

**Do early competitive outcomes have long-term consequences for cognitive ability in the  
Mangrove Rivulus?**

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## ABSTRACT

Individuals vary in their cognitive abilities for a number of reasons, one of which includes the influence of their early environment. Early social experiences and stressors can affect brain development, resulting in cognitive differences later on in life. This study examined whether competitive interactions early in life affected later associative learning in the mangrove rivulus (*Kryptolebias marmoratus*), a self-fertilizing hermaphroditic fish that essentially produces genetic clones of itself. Genetically identical siblings that hatched on the same day were paired together and grew up in a competitive environment for approximately five months with one fish eventually becoming larger (and presumably dominant) over its smaller, subordinate partner. Siblings were then separated and after five additional months, each individual was trained to associate a particularly patterned wall with a food reward over several days. At the time of these learning trials, subordinate partners had recovered from their initially smaller size and there was no remaining size difference between partners. Despite this compensatory growth, the consequences of the early competitive social environment continued to affect performance. Specifically, the initially larger dominant partner tended to successfully reach the rewarded feeder quicker than their initially smaller subordinate partner. Interestingly, no evidence was found that individuals improved in their performance over time (i.e. no learning), but instead these underlying differences between the initially large and small individuals seemed to be present across all of the trials. Overall, these findings suggest that early social and competitive experiences can have lingering effects on individuals and result in subtle differences on their performance and cognitive abilities.

## INTRODUCTION

Within a population, individuals often vary dramatically in their cognitive or brain-based performance and ability to learn associations with particular cues in the environment (Boogert et al. 2018). This variation in cognition among individuals has been tied to differences in individuals' morphology and physiology (Wallace et al. 2022), personality (Belgrad and Griffen 2016), and dominance rank (Barnard and Luo 2002, Pongracz et al. 2012). For example, mice who established a high-ranking status performed better in a maze learning task than low-ranking mice (Barnard and Luo 2002). In some cases, cognitive performance can be linked to underlying differences in hormones associated with dominance status (Wallace et al. 2022). While current differences among individuals might contribute to cognitive differences, differences that emerge earlier in life can also play a role.

A variety of experiences early in life can shape an individual's learning performance in adulthood. Early enrichment, access to exercise, larger housing space and social grouping can have strong effects on learning performance later in life (Lambert and Guillette 2021, Montalbano et al. 2022, Salvanes et al. 2013). Social experiences in particular can be a major component of an individual's development. In fishes, environmental enrichment that includes social interactions improves learning ability (Montalbano et al. 2022, Salvanes et al. 2013, Carbia et al. 2019). For example, in group-living cichlids, a large portion of many animals' lives consist of interacting with group members. Their social competency and performance in these interactions can strongly depend on whether individuals interact with siblings versus parents early in life. In addition, individuals raised by parents behave better in social interactions than when they are reared only with siblings (Taborsky et al. 2012). Accounting for an individual's

early environment and social experiences can give insight into an individual's behavioral responses later on.

Stressful experiences early in life might be particularly influential because they have the potential to shape the development of the Hypothalamic-Pituitary-Adrenal (HPA) axis (or in the case of fishes, Hypothalamic-Pituitary-Interrenal axis) which is the set of structures in the brain which deal with stress (Pijanowski et al. 2015). Developmental changes to the HPA (or HPI) axis can affect how individuals respond to stress for the rest of their lives. For example, mice who experienced social isolation in their early environment have an overactive HPA axis and stress response when reacting to current stressful situations (Ros-Simó and Valverde 2012). Similarly, experiencing unpredictable stressors early in life induces a variety of long term physiological changes consistent with chronic stress in lambs (Destrez et al. 2013). Furthermore, these early stressful experiences can have consequences for brain development and learning. For example, chronic stress during early development in mice affects their performance on many cognitive and memory tasks as adults (Naninck et al. 2015). Even milder stressors during early life, such as a lower food intake, can result in lower neuronal concentration and development of smaller areas of the brain that are important for song learning in zebra finches (Mishra, et al. 2020). A variety of early life stressors can have downstream effects on cognition later on.

In addition to the strong effect of the early environment on behavior, we also know that there is a genetic component to behavior. General cognitive ability in mice has substantial heritability but is also malleable to the environmental conditions experienced during adolescence (Sauce and Matzel 2018). The quality of song learning in birds is also highly heritable but less so

under enriched learning environments (Mets and Brainard 2017). In addition to these genetic effects, maternal effects and the experiences of mothers can also contribute substantially to offspring traits, including offspring cognition (Moore et al. 2019, Nettle and Bateson 2015, Boyce 2016). For example, maternal experiences with predators during reproduction can have life-long effects on associative learning in offspring (Roche et al. 2012). Differential resilience or vulnerability of particular families to early life stress could be an underlying factor contributing to variation among offspring in cognition, but it is often impossible to control for genetics and maternal effects in most systems.

This study explores how competitive interactions early in life affect later learning in the mangrove rivulus, *Kryptolebias marmoratus*. The mangrove rivulus is a self-fertilizing hermaphroditic fish that produces genetically identical offspring due to many generations of selfing, resulting in distinct genetic lineages (Awise and Tatarenkov 2015). Additionally, parents can produce multiple embryos, controlling for parental effects and experiences. By controlling genotype and parental effects, the direct effects of an individual's early social experience and competitive rank on its later cognitive ability can be examined. In this study, I hypothesized that individuals who experienced greater stress from early competitive interactions will perform better in a learning assay compared to individuals who experienced less stress.

## **METHODS AND MATERIALS**

### ***Overview***

The effect of genotype can be controlled in any developmental plasticity manipulation (Earley et al. 2012). In this study, early social experience was manipulated by pairing genetically identical siblings hatched on the same day for approximately 5 months. This pairing and the resulting competitive environment causes one fish to become larger (and presumably dominant) over its smaller partner. Siblings from the same parent were then separated and reared apart. After five additional months (at approximately ten months of age), individuals were trained to associate a particularly patterned wall with a food reward over several days.

