the 75 genes, we observed similar coding sequence changes in both Pachón and Tinaja cave populations. In addition to the 75 genes with coding region changes, we discovered several genes with intact coding sequences that have significant mRNA expression level changes. Based on the literature, many of these identified genes play a role in neural crest migration, melanophore lineage determination, and melanoblast survival, migration and proliferation. Thus, the candidate genes identified from this analysis are involved in many diverse aspects of pigmentation development. Further, the presence of both coding and expression-level differences implies that diverse genetic processes collectively underlie the convergent loss of pigmentation in *Astyanax* cavefish. Future studies will include developmental analyses of gene expression based on in situ hybridization and pigmentation phenotypic assays. By better understanding the temporal and spatial aspects of pigment gene expression across differentiation, migration, and proliferation stages, we hope to better understand the molecular mechanisms underlying the evolution of depigmentation in *Astyanax* cavefish.

Morphological and ecological variation in contrasting environments in the Mexican sardine (*Astyanax mexicanus* de Filippi 1853)

Ma. Pamela Bermúdez-González, Carlos Pedraza-Lara , Joshua B. Gross y Claudia Patricia Ornelas-García

Astyanax mexicanus species is distributed at the northeastern part of Mexico. Within this distribution range, it has been found a convergent evolution of troglobitic forms in multiple and independent evolutionary lineages of the species, giving evidence of the capability of the species to adapt to contrasting environments. There is a growing number of studies in order to associated the genetic basis some cave adapted traits (ie evolutionary regression traits). Based on the previous evolutionary studies we consider *Astyanax mexicanus* species correspond to a good model to evaluate the morphological evolution, testing the phylogenetic inertia imposed over the body shape and in those characters associated to food intake in the independent linages present in the caves. We also are interested to carried out the characterization of the food web networks between the surface and cave systems in order to determine biotic and abiotic interactions associated to the cave adapted populations. We have collected 16 locations (7caves, 9 surface) in the region of the Sierra de El Abra, Sierra the Guatemala, and Micos. A total of 25 landmarks have been digitalized for 253 specimens and perform a geometric morphometric analysis to determine the morphological variation. For the food web network characterization, we have collected 103 samples for both habitats (troglobitic and surface habitats), including the basal material (benthic sediments and periphyton), crustaceans, arachnids, among other fauna associated to each habitat. A total of 75 muscle tissues of *Astyanax mexicanus* (troglobitic and surface) were obtained for further stable isotope analyses.

How to build a DIY water-flow system that successfully makes cavefish spawn

Masato Yoshizawa and Christian Macaspac

Astyanax mexicanus is a model cave-dwelling fish with sighted surface-dwelling (surface fish) and blind cave-dwelling (cavefish) forms. Although it is relatively easy to make them spawn in the lab comparing with other non-model system, it is sometime hard to do so especially at the newly established system. Here I present a simple and a cheap way to build the system. The key features required for the system are: (1) water-flow, connecting multiple tanks into one, (2) tight temperature control by a heater and a chiller, (3) filtered water from reverse-osmosis system, and (4) rough surface at the bottom. For (1), female *Astyanax* seem to release chemical (pheromone) when they are ready to spawn, that stimulate other fish. If you have multiple tanks connected to each other, it will raise the chance of spawning events. Also, you need one sump for such water flow system, making it easy to regulate water chemicals. In addition, I will talk about a hand-made sump system, which filter and maintain the water quality enough. For (2) the tight temperature control, *Astyanax mexicanus* are tropical fish, sensitive to the change of the water temperature as low as 1°C shift. Therefore, the combination of heater and chiller stabilize the temperature better at 22°C, which is a maintaining temperature for the *Astyanax*. For (3) about the water source, the reverse osmosis system stabilizes water chemicals. We add the pH conditioner and sea salt for conditioning water at pH ~7.0 and conductivity at 600 µS. As for (4), the rough surface at the bottom of the tank has not been thought as an important factor. In our observation, surface fish spawn after introducing the spawning net, which has a course net on the top and fine net at the bottom, making easy to recover spawned embryos. Perhaps female prefers the rough bottom surface because it can give embryos a better chance to avoid to be eaten by conspecific or others. Our current system houses 18 of 10 gallon (38 L) tanks connected to a 40 gallon (150 L) sump tank. We feed TetraColor Tropical Granules and dried shrimp, and our fish routinely spawn every other week. We also provide the technique how to make spawning nets.

Phylogeography of *Astyanax* aff. *mexicanus* (De Fillipi, 1835) (Characidae) in surface and cave populations.

