USING HIGH SPEED KINEMATICS TO INVESTIGATE KINEMATIC VARIATION ACROSS POPULATIONS OF *ASTYANAX MEXICANUS*

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ABSTRACT

USING HIGH SPEED KINEMATICS TO INVESTIGATE KINEMATIC VARIATION ACROSS POPULATIONS OF *ASTYANAX MEXICANUS*

Agniva Sinha

Kinematic analysis pipelines now provide temporal and geometric resolution for determining a variety of body movements. These machine-learning trained programs allow behavioral neuroscientists to dissect complex behaviors and relate changes in those movements to conserved neural assemblages. These analyses are common in model organisms, but a lack of ethologically driven kinematic work in non-models has hampere d our understanding of how evolution shapes behavior through changes in sensorimotor circuits. *Astyanax Mexicanus* is a unique model organism for investigating how the brain adapts to changes in environmental conditions and how evolutionary divergence at the genotypic and phenotypic level impacts behavior. In this study, we analyzed the kinematic movements in open-arena assays of 1- to 2-week-old larvae of wildtype and hybrid genotypes of surface fish and cavefish populations (Molino, Tinaja, Pachon). 10-15 second videos of open arena conditions were recorded at 200 fps, specifically aimed to identify behavioral differences in light (white light) and dark (infrared light) conditions. Bout transition probabilities were analyzed using spectral gap and spectral entropy metrics. Analysis of bout transition probabilities in cave and surface populations reveals that all populations exhibit a conserved dominant exploratory scaffold, but differ in how they allocate probability

mass across subsidiary motor programs. Baseline flexibility across all conditions for surface fish is generally higher while cavefish depict more stereotypy in behaviors. In general, transitions from IR to white light resulted in increased mixing of behavioral states. These results suggest that central sensorimotor architectures are preserved, with the process of evolution exerting change upon bout dynamics through genetic variation and environmental variability.

DEDICATION

This thesis is dedicated to the members of the Kozol lab, especially prior members who helped establish the necessary groundwork for performing the behavioral work we were able to do across *Astyanax* populations. I am also extremely grateful for the patience and persistent support provided by the Sinha family in encouraging me to pursuit my higher education.

ACKNOWLEDGEMENTS

I want to express my gratitude to the current and former members of the Kozol lab in aiding in duties such as fish husbandry, video recording/tracking, and providing useful insights into other aspects of *Astyanax* as a model organism. I would also like to express my gratitude for the thesis committee in providing guidance through these research efforts. Finally, I would like to extend by gratitude for Dr. Kozol, who helped conceptualize the background for this project and its evolutionary context.

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INTRODUCTION

Behavioral kinematics utilizes the classification and stereotypy of body movements to investigate biological mechanisms driving complex behavior (*1*). Kinematic bouts are typically described as discrete movements that can be generalized based on motivation, such as pursuit (forward motion) or reorientation (turns) (*2*). Complex behaviors emerge from these discrete movements and behavioral adaptation is only limited by the potential frequency and concatenation of this predefined set of bout types. While pioneering studies in model organisms have helped us understand how the vertebrate brain selects movements (*3-5*), we know little about how changes in environmental composition and standing genetic variation influences the selection, order and frequency of these bout types (*6*). In this regard, much work is needed to determine how the internal processes (neurogenetics) and external processes (environmental variability) collide to govern movement selection and generate novel behavior.

Zebrafish larvae have provided an ideal model for studying how premotor and motor regions of the brain control kinematics in relation to numerous behavioral states (*3, 5- 8*). Early kinematic studies helped define bout types that are produced by larval zebrafish, including slow swims, turns (e.g. j-turn) and escape responses (e.g. slc) (*8*). These bout definitions were then neurologically detailed via precise developmental and neurogenetic mechanisms that shape the local (hindbrain to brainstem) and global (descending reticulospinal) descending drive that governs complex behavior (*3, 5*). More recently, zebrafish studies have

turned towards machine-learning based kinematic pipelines, including ZebraZoom (*2*) and MegaBouts (*9*), which defines 13 different swim bout categories based on distinct changes in body angle (*9*). While this infrastructure provides state-of-the-art kinematic tools for the model community, significant efforts have not been made to characterize kinematic variation for emerging behavior across the over 50,000 described bony fish species (*1, 10*). Therefore, non-model teleost species can be used to determine how genome-wide variation across closely related groups and ecotype variability shapes behavioral evolution through changes in kinematic dynamics.

The blind Mexican cavefish (Astyanax mexicanus) is a species with ancestral surface-dwelling populations and over 30 independently derived cave-dwelling populations (*11, 12*). Cavefish populations have evolved in an environment that is unique from their surface-dwelling counterparts that has led to behavioral adaptations, such as loss of vision (*13*), enhanced sensitivity of the lateral line (*14*), and novel behaviors related to light (*15*) and olfactory stimuli (*16*). Importantly, these populations exhibit genome-wide variation that can be harnessed through surface to cave hybrids for making genotype-phenotype discoveries (*17*). Overall, cavefish provide an exceptional model for investigating how genetic variation and environmental variability drive changes in larval kinematics and the selection of movements that govern motivated behavior.