### ***Study Species***

The mangrove rivulus, *Kryptolebias marmoratus*, is a self-fertilizing hermaphroditic fish that is found in mangrove swamps in subtropical and tropical climates (Taylor 2000). Furthermore, it is amphibious and can tolerate a wide range of water qualities and periods of drying (Taylor 2012). Although males are produced occasionally and out-crossing can occur, most reproduction is via self-fertilization of hermaphrodites in nature (Avisé and Tatarenkov 2015). Generations of selfing have resulted in distinct genetic lineages that produce genetically identical offspring (Avisé and Tatarenkov 2015). This presents a powerful natural system in which to examine plasticity while controlling for underlying genotype (Earley et al. 2012). In this study, individuals from nine distinct genetic lineages were used (chosen based on embryo availability - see below). All lineages selfed in the lab for over 9 generations. Five lineages were originally collected by R. Earley from Belize (BWS34, BWS37, BWS38, CROC27, CROC 31)

and four lineages were originally collected by R. Earley from Florida (BP3, BP11, BP18-20, BP21).

### ***Housing and Maintenance***

Mangrove rivulus live in brackish water and in the lab, all fish are housed in 15 parts per thousand (ppt) water made with Instant Ocean® from tap water treated with CloramX®. Fish were fed >1 ml live *Artemia* nauplii every 1-2 days and in the brackish water of the fish containers, nauplii continue swimming for >24 hours providing individuals with live food over an extended period. Food levels were adjusted as individuals grew. All individuals were on a 12-hour light cycle between 700 to 1900 hours, and the temperature of the fish room was maintained at 25-27 °C. All assays and maintenance were approved by the Animal Care and Use Committee at the University of the South (Protocols: McGhee 1-2021 and 13-2022) and adhered carefully to guidelines for ethical animal treatment (ASAB/ABS 2018).

### ***Offspring Collection***

In December 2021 and January 2022, embryos were collected from the nests in adult containers and housed until hatching in small deli cups (bottom diameter = 2.5 cm, height = 3 cm) with 15 ppt saltwater. Two genetically identical siblings from the same parent that hatched on the same day were transferred into single deli cups (bottom diameter = 2.5 cm, height = 6 cm) filled with 15 ppt water and with a 3 cm<sup>3</sup> ball of polyester floss (Poly-Fil®), typically used for stuffing pillows, as cover. Each pair was given a random number unrelated to their genetic lineage. A total of 17 pairs were set up from 9 distinct genetic lineages.



### ***Rearing and Social Treatment***

After 8 weeks, pairs were transferred together into a 750 ml Rubbermaid® TakeAlongs® Deep Square container filled with clean 15 ppt saltwater and their ball of polyester floss. At approximately 5 months of age, pairs were separated and transferred individually to new 750 ml Rubbermaid® TakeAlongs® Deep Square containers filled with clean 15 ppt saltwater and a new polyester floss nest (Poly-Fil®). At this time, the standard length of individuals was measured using a laminated ruler and each individual was given a random number unrelated to their large-small status within the pair and their genetic lineage so that future observations were blind to the pair identity, their status within the pair (large versus small), and genetic lineage. Fish were maintained separately in these individual containers for another 5 months.

Before conducting the learning trials, all individuals experienced a novel tank to measure exploratory behavior (at approximately 5 months of age) and were shown a mirror in their home container to measure aggressive behavior (at approximately 10 months of age). These novel tank and mirror assays were part of a separate experiment. Note that all fish used in this study experienced these same assays prior to being tested in the learning assay.

### ***Learning Assay Set-up***

To measure learning in the mangrove rivulus, a pattern-reward association assay was conducted in the fall of 2022 when the fish were approximately 10 months old. Fish were tested singly and were repeatedly presented with two options in a new tank, a patterned wall on one side with a food reward or a different patterned wall on the other side without a food reward (Figure 1). Trials occurred in a plastic tank (35 cm x 21 cm x 21 cm) filled with 4 L of new 15 ppt saltwater with two small artificial plants in the middle, and all four sides were covered with

black plastic. The two patterned walls consisted of laminated white paper and were positioned on either end of the tank (Figure 1). Each wall was taped to a wooden skewer that could be rested on the top of the tank, letting the wall sit level with the tank bottom and sides of tank walls. Each wall had a different black and white pattern (A and B) surrounding an entrance and fish could enter each entrance freely and move along the corridor to get a food reward (or not) (Figure 1). The pattern around each entrance was drawn with black sharpie on both sides of the paper prior to being laminated and extended approximately 2 cm around the entrance. The entrance was approximately 3 cm x 3 cm and located on one side of the wall, 3 cm away from the wall's edge. A small hexagonal plastic weighboat (4.5 cm x 5cm) with a hole poked through the middle floated on the surface of the water and was positioned on the opposite and furthest side of the entrance for each wall (Figure 1). If behind the rewarded wall, the weighboat contained a food reward (live brine shrimp nauplii), and if behind the unrewarded wall, it did not. Note that four different equivalent testing tanks and walls were used repeatedly in order to observe fish simultaneously when there were multiple observers.

To account for any possible bias for a particular wall type, fish were assigned their rewarded wall type randomly based on a coin flip (heads = wall A was rewarded; tails = wall B was rewarded). Once assigned, the rewarded wall for each fish remained constant throughout their trials. To ensure that wall type was not confounded with tank side/location, a coin was flipped in the first trial to determine on which side wall A should be located (heads = left; tails = right), and for each subsequent trial, the sides were swapped and then alternated. For example, if wall A was on the left side of the tank for trial 1, it would be on the right side in trial 2, and then the left on trial 3, etc. The fish to be tested in a given week were not fed for at least 24 hours

prior to their first trial. Fish were also not fed outside of these learning trials to increase their motivation to find the rewarded feeder.