Herrera García Andrea, Pedraza-Lara Carlos and Ornelas García C. Patricia

The mexican sardine, *Astyanax* aff. *mexicanus* (De Filippi, 1835), is the only species of the family Characidae widely distribute in the Nearctic Region, especially north of the Trans Mexican Volcanic Belt (TMVB). The times for divergence between different lineages (i.e., “old” and “new”) and the number of species inhabiting this area are topics still under debate. Therefore, the main target of this study is to include an exhaustive sampling of the species in this region in to propose robust phylogenetic and biogeographic hypotheses to explain the occurrence of such lineages. Under the scenario of several demographic expansions to the north of the TMVB, it is expected that events would resulted in multiples isolated lineages occurring today. We obtained 1,063 bp of the mitochondrial gen cytochrome b for 86 individuals, which joined to previous data recovered 95 haplotypes for the Region, which distributed in eight haplotype groups. In correspondence with previous jobs, multiples lineages were observed for the North of México. We found the caves of the Sierra del Abra (Arroyo y Sabinos) belonging to the “old lineage”, while Chica y Pachón showed signs of mitochondrial introgression with surface populations of the new lineage and even shared haplotypes between one cave population, and surface populations Arroyo Lagartos and Huichihuayán. The caves of Molinos (Sierra de Guatemala) and Subterráneo (Micos) were include in the “new linage” and Subterráneo shared haplotypes with Chica and several populations of surface (-h56-). Finally, we surveyed for the first time several surface populations harboring the old lineage (Río Nazas-Aguanaval en Zacatecas -h29-), likewise they were included populations old the new linage (Sabinas, Salado, San Fernando y Texas). Also, a lineage not previously recored has been detected that for the case of the basin of Río San Fernando.

Endohelminth parasites of the genus *Astyanax* (Characidae) through its geographical distribution in Mexico

David Iván Hernández Mena, Ana Ofelia Santacruz Vázquez, Patricia Ornelas García,
Berenit Mendoza Garfias, Gerardo Pérez Ponce de León

The *Astyanax* genus is geographically distributed from the southern of United States of America to the Negro River in Patagonia. Particularly in Mexico two species are recognized: *A. mexicanus* and *A. aeneus*. These species have been studied for helminths, and apparently are infected by an exclusively group of parasites, and also by species with wide host range (generalists). The main objective of this study is to establish a more precise list of endoparasites species in *Astyanax* spp along its distribution in Mexico. To meet this objective, between October 2013 and May 2014, we conducted a survey of *Astyanax* spp specimens in 27 locations from 11 states: Campeche, Chiapas, Jalisco, Michoacán, Morelos, Querétaro, Oaxaca, San Luis Potosí, Tabasco, Veracruz and Yucatan. The parasites obtained were fixed for morphological and molecular study. The results suggest that species of *Astyanax* in Mexico are parasitized by species of the genera of trematodes *Auriculostoma*, *Clinostomum*, *Creptotermatina*, *Magnivitelinum*, *Prosthenhystera* and *Wallinia* (Platyhelminthes), as well as by nematodes like *Contracaecum*, *Procamallanus*, *Rhabdochona* and *Spinitectus*.

Analysis of stomach content in population of *Astyanax aeneus* (günter, 1860) from highlands on basin rio Papaloapan in Oaxacan area of the Tehuacán-Cuicatlán Biosphere Reserve.

Gabriel Isaías Cruz-Ruiz, Eufemia Cruz-Arenas, Emilio Martínez-Ramírez, Rubí Marcos-Gómez y Rosa María Gómez Ugalde

There are few jobs that contribute to the biology of native fish species, giving priority to introduced species for commercial use, leaving aside the natives, endemics or those that, because of the small sizes, fall outside the consideration of commercial species. Therefore this study was conducted, with the aim of analyzing the food components of the diet of this population of *A. aeneus*, which allowed us to know their status in the community of fishes (trophic level), since most of fishes adapt well to their feeding habits and take advantage of foods that are available. For this, were used specimens collected in 30 sampling stations during the period September / 2005 to March / 2006, with electrical and craft fishing (seine and cast nets), which formed part of the project "Fishes from the Oaxacan area of the Tehuacan-Cuicatlán Biosphere Reserve ". 143 digestive tracts were examined using the frequency-occurrence and numerical methods, with which 47 types of food preys were determined, the most important were Diptera, Ephemeroptera, Hemiptera, knuckles and detritus of animal origin (arthropods); is an omnivorous species, with a tendency to be planktivorous in juveniles and adults mesopredator carnivores, and is located in the trophic level of tertiary consumer.

Cavefish *Astyanax mexicanus* as a model to study genetic expression related to shoaling and aggressiveness