This study integrated zebrafish-based machine learning programs into a kinematic pipeline for *A. mexicanus* to determine how kinematic dynamics vary across populations. Open-arena behavior from surface fish and three independently

derived cave populations were analyzed in relation to ecotype, light (surface) and dark (cave) conditions. We found that cavefish retain all bout categories but exhibit a reduction in approach swims that are distributed across slow swims and turn bouts in open arena paradigms. Analysis of bout transition probabilities in surface fish and cavefish reveals that all populations exhibit a conserved dominant exploratory scaffold, but differ in how they allocate probability mass across subsidiary motor programs. Treating transition probabilities as a Markov chain model, transition dynamics were analyzed using spectral entropy and spectral gap methods. Baseline flexibility across all conditions for surface fish is generally higher while cavefish depict more stereotypy in behaviors. In relation to lighting conditions, transition from IR to white light resulted in increased mixing of behavioral states. In total our study reveals evolutionary shifts in bout type choice and deployment for resting state exploration and homeostasis.

METHODS

Animal Care and Husbandry

Larvae from wildtype and hybrid populations were generated by induced spawning in adult populations of *A. mexicanus*. Adult populations of *Astyanax mexicanus* were housed in the cavefish facility and cared for according to the IACUC approved protocol 2073. Spawning populations are maintained in a Pentair recirculating rack and 20-gallon stand-alone tanks in the St. John's University cavefish facility. Fish were bred by raising the water temperature from 23 °C to 28 °C and feeding extra meals of bloodworms. Fertilized embryos were cleaned and maintained for two weeks on a 14:10 hour light and dark light cycle in a 23 °C incubator and fed from day 6 to day 14.

Behavioral paradigm and Video Capture

Larvae were assayed between 6-14 dpf and food was withheld until after assessing behavior. All behavioral videos were captured between 10 am – 2 pm. The behaviors of 1-2 week old surface fish and cavefish populations (Molino, Pachon, and Tinaja) (n=40 each) were recorded in intervals of 10-15 seconds with an initial 5 second acclimation period using a 200 frames per second camera. A 33x37 mm square arena was constructed out of a petri dish using sylgard gel. The depth of the arena was kept at 2 mm. Strips of white and infrared light were used to generate light and dark conditions, respectively. In addition to wildtype populations of surface fish and cavefish, surface to cave F_2 hybrids were used (n = 80) to evaluate bout dynamics in comparison to wildtype populations how genetic variation influences decision making and bout tendencies of the hybrid

population might cluster relative to wildtype populations.

Kinematic software and analyses

Following recordings, videos were computationally grayscaled and analyzed using the behavioral software Zebrazoom (*2*) and SLEAP (*18*). Both software required annotations of up to 6 points of the fish along the head up to the tail of the fish. Due to computational limitations in implementing a full body tracking system, we used the more tested and safer option of utilizing head and swim bladder anatomical key points to identify movement bouts using head and swim bladder angular displacements. The database MEGABOUTS (*9*) was used to identify discrete bout categories within the swim videos after anatomical key points were computationally identified. The most accurate tracking methods required between 7-9 key point identifications.

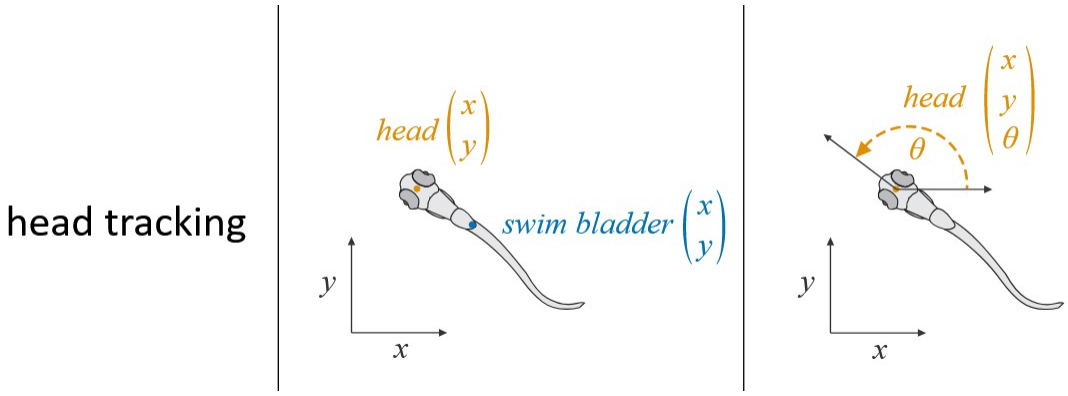

Figure 1: Anatomical Annotation for Tracking
Sample annotations for how fish larvae are anatomically tracked within a behavioral space. Outputs of anatomical key points are then used for kinematic analysis and converted to bout categories using MEGABOUTS.