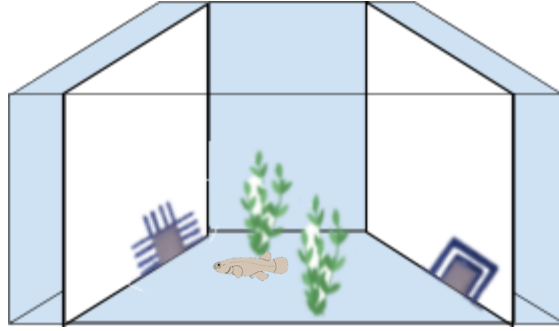
At the start of each trial, a transparent acclimation cup was positioned (height = 12 cm, 16 oz) in the middle of the tank between the two artificial plants (Figure 1B). The focal fish to be tested was transferred in a water-filled cup and gently poured into the transparent acclimation cup, where it could observe both patterned walls for 1 min. While the fish was inside of the acclimation cup, the feeder behind the rewarded side was gently filled with the reward of 0.25 mL of live brine shrimp nauplii. Note that over the course of the 10 min assay, the brine shrimp slowly escape from the small hole at the bottom of the weighboat and disperse slightly below (approximately 1 to 2 cm) the weighboat, allowing fish to feed on the reward within a body length of the feeder. After 1 min, the acclimation cup was slowly removed and the stopwatch was started. During the 10 min trial, the following were recorded: the time the focal fish entered either the rewarded or unrewarded wall (head goes into doorway), the time the fish got to the rewarded feeder (within a body length of feeder), and the number of times that the fish entered both the rewarded or unrewarded walls. After a trial, the focal fish was returned to their home container. Between each trial, the test tank was drained, rinsed with hot water, and refilled with new 15 ppt saltwater.

Each focal fish underwent four learning trials and one test trial, for a total of five sequential trials. In all trials, data was collected for a maximum of 10 min but if the fish came within a body length of the rewarded feeder and had fed on the reward for 30 sec, the trial was ended. Although data was collected for 10 min on all trials, trials 1 and 2 lasted a maximum of

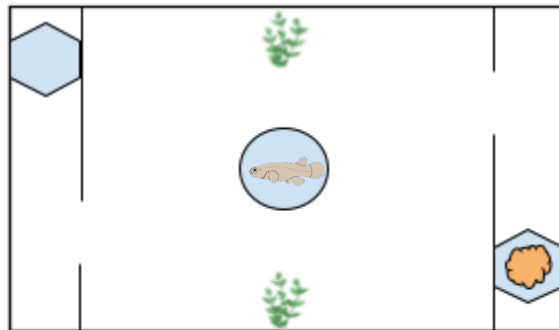
15 min, so the fish could be directed to the rewarded feeder (and the food reward) if they had not found it during the 10 min trial period. Specifically, if the fish had not entered the rewarded side after 10 min, a wooden skewer was used to guide the fish into the rewarded wall. If the fish had not reached the rewarded feeder within 15 min, the fish was guided with the wooden skewer all the way to the rewarded feeder and they could feed for 30 sec. Trials 1 and 2 were the only trials that lasted longer than 10 min and where any guidance was provided. After these trials, it was assumed that the fish was aware of the food reward and the wall patterns. Trials 3 through 5 lasted a maximum of 10 min and there was no guidance to the rewarded side. In all trials, fish who did not get to the rewarded feeder within the 10 min trial were given the maximum time of 600 sec.

A total of four training trials and one test trial were run for each fish. In all the training trials, the rewarded feeder contained live brine shrimp. To ensure that the fish were not simply finding the food reward based on some chemical cue from the brine shrimp, the final test trial did not have a brine shrimp reward and both weighboats were empty. In terms of timing throughout the day and across days, training trial 1 took place between 8 am and 1 pm on day 1. Training trial 2 took place in the afternoon between 2 pm and 7 pm on day 1, with at least 2 hours between trials 1 and 2 for any one individual. Training trial 3 took place between 8 am and 1 pm on day 2. Training trial 4 took place between 8 am and 1 pm on day 3. The test trial (trial 5) took place between 8 am and 1 pm on day 4. After all five trials, the standard length of each fish was measured with a laminated ruler. Four to five fish were tested each week, with a total of 34 individuals tested over the course of 6 weeks.

**A**



**B**



**Figure 1.** Experimental set-up for the learning assay from a (A) head-on view with wall A on the right and wall B on the left and an (B) aerial view. These images are not exactly proportionate to the actual tank set-up. The tanks were 35 cm in length with both walls holding the 4.5 cm floating feeder in place, making the corridors 4.5 cm wide and the walls 26 cm away from each other. Walls touched the sides of the tank and were level with the bottom of the tank. Both walls had a feeder tray located on the opposite end of the wall entrance. The feeder tray behind the rewarded wall contained a food reward while the other did not. The side on which the rewarded wall was positioned was alternated between trials. All sides of the tank were covered with black plastic during training and testing assays, and the tank was observed from above.

## ***Data Analysis***

First, we wanted to confirm that being reared with a sibling and experiencing competitive interactions resulted in a size difference between the partners, with one becoming the larger, dominant partner and one becoming the smaller, subordinate partner. We compared sizes between the larger and smaller partners within a pair at the time that they were separated into their single containers (at approximately 5 months of age). Second, we wanted to examine whether being reared individually for five more months caused these initial size differences to disappear (indicative of compensatory growth by the smaller partner) or whether these initial size differences persisted despite 5 months of a non-competitive environment. For both of these analyses, we used mixed models testing for the effect of initial rank (large versus small) and including pair identity and genetic lineage as random effects.

Next, we explored whether there was a bias for (or against) a particular wall pattern. Although fish were randomly assigned wall A or wall B as their rewarded wall, with 17 fish having rewarded wall A and 17 fish having rewarded wall B, any bias for (or against) a particular wall type would affect their performance on the learning assay. First, we used Chi-square tests of independence to examine whether the first wall they entered was independent of the wall type they had been assigned, at the first trial and then at the final test trial. Second, we examined whether how quickly they arrived at the rewarded feeder across the five trials was affected by the wall type they had been assigned as rewarded (A or B) using a mixed model with individual identity, pair identity, and genetic lineage as random effects. This analysis would reveal whether being assigned one of the wall types as rewarded resulted in a faster (or slower) arrival to the food reward.