G. A. Peña-Herrejón, C.P. Ornelas-García, A. Cruz-Hernández, J. Caballero-Pérez, J. F. García-Trejo

The importance to study fish behavior in aquaculture is becoming increasingly apparent since behaviors such as aggressiveness and shoaling affect domestication and welfare of fish. The study of behavior on major aquaculture species is difficult because of their generation times and the necessary space to their handling. To perform these researches, it is necessary to use model organisms, such as the cave fish *Astyanax mexicanus*, a model for evolution and development studies. It presents surface (SF) and cave (CF) populations with different phenotypes and the ability to interbreed. Among the changes generated by its independent evolution, CF populations presents a reduction on their aggressiveness and shoaling behavior, caused in part by the modification of their serotonin (5-HT) levels through a mutation of the monoamine oxidase, however there could be more than one gene involved on these behavior changes. We propose a direct study of shoaling and aggressiveness by obtaining *A. mexicanus* lineages, with different stable behaviors, through the crosses of SF, CF, and their progeny. We will seek to obtain an aggressive fish lineage with full visual capabilities, as well as a non-aggressive one. A shoaling and non-shoaling lineage will be also sought. After obtaining the lineages, a high aggressive organism will be crossed with a non-aggressive one and a backcross will be performed. The same methodology will be done on the shoaling and non-shoaling organisms. Each generation will be maintained under a rack system for 3 months, until the identification of aggressive and gregarious behaviors, through an experimental design considering the numbers of attacks, the nearest neighbor distance and the inter-individual distances. After the backcross experiment, a pool of each offspring expressing the aggressive, non-aggressive or shoaling, non-shoaling behaviors will be used to perform a transcriptome analysis in order to identify differences in gene expression. All behavior results will be analyzed with an alpha of 0.05 with an ANOVA and LSD test or a Kruskal Wallis depending on normality. The establishment of these *Astyanax mexicanus* lineages and the identification of differences in the genetic expression will help to search conserved genes in species of importance for aquaculture.

The evolution of temperature in *Astyanax*

Julius Tabin, Nicolas Rohner, Alexander Haro, Johanna Kowalko, Brian Martineau,
Richard Borowsky, Cliff Tabin

Even though a human can survive equally well in both fifty degree weather and seventy degree weather, why is it that most would prefer to be in the latter? Everybody has preferences, but not much is known about the genetics of preferences or how they evolve. We have been studying temperature preference of *Astyanax mexicanus* populations from different caves and of surface population living in adjacent rivers. In principle, the temperature in the caves differs from, and is more stable than, the temperature in rivers, and we hope to measure this during our time in Mexico, which could lead to selection for different preferences. It would make sense that the caves have different temperatures, as we have indeed found that the cave populations have different temperature preferences. In particular, the fish from the Molino cave like warmer temperatures, the Pachon fish like cooler temperatures, and the Tinaja fish and river fish both seemed to like moderate temperatures. Genetic analysis indicates that these are inherited traits. For example, when we crossed the Molino and Pachon fish, the hybrids overwhelmingly went to the warmer temperatures like the Molino fish. We are now analysing F2s to determine if the trait is Mendelian or if it is more complex.

Distinct neural mechanisms underlie the convergent evolution of sleep loss in the Mexican cavefish

James Jaggard, Beatriz Robinson, Ian Oh, Pavel Masek, Masato Yoshizawa, Alex Keene

Sleep is a fundamental behavior found nearly ubiquitously throughout the animal kingdom, yet the function of sleep remains largely unknown. A defining characteristic of sleep is elevated arousal threshold, where a greater sensory stimulus is required to induce response during sleep relative to waking states. While this feature of sleep is conserved across phyla, the biological basis underlying interactions between sleep and sensory processing remains poorly understood. In the Mexican cavefish, *Astyanax mexicanus*, the evolution of cave populations has resulted in the convergent evolution of sleep loss, providing a unique opportunity to examine the evolution of sleep in response to a changing environment. In addition to sleep changes, *A. mexicanus* cave populations have enhanced non-visual sensory systems including a greater number of mechanoreceptive lateral line neuromasts that detect water motion and vibration. Here, we investigate the relationship between sensory systems and sleep. Ablation of the lateral line with gentamicin restores surface fish levels of sleep in Pachón cavefish, suggesting that increased sensory input from the lateral line underlies sleep loss. Localized lateral line ablations, and analysis of surface-cave hybrids reveal superficial neuromasts in the trunk and cheek are required for sleep loss. Strikingly, no effect of lateral line ablation on sleep is observed in four different cave populations tested. Therefore, these findings suggest enhanced sensory input underlies evolutionarily-derived sleep loss in Pachón cavefish, and also, suggest that distinct sensory bases regulate the evolution of sleep loss in independently derived populations of Mexican cavefish.

Caves, infectious diseases and its reservoirs in México

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Caves have been important for humans since our beginnings, as refuge and as sacred sites. With time cave uses have changed, and are now frequently used for research as well as recreational activities. Caves present unique and very stable environmental conditions that provide habitat or shelter to a varied fauna, including vertebrates (like bats and rodents) and invertebrates (like mosquitoes and fleas), with high numbers of endemic taxa. Some of these animals can act as vector of diseases that can infect humans. In this work I present a list of the infectious diseases that can be transmitted to humans in cave environments in Mexico, their animal vectors and some preventive measures to avoid infection. The main infectious diseases in Mexico are: rabies, histoplasmosis, leptospirosis, leishmaniasis, tetanus and malaria. Their main vectors are bats, rodents, mosquitoes and fleas. In Mexico there are reported over 30 species of bats infected with rabies and recently leishmaniasis have been reported in 13 different bat species. A little known way to get rabies in caves is by aerosols, although reported cases are infrequent; in this case the virus keeps floating in the air and infection can occur through the respiratory system, eyes and/or superficial wounds. Some preventive measures that we can implement are the use of protective masks and glasses, insect repellents, to wear resistant clothes and leather gloves against bites, and get vaccinated against tetanus and rabies.