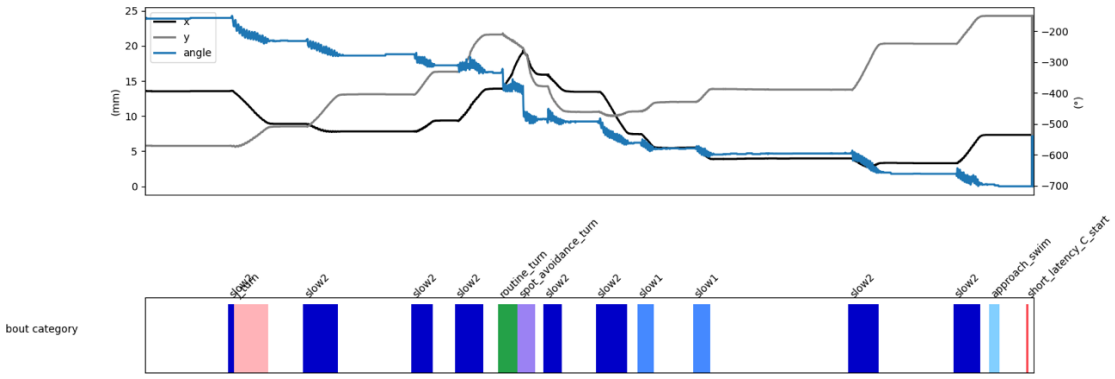

Figure 2: Angular Displacements and Bout Categories
Readouts of head and swim bladder displacement are converted into bout categories using MEGABOUTS software. Across video samples, angular displacements are identified as discrete bout categories which can be analyzed across populations.

After bout categories were identified, a transition matrix was created to identify frequency of transition from one bout state to another across populations. Raw counts were transformed into normalized frequencies. Transition matrices with normalized probabilities were then analyzed using spectral gap and spectral entropy metrics to identify extent of behavioral mixing across bout states or circuit stereotypy. Bout transition probabilities were structurally visualized and analyzed as decision tress with edge weights representing transition probabilities. Directed graph properties such as unilaterality or bilaterality across transition states, extent of symmetry or extent of stereotypy were visualized for analysis.

Mathematical Modeling

We model behavior as a **Markov process**:

$$P_{ij} = \Pr(\text{next behavior} = j \mid \text{current behavior} = i)$$

This defines a transition matrix P, which encodes how behavior flows through state space. Spectral gap analyzes how quickly an organism settles into a

behavioral pattern while spectral entropy looks at how behavioral patterns are spread. Let $\lambda_1 = 1$ and λ_2 be the second-largest eigenvalue (in magnitude) of P. The spectral gap ($1 - |\lambda_2|$) indexes the rate at which behavioral sequences converge toward equilibrium, with larger gaps reflecting greater flexibility and exploratory routing.

Metric	Value	Mathematical Meaning	Behavioral Correlate	Intuition
Spectral Gap	High	Fast convergence to stationary distribution ($1 - \lambda_2$ large)	Strong, directed transition structure; behavior quickly settles into dominant pathways	Coherent, structured flow
Spectral Gap	Low	Slow convergence; λ_2 close to 1	Persistent switching, weak structure, or long-lived loops	Diffuse or "wandering" dynamics
Spectral Entropy	High	Stationary distribution spread across many states	Broad behavioral repertoire; many states used	Diverse, exploratory behavior
Spectral Entropy	Low	Stationary distribution concentrated in few states	Restricted repertoire; few dominant behaviors	Stereotyped or constrained behavior

Table 1: Explication of Spectral Metrics

Spectral entropy looks at the extent to which behaviors through a period of time are distributed across each behavioral state. High entropy suggests a large

behavioral repertoire while low entropy shows a reliance on few dominant behaviors. High spectral gap suggests that there are strong behavioral motifs or loops present while low spectral gap is indicative of diffuse behaviors. First compute the **stationary distribution** π, where:

$$\pi = \pi P$$

Then compute entropy:

$$H = -\sum_i \pi_i \log \pi_i$$

Often normalized:

$$\frac{H}{\log (N)}$$

RESULTS

Cross-Population Comparison of Bout Probabilities

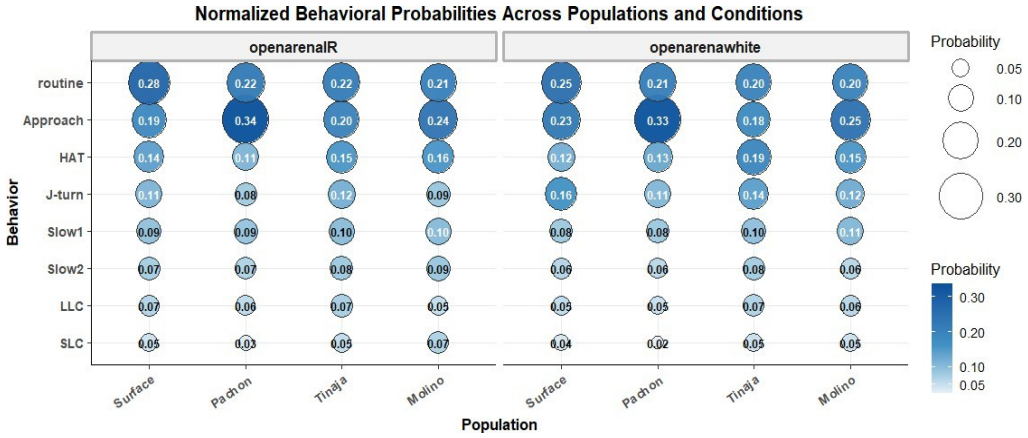

Figure 3: Cross Population Bout Probabilities
Balloon plots representing proportions (plot sizes and shade represent probability of transition) for each bout category, comparing lighting conditions (infrared versus white light) and populations (all cave populations, and surface populations).

Normalized behavioral probabilities revealed a conserved hierarchy of state occupancy across populations and lighting conditions (Fig. 3). Across all groups, behavior was dominated by **Routine** (≈0.20–0.28) and **Approach** (≈0.18–0.34) **bouts**, followed by intermediate states (**HAT**, **J-turn**), with **Slow1**, **Slow2**, **LLC**, and **SLC bouts** consistently occupying lower proportions. This indicates a shared behavioral repertoire with stable baseline structure. Population differences emerged as shifts in relative weighting within this conserved hierarchy. **Pachon** exhibited elevated **approach** probabilities across both conditions, whereas **surface fish** showed greater allocation to **Routine** behavior. **Tinaja** displayed intermediate distributions,

while **Molino** exhibited a more uniform allocation across states, lacking strong dominance of any single behavior. Lighting induced modest but consistent redistribution of behavioral probabilities. Under white light, **Approach**, **J- turn**, and **HAT** were generally enhanced, accompanied by slight reductions in routine behavior, suggesting increased engagement of orientation and sensorimotor processes. In contrast, IR conditions favored more routine-dominated allocation. Together, these results demonstrate that behavioral variation across populations arises from **quantitative reweighting of conserved states**, with environmental context modulating their relative occupancy rather than altering the underlying behavioral repertoire.

Spectral Analyses

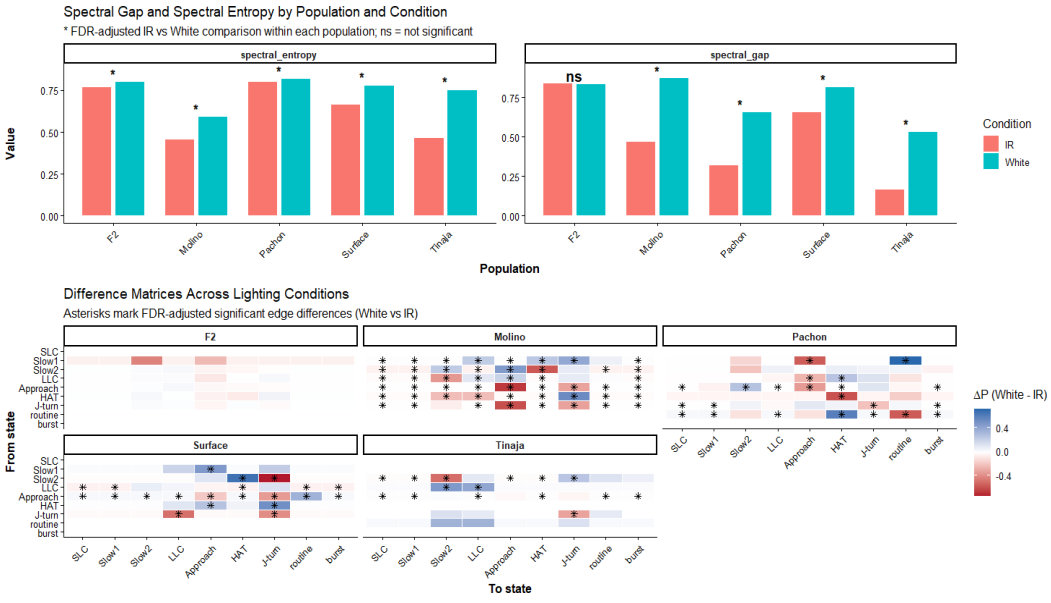

Figure 4: Spectral Analyses and Difference Matrix

Comparison of spectral gap and spectral entropy measures across populations of larval fish and comparing lighting conditions. Previously

normalized transition probabilities are visualized as a difference matrix to contrast lighting conditions (delta = white light – IR light).