Next, to assess performance in the learning assay, we examined the effects of the initial rank of the partner (large versus small) and trial (over the four training trials and final test trial) on the latency to get to the rewarded feeder ( $\ln(x+1)$  based on model residuals). A significant effect of trial would show evidence of change in how quickly individuals reached the rewarded feeder over time and potentially learning. The interaction between initial rank and trial was also included because a significant interaction would be indicative of differences between ranks in learning over time. Because fish had a tendency to enter wall B (or avoid entering wall A) regardless of its rewarded status, bias was accounted for in the analyses by including the assigned rewarded wall as a factor. Because individuals were tested repeatedly across five learning trials, the individual ID was included as a random factor (making these repeated measures analyses). Furthermore, pair identity (N=17) and the genetic lineage of the pair (N=9 lineages) were included as random factors to account for additional sources of non-independence in the data.

Finally, any differences in performance in the learning assay and arrival to the rewarded feeder between the two initially large and small fish might be due to underlying differences in boldness, with the early environment affecting whether individuals are more (or less) willing to explore and enter a wall (regardless of the food reward). Thus, we examined how latency to enter any wall ( $\ln(x+1)$  transformed based on model residuals), regardless of whether it was the rewarded or unrewarded wall, was affected by the initial rank of the partner (large vs small) and trial (over the four training trials and final test trial). As above, the individual identity, pair identity, and genetic lineage were included as random effects. The level of statistical significance was  $\alpha = 0.05$ .

### ***Data Analysis Software***

All analyses were conducted in RStudio version 2023.03.0+386 (Posit Software, PBC, 2022) in R version 4.2.2 (2022-10-31) using dplyr (Wickham et al. 2022), lme4 (Bates et al. 2015), lmerTest (Kuznetsova et al.2017), effectsize (Ben-Shachar et al. 2020) packages with figures made in ggplot2 (Wickham 2016) and ggpubr (Kassambara 2022).



## RESULTS

### *Size Differences*

The manipulation of the early competitive environment was effective at inducing differences in size. At approximately 5 months of age, large individuals were 22.3 +/- 0.3 mm (mean +/- SE) while small individuals were 20.6 +/- 0.3 mm (mean +/- SE) (Figure 2A, Table 1A). After five additional months of being reared separately without their partner and at about 10 months of age, these initial differences had disappeared. At the time of the learning assays, the sizes of partners were not significantly different from one another, the initially large individuals were 26.5 +/- 0.3 mm (mean +/- SE) and the small individuals were 26.3 +/- 0.4 mm (mean +/- SE) (Figure 2B, Table 1B).

### *Bias for Wall Types*

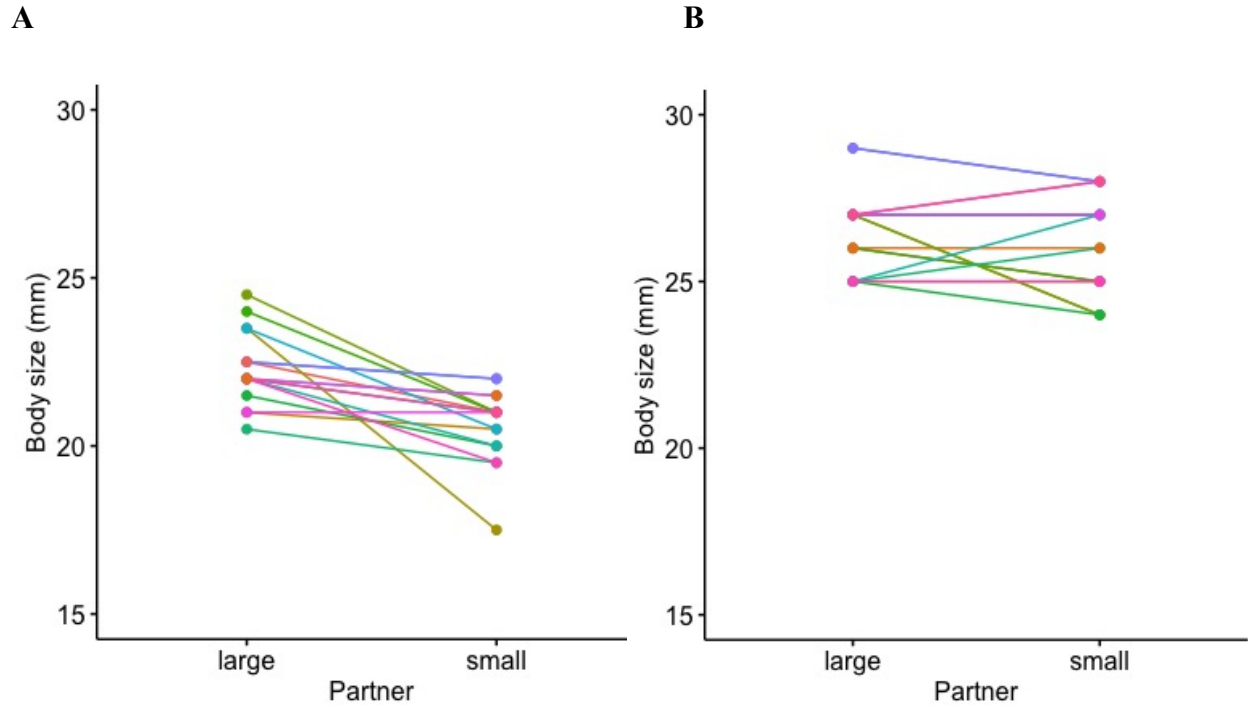
Regardless of whether wall A or wall B was assigned as the rewarded wall, all fish showed a slight preference to enter wall type B (or an avoidance to enter wall type A) initially in trial 1 (Figure 3; wall B: 17 out of 34 individuals;  $\chi^2 = 2.94$ ,  $P = 0.0863$ ,  $df = 1$ ,  $N = 34$ ). This bias persisted even after training and fish continued to show a preference to enter wall type B in the final test trial (Figure 3; wall B: 23 out of 34 individuals;  $\chi^2 = 4.3714$ ,  $P = 0.0365$ ,  $df = 1$ ,  $N = 34$ ). Note that approximately equal numbers of large and small individuals were assigned each wall type as rewarded (9 large and 8 small individuals were assigned wall A; 8 large and 9 small individuals were assigned wall B).