We utilized spectral analysis to investigate the predictability and complexity of kinematic movements across surface fish and cavefish populations. The spectral gap ($1 - |\lambda_2|$) quantifies how quickly a behavioral sequence "forgets" its past and converges to a stable pattern. In behavioral terms, a larger spectral gap indicates faster switching and greater flexibility, whereas a smaller gap reflects persistent loops or stereotyped behavioral sequences. Spectral entropy measures how evenly behavioral activity is distributed across available states. In behavioral terms, high entropy indicates a diverse, exploratory repertoire with many states used, whereas low entropy indicates dominance of a few behaviors and a more restricted, stereotyped repertoire.

Spectral analysis revealed population- and context-dependent reorganization of behavioral dynamics (Fig. 4). Across populations, spectral entropy varied systematically, with Pachon and F_2 hybrids exhibiting the highest values, whereas Molino and Tinaja showed reduced entropy. Entropy was significantly increased under white light across all populations (FDR-adjusted, $q < 0.05$), indicating a consistent expansion of behavioral diversity under illuminated conditions.

In contrast, spectral gap showed strong sensitivity to both population and environmental context. White light significantly increased spectral gap in all populations except F_2 hybrids (FDR-adjusted, $q < 0.05$), indicating enhanced convergence toward structured transition dynamics. This effect was

most pronounced in Molino, Pachon, and Tinaja, which exhibited low spectral gap under dark conditions but markedly higher values under white light. F_2 hybrids uniquely showed no significant modulation by lighting, maintaining high spectral gap across conditions.

Edge-level permutation testing further revealed that these global changes arise from specific, non-uniform rewiring of transition probabilities. Edge-level permutation testing is a statistical approach used to determine whether changes in individual transition probabilities (edges) between conditions are greater than expected by chance. For each transition, behavioral sequences are randomly shuffled across conditions to generate a null distribution of expected differences. The observed change in transition probability (e.g., White − IR) is then compared to this null distribution. Transitions whose observed differences exceed this random expectation are identified as significantly altered, after correction for multiple comparisons. Difference matrices (ΔP = White − IR) identified FDR-corrected significant edge differences ($q < 0.05$) concentrated in transitions involving Approach, J-turn, and LLC-associated pathways, consistent with enhanced orientation and engagement under white light. These effects were population-specific, with Surface and Pachon showing widespread significant changes, whereas F2 hybrids exhibited fewer significant edges, indicating reduced sensitivity to environmental modulation.

Together, these results demonstrate that behavioral variation across populations is governed by coordinated changes in both state-space

distribution (entropy) and transition structure (spectral gap), with environmental input selectively reshaping transition pathways in a statistically supported, population-dependent manner.

Significant Comparisons

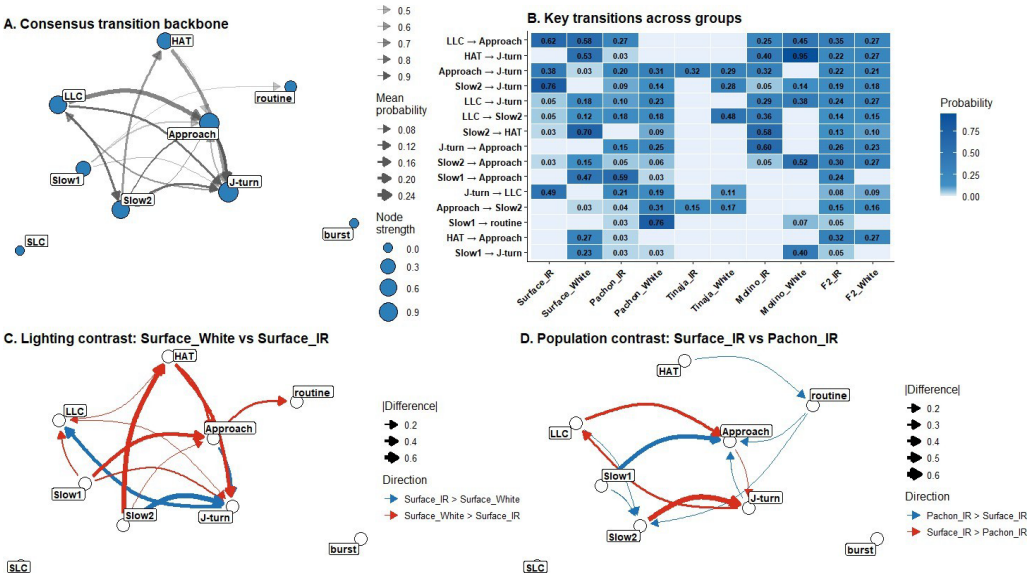

Fig. 5: Significant Comparisons

Conserved transition architecture and population- and condition-specific rewiring of behavioral dynamics. A) Consensus transition backbone derived from all populations and conditions, illustrating a conserved core network centered on Approach, J-turn, Slow2, and LLC states. Edge thickness represents mean transition probability and node size reflects overall state strength, indicating a shared dynamical scaffold underlying behavioral organization. B) Key transition probabilities across populations and conditions, highlighting conserved pathways alongside population-specific differences in edge weighting. Values denote transition probabilities, revealing systematic reallocation of flow through the shared network. C) Within-population contrast (Surface, White − IR) showing directional rewiring of transitions under sensory modulation. Red edges indicate strengthened transitions in white light, whereas blue edges indicate reduced transitions relative to infrared, demonstrating selective enhancement of orientation- and approach-related pathways. D) Between-population contrast (Surface

vs Pachon under IR) illustrating divergence in transition structure within the conserved network. Edge direction and magnitude reflect relative strengthening of pathways, indicating population-specific routing biases despite shared topology.