The preference for wall B (and avoidance of wall A) affected how quickly individuals arrived to the food reward across all trials (rewarded wall:  $F_{1,163.5} = 7.065$ ,  $P = 0.0086$ ; trial:  $F_{4,162.8} = 1.186$ ,  $P = 0.3190$ ). Specifically, having wall A as the rewarded wall delayed arrival at the rewarded feeder compared to having wall B as the rewarded wall (Figure 4).

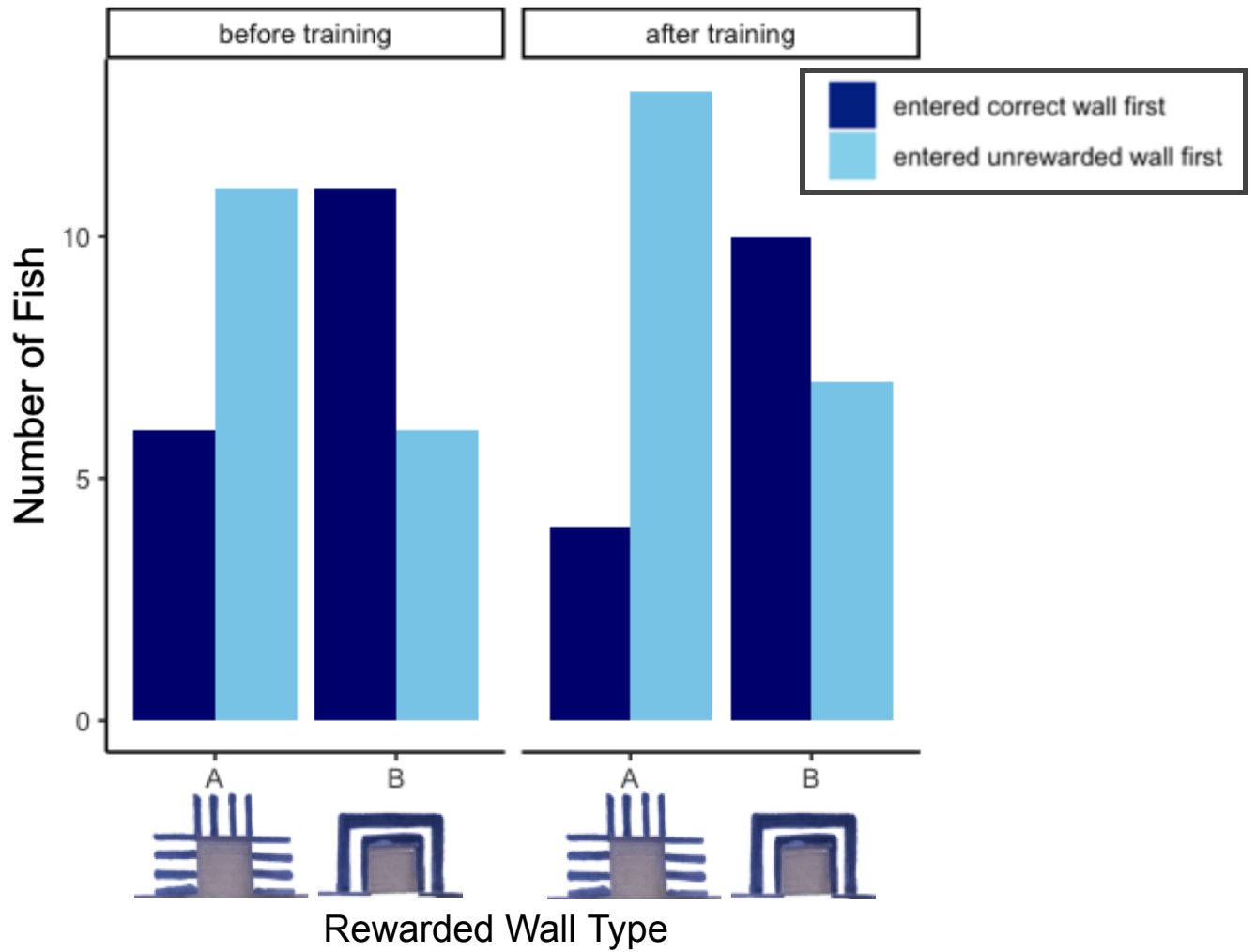
### ***Differences in Performance on the Learning Assay***

At test trial 5, 26 individuals arrived at the rewarded feeder, and 8 did not arrive at the rewarded feeder. Fish did not improve in their ability to find the rewarded feeder across the four training trials and the final test trial, suggesting that no associative learning had occurred (Figure 5, Table 2A). Additionally, the bias for wall B significantly affected how quickly they arrived at the rewarded feeder (Figure 4, Table 2A). After accounting for this wall type bias, the initially large individuals arrived at the rewarded feeder quicker than the initially small individuals across all five trials (Figure 5, Table 2A). This difference between the initially large and small partners was present from the initial training trial until the last test trial (without the food reward).

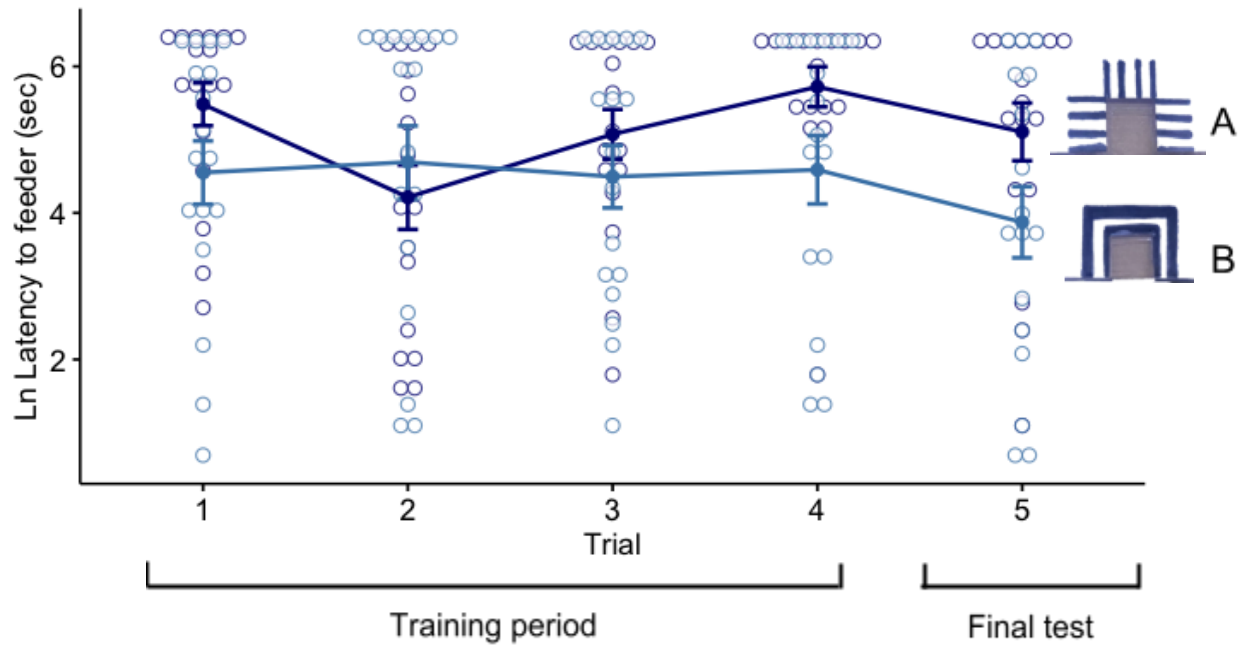
Although there was a tendency for the initially larger individuals to enter any wall (regardless of whether it was rewarded or not) faster than the initially smaller individuals, this was not quite statistically significant (Figure 6, Table 2B). Similar to the latency to arrive at the rewarded feeder, the fish did not improve in how quickly they entered a wall over time (Table 2B).



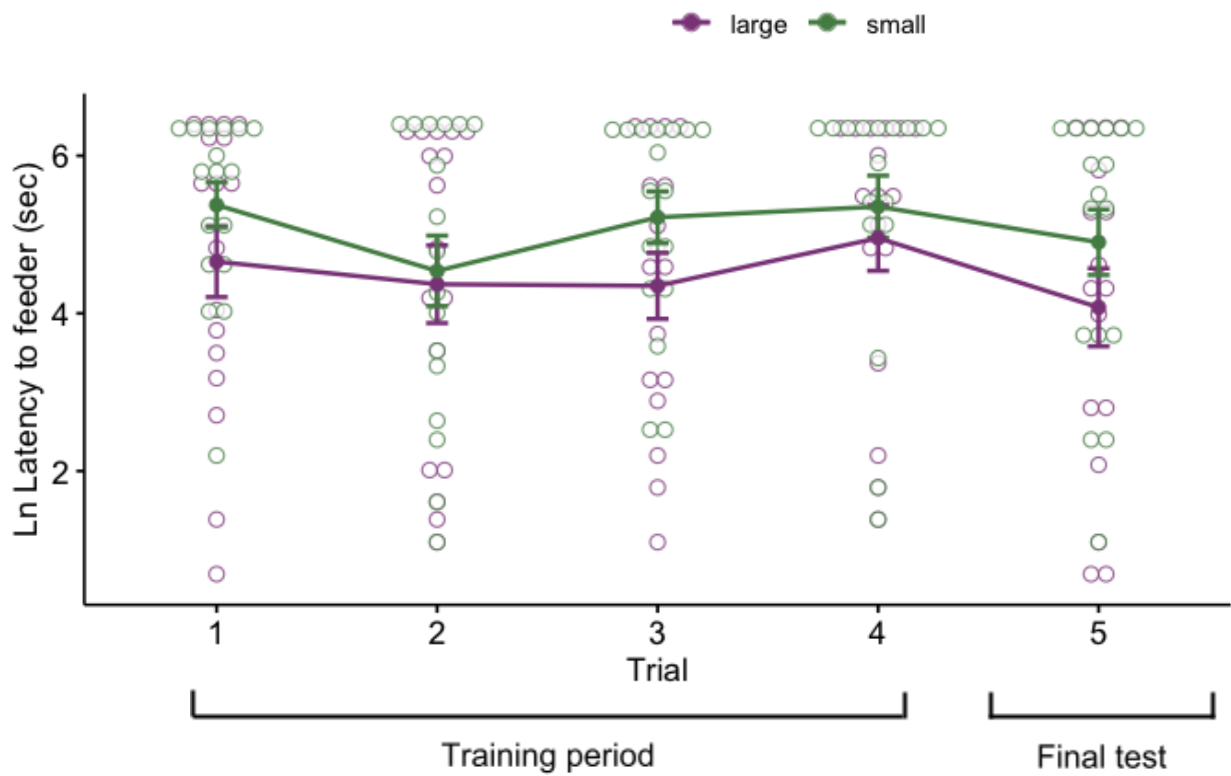
**Figure 2.** Standard length (mm) of fish measured (A) at approximately 5 months of age when they were separated and (B) at approximately 10 months of age after completion of the learning assays. Colors indicate different pairs of individuals with partners connected by a line (N=17 pairs of 34 individuals).