Behavioral dynamics across populations were organized around a conserved transition backbone centered on Approach, J-turn, Slow2, and LLC, forming a recurrent core that constrained behavioral flow (Fig. 5). Despite this shared architecture, transition strengths were differentially reweighted across populations and conditions. Key transitions involving Approach, J-turn, and Slow2 varied systematically, indicating that behavioral divergence arises from modulation within a common network rather than the emergence of novel states.

Contrast analyses revealed targeted, directional rewiring of transitions. Within populations, white light selectively strengthened pathways into Approach and HAT and enhanced Slow2–J-turn coupling, while weakening alternative routes (FDR-adjusted, $q < 0.05$). Between populations under identical conditions, Surface fish showed stronger routing through Approach and J-turn, whereas Pachon exhibited relatively greater engagement of slower and intermediate states. Together, these results demonstrate that both environmental and evolutionary effects reshape behavior by reweighting edges within a conserved transition network, linking local transition changes to global dynamical differences.

Clustering and Behavioral Space

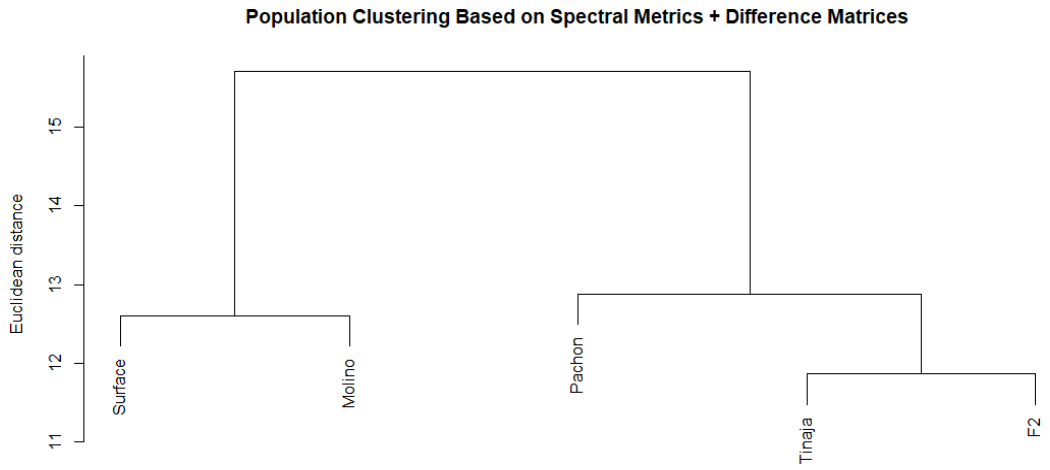

Figure 6: Hierarchical Clustering
Population-level clustering reveals higher-order organization of behavioral dynamics. Hierarchical clustering of populations based on combined spectral metrics (spectral gap and entropy) and lighting-dependent difference matrices (White − IR). Distances reflect Euclidean dissimilarity in global dynamical structure and transition reweighting.

Hierarchical clustering of populations based on combined spectral metrics and White–IR difference matrices revealed two major dynamical groupings (Fig. 6). Surface and Molino clustered together, indicating similar overall organization of transition structure and lighting-dependent reweighting despite differences in individual behavioral distributions. In contrast, Tinaja and F2 formed a second, closely related cluster, consistent with shared intermediate or less specialized transition dynamics. Pachon grouped with the Tinaja–F2 branch but remained more distinct, suggesting partial overlap with this dynamical regime while retaining population-specific transition structure.Together, these results indicate that populations segregate not simply by single behavioral features, but by higher-order similarity in both global spectral organization and environmentally induced rewiring of transition probabilities.

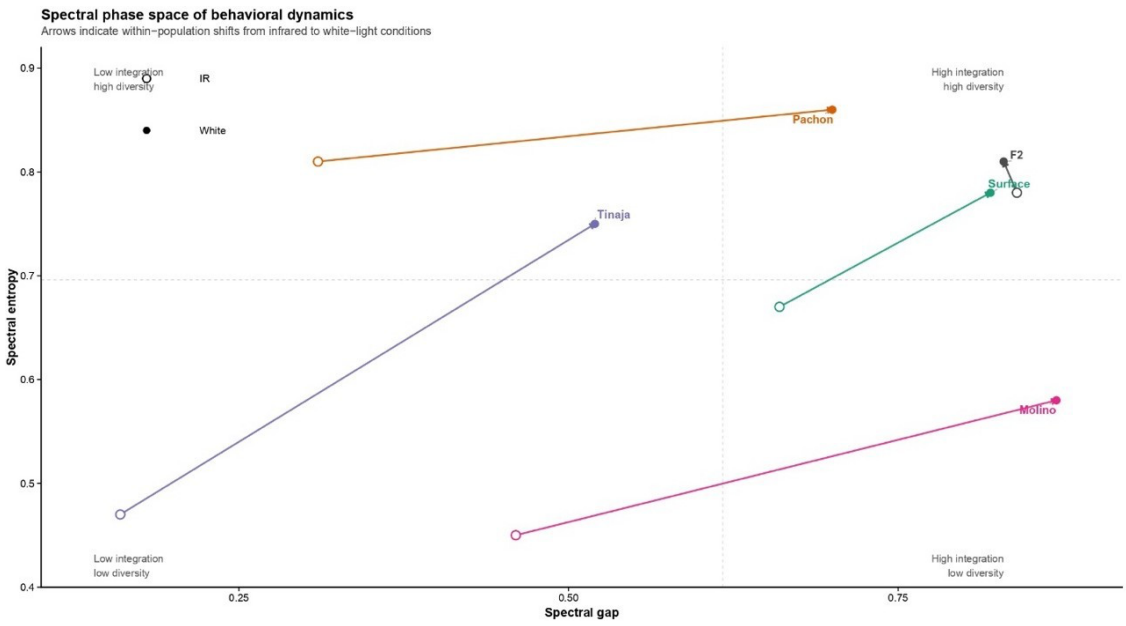

Figure 7: Behavioral Space Analysis
Spectral phase-space representation of behavioral dynamics showing population-specific shifts in integration (spectral gap) and diversity (entropy) from infrared to white-light conditions, with arrows indicating direction and magnitude of reorganization.

Projection of population-specific transition dynamics into spectral phase space revealed structured, condition-dependent reorganization of behavioral regimes (Fig. 7). Across all populations, white-light exposure induced a consistent rightward shift along the spectral gap axis, indicating increased dynamical integration and faster convergence of behavioral sequences. This effect was most pronounced in Tinaja and Molino, which exhibited large displacements from low-gap, low-entropy regimes under infrared conditions toward more integrated states under illumination. In parallel, spectral entropy increased across populations, reflecting a generalized expansion of behavioral diversity, with Pachon and F_2 hybrids

occupying the highest-entropy regions of the phase space.

Despite these shared directional trends, populations remained segregated within distinct regions of the phase space, indicating persistent differences in underlying transition structure. Surface and F_2 hybrid populations were localized within a high-gap, high- entropy regime across both conditions, consistent with intrinsically flexible and diversified behavioral dynamics. In contrast, Molino remained confined to a lower-entropy regime despite increased integration, suggesting constrained diversification of state usage. Pachon exhibited high entropy with moderate-to-high integration, reflecting distributed yet structured transition dynamics.

Notably, the magnitude and direction of IR-to-white trajectories differed across populations, indicating non-uniform sensitivity to environmental modulation. Tinaja displayed the largest combined increase in both spectral gap and entropy, consistent with a strong release from constrained dynamics under illumination. In contrast, F_2 hybrids exhibited relatively modest displacement despite occupying a high-integration regime, suggesting reduced responsiveness to sensory context. Together, these results demonstrate that environmental input drives a coherent shift toward more integrated and diverse behavioral dynamics, while preserving population-specific organization within the underlying state space.

DISCUSSION

A Conserved Behavioral Backbone with Environment-Dependent Modulation

Kinematic studies provide behavioral context for how the brain selects movements from predefined neuronal assemblages (*1, 4*). We know that these conserved circuits are essential to provide adaptive behavior across generations, but they also constrain behavioral evolution by limiting the potential for novel movements or bout types (*19*). Therefore, behavioral evolution is more likely to emerge from changes in bout selection and the dynamics that underlie bout stasis and flow. Our study utilized this notion to investigate how genetic diversity and environmental ecotype impacts the evolution of larval kinematics. We hypothesized that kinematic repertoires would vary depending on lighting conditions and genetic backgrounds. Overall, we found that all populations generate behaviors based on a conserved kinematic backbone and that the bout transition dynamics varied across surface and cave populations. Furthermore, light as an environmental cue impacted all populations through changes in behavioral flow, while surface to cave hybrids exhibited a muted response to light changes and a flattening of behavioral dynamics that may reflect kinematic dissonance related to wildtype genetic variation.