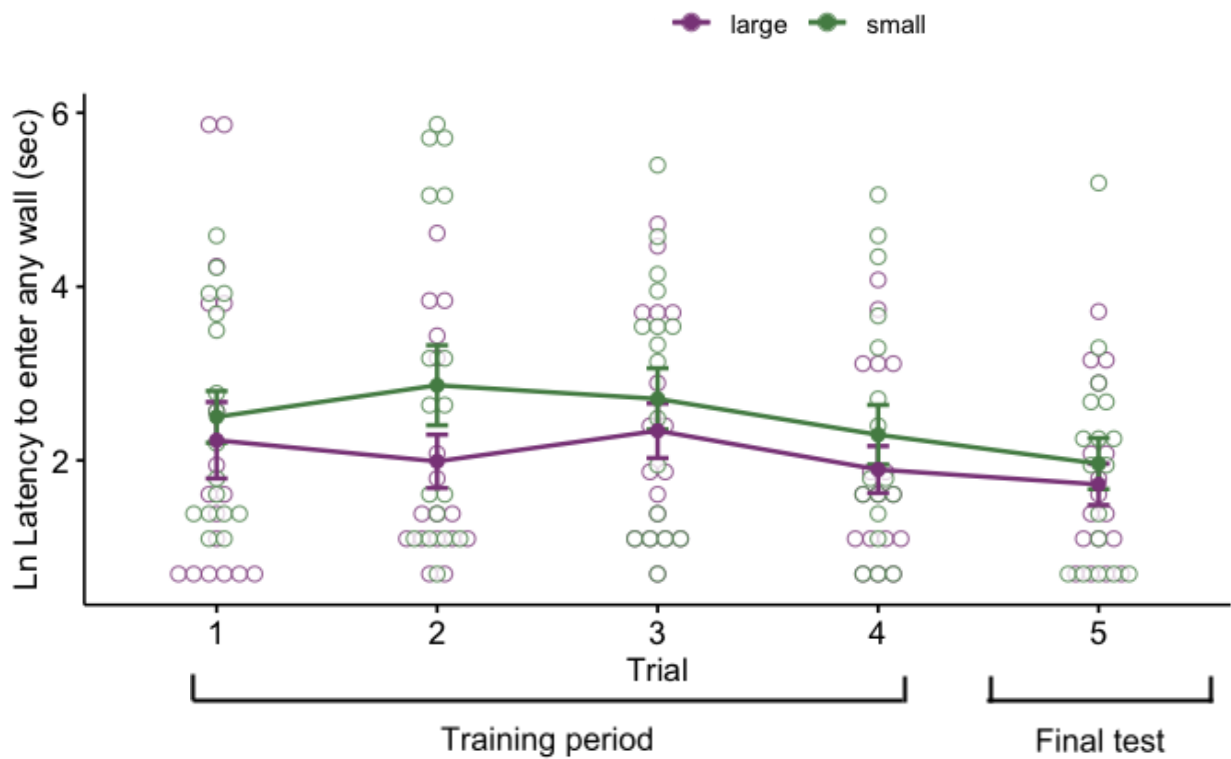
**Figure 3.** At both the first training trial and the final testing trial, fish preferred to enter wall type B regardless of whether it was the rewarded wall or not. The rewarded wall type was randomly assigned with 17 fish having wall A rewarded and 17 fish having wall B rewarded (n=34).



**Figure 4.** The bias to enter wall B (or to avoid entering wall A) had consequences for how quickly individuals got to the rewarded feeder both over the training period and in the final test trial (latency is  $\ln(x+1)$  transformed). Means (+/- standard errors) are shown as well as the individual data points for fish with wall A as rewarded (dark blue) and wall B as rewarded (light blue) (n=34 fish).



**Figure 5.** Latency of initially large and small partners to successfully reach the rewarded feeder over the training period and on the final test (latency is  $\ln(x+1)$  transformed). Means (+/- standard errors) are shown as well as the individual data points for initially large (purple) and small (green) partners ( $n=34$ ,  $n$  pairs = 17).



**Figure 6.** Latency of initially large and small partners to enter any wall regardless of whether or not that wall was rewarded across all four training trials and the final test trial (latency is  $\ln(x+1)$  transformed). Means ( $\pm$  standard errors) are shown as well as the individual data points for initially large (purple) and small (green) partners ( $n=34$ ,  $n$  pairs = 17).

**Table 1.** Results examining how early social interactions and dominance rank affects body size (A) after five months of living together (5 months of age) and (B) after an additional five months of living singly (10 months of age) (N= 17 pairs, 34 individuals). Factors that are statistically significant are in bold.

Parameter	Estimate (SE)	Eta <sup>2</sup> (partial)	SS	D.F.	F-value	P-value
<i>(A) Standard length at approximately 5 months</i>						
(Intercept)	22.302 (0.262)					
<b>Partner rank (large vs small)</b>	<b>-1.676 (0.353)</b>	<b>0.47</b>	<b>23.89</b>	<b>1, 25.49</b>	<b>22.508</b>	<b>&lt;0.0001</b>
Random effect of pair	Variance = 0		LRT = 0		1.0	
Random effect of genetic lineage	Variance = 1.06		LRT = 0.097		0.7558	
<i>(B) Standard length at approximately 10 months</i>						
(Intercept)	26.457 (0.398)					
Partner rank (large vs small)	-0.353 (0.311)	0.07	1.059	1, 17	1.291	0.2716
Random effect of pair	Variance = 0.0815		LRT = 0.0932		0.7602	
<b>Random effect of genetic lineage</b>	<b>Variance = 4.590</b>		<b>LRT = 4.59</b>		<b>0.0322</b>	



**Table 2.** Results examining how early social interactions and dominance rank affects later (A) latency to get to the rewarded feeder across all five learning trials (training period and the final test trial) while accounting for the bias to wall B, and (B) willingness to enter any wall (i.e. boldness) (N= 17 pairs, 34 individuals). Factors that are statistically significant are in bold.