Evolution Within a Constrained Behavioral State Space

Behavioral dynamics across populations were organized around a conserved transition backbone, indicating that all groups operate within a shared behavioral state space. Behavioral dynamics across populations were

organized around a conserved transition backbone, indicating that all groups operate within a shared behavioral state space. This observation is consistent with longstanding ethological and systems-level work demonstrating that animal behavior is composed of conserved motor modules and stereotyped action patterns, as originally described in studies of fixed action patterns (*20, 21*). More recent quantitative analyses further show that even complex, freely behaving animals occupy low-dimensional behavioral manifolds defined by a limited repertoire of movements, rather than continuously generating novel actions (*22, 23*). In zebrafish specifically, larval locomotion is structured around a conserved set of bout types, with variability arising from differences in selection and sequencing rather than the introduction of new kinematic states (*24*). Within this framework, evolutionary change is thought to act primarily on the organization of transitions between behavioral states, consistent with dynamical systems perspectives in which behavior is governed by trajectories through a constrained state space shaped by underlying neural circuitry (*25*). Thus, rather than representing a trivial consequence of shared species identity, the conserved backbone observed here provides a critical constraint within which population-specific differences emerge. Our results show that divergence occurs primarily in the structure and coordination of transitions within this shared space, as reflected in differences in spectral gap, entropy, and edge-level reorganization. This supports a model in which behavioral evolution proceeds through reweighting of existing transitions and modulation of

dynamical flow, rather than expansion of the underlying behavioral repertoire.

Spectral Signatures of Population-Specific Dynamics

However, spectral analysis revealed that populations diverge in how transitions are structured within this space. Cave-adapted populations exhibited distinct configurations of entropy and spectral gap, reflecting differences in both the diversity of behavioral states and the strength of dynamical constraints. Molino displayed low-entropy, high-gap dynamics consistent with strongly constrained attractor behavior, whereas Pachon maintained high entropy with structured transitions, indicating a more distributed but organized behavioral repertoire. Surface fish exhibited intermediate characteristics, while Tinaja showed reduced dynamical stability. Notably, hybrid individuals exhibited flattened behavioral distributions and diminished sensitivity to environmental modulation, suggesting a disruption of coordinated transition architectures. Across all populations, lighting conditions altered specific transitions within the network, particularly those involving orientation and approach behaviors, indicating that environmental context reshapes behavioral flow rather than overall activity levels. Together, these findings demonstrate that behavioral evolution operates through reorganization of transition dynamics within a conserved state space.

Behavioral Evolution as Dynamical Reorganization

The present results demonstrate that behavioral evolution in this system is governed by reorganization of transition dynamics within a

conserved sensorimotor architecture. This interpretation is consistent with a growing body of work showing that animal behavior is composed of conserved motor primitives organized within low-dimensional dynamical manifolds, with variability arising primarily from changes in selection and sequencing rather than the emergence of novel behavioral states (*20, 26*). At the neural level, population dynamics studies further support the idea that behavior reflects trajectories through constrained state spaces shaped by underlying circuitry (*27*), reinforcing the view that functional diversity emerges from modulation of dynamical flow rather than expansion of the representational space itself. Similarly, across species and contexts, perturbations such as genetic variation or environmental change have been shown to alter behavioral distributions and transition structure without introducing new behavioral categories (*23, 24*). Within this broader framework, the present findings extend existing theory by demonstrating that such transition-level reorganization can be quantitatively captured through spectral properties of behavioral networks, revealing coordinated changes in integration (spectral gap), state distribution (entropy), and edge-level structure. Notably, the F2 hybrid population departs from both parental patterns, exhibiting a dissociation between global dynamical organization and local transition plasticity. This suggests that evolutionary and genetic perturbations can decouple baseline behavioral structure from adaptive reconfiguration, a phenomenon not predicted by classical models of gradual or intermediate inheritance. Together, these results provide empirical support for

a dynamical systems view of behavioral evolution, in which conserved sensorimotor architectures constrain the space of possible behaviors while evolutionary processes reshape the flow of activity within that space.

CONCLUSION

This study shows that behavioral differences across Astyanax mexicanus populations arise from reorganization of transition dynamics within a conserved kinematic framework, rather than the emergence of novel behaviors. Across all groups, a shared transition backbone constrained behavioral structure, while population-specific differences were driven by quantitative reweighting of transition probabilities.

Spectral analyses demonstrated that these differences manifest as coordinated changes in behavioral flexibility (spectral gap) and repertoire diversity (spectral entropy), linking local transition structure to global dynamical organization. Environmental modulation via lighting consistently increased both integration and diversity, but did so in a population-dependent manner, preserving distinct dynamical regimes.

Notably, hybrid individuals exhibited reduced sensitivity to environmental change and altered transition organization, suggesting that genetic recombination can disrupt the coupling between baseline behavioral structure and adaptive plasticity. Together, these results support a framework in which behavioral evolution operates through modification of transition structure within conserved sensorimotor constraints, providing a quantitative basis for comparing behavioral dynamics across populations and conditions.

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