Parameter	Estimate (SE)	Eta <sup>2</sup> (partial)	SS	D.F.	F-value	P-value
<i>(A) Latency to arrive at the rewarded feeder (ln(x+1))</i>						
(Intercept)	5.0084 (0.4166)					
<b>Partner rank (large vs small)</b>	<b>0.7614 (0.5584)</b>	<b>0.04</b>	<b>17.2784</b>	<b>1, 162.8</b>	<b>6.5266</b>	<b>0.0115</b>
Trial (4 training trials + 1 test trial)		0.03	13.1549	4, 162.7	1.2423	0.2951
Rank X Trial		< 0.001	3.0978	1, 162.7	0.2925	0.8825
<b>Rewarded wall (A vs B)</b>	<b>-0.7254 (0.2529)</b>	<b>0.05</b>	<b>21.7816</b>	<b>1, 164.5</b>	<b>8.2276</b>	<b>0.0047</b>
Random effect of individual		Variance = 0.0		LRT = 0		1.0
Random effect of pair		Variance < 0.001		LRT = 0		1.0
Random effect of genetic lineage		Variance < 0.01		LRT = 0.2486		0.618
<i>(B) Latency to enter into any wall, regardless of whether it is rewarded or not (ln(x+1))</i>						
(Intercept)	2.2307 (0.3391)					
Partner rank (large vs small)	0.2686 (0.4461)	0.12	5.5377	1, 25.6	3.5486	0.0710
Trial (4 training trials + 1 test trial)		0.05	10.5232	4, 136.0	1.6858	0.1568
Rank X Trial		0.01	2.2611	4, 136.0	0.3622	0.8351
Random effect of individual		Variance = 0.1310		LRT = 1.4042		0.2360
Random effect of pair		Variance = 0.0		LRT = 0.0		1.0
Random effect of genetic lineage		Variance = 0.1363		LRT = 1.9939		0.1579

## DISCUSSION

Overall, I found that early social experiences in the mangrove rivulus carry-over to affect their performance in a foraging assay many months later. First, rearing individuals with a sibling was effective in inducing a competitive environment and causing size differences between partners. However, once individuals were moved to single containers, smaller individuals showed compensatory growth, and at the time of testing, all individuals were roughly the same size regardless of their initial size at five months (Figure 2). Second, fish had a preference for wall B regardless of whether wall B was their rewarded wall or not (Figure 3). This bias for wall B had consequences for how individuals performed in the learning assay (Figure 4). Third, despite the lack of learning, I found that initially larger individuals reached the rewarded feeder quicker than initially smaller individuals across all trials (Figure 5), potentially due to slight differences in boldness (Figure 6). Notably, these behavioral differences between initial ranks are present despite controlling for genetics and there being no persistent differences in body size at the time of testing.

Surprisingly, fish did not show any evidence of learning and they did not improve in how quickly they entered the correct wall or arrived at the rewarded feeder. It is possible that fish may have needed more training trials to achieve learning. However, even within the five trials, there was not any evidence of improvement suggesting that the fish were simply not associating the wall pattern with the reward. An alternative explanation is that the particular type of associative learning assay I used was not ecologically relevant to the mangrove rivulus. For example, the mangrove rivulus may use spatial cues rather than pattern cues to form associations with food or other rewards. Due to the highly dynamic nature of the mangrove swamps they occupy, these

fish have the capacity to understand and map their spatial surroundings. As areas dry, these amphibious fish are able to use sensory cues when on land to position themselves towards water (Bressman, et al. 2018). Other fish show similar reliance on spatial cues. For example, cichlids show improved learning after 5 days when they are trained to associate a place with a reward (Long and Fu 2022). Examining whether mangrove rivulus show evidence of learning in spatial assays where a location is associated with a reward would allow me to explore this possibility.

Another unexpected finding was the underlying preference for wall type B and/or the avoidance of wall type A. I had intentionally used black-and-white patterns rather than two different colors since other fish species are known to see color and have biases for particular colors (e.g. Pintor et al. 2014, Spence and Smith 2008). It is possible that to the mangrove rivulus, wall B's design had a more apparent opening than wall A, or that wall B was more approachable (less intimidating) than wall A. To understand the particulars of this bias, follow-up studies would have to be conducted manipulating different aspects of the pattern (e.g. spacing, line direction, etc) and examining how these affect the likelihood of fish entering the wall. Interestingly, lizards show a bias for vertical stripes over horizontal stripes when choosing a refuge (Paulissen 2021). Exploring how these different patterns are tied to the landmarks or structure found in the natural habitat of the mangrove rivulus would be worthwhile for designing future, more ecological relevant, learning assays.

Despite this lack of learning and the presence of the wall-type bias, these results still suggest that size (and status) differences due to early competitive interactions can shape behavioral traits later on in life. Specifically, initially larger, presumably dominant, individuals

tend to arrive at the feeder quicker than initially smaller, presumably subordinate, individuals. Other studies have found that higher ranked individuals also perform better in a variety of performance tasks (Barnard and Luo 2002; Pongracz et al. 2012; Wallace et al. 2022). In addition, dominance status can alter an individual's activity patterns. For example, subordinate fish tend to relocate more frequently than dominant fish, possibly due to being excluded from resources by dominant individuals and having to find new resources (Akbaripasand et al. 2014). My results suggest that the initially larger dominant fish is slightly bolder and more likely to enter walls in general compared to the smaller subordinate fish. Because there was no evidence for learning, initially larger individuals seem inherently bolder and faster at finding the rewarded feeder, compared to their smaller partner, even at the first exposure to the assay. It is important to remember that these behavioral differences reflect the outcome of a competitive environment and size differences that occurred many months ago, and that the consequences of this early environment are still detectable even after months of being reared alone in a non-competitive environment.

What might be the mechanism underlying the long-term persistence of the early social environment? Experience effects such as winner/loser effects shape the hormonal state of individuals and potentially an individuals' future behavior (Hsu et al. 2006). In mangrove killifish, the "losers" of competitive interactions are more likely to change their strategies in future interactions than "winners" due to their hormonal state (Earley et al. 2013). Specifically, fish with lower hormone levels of cortisol, testosterone, and 11-ketotestosterone are more receptive to their contest experience and are more likely to change their actions in the future. Hormone levels in the mangrove rivulus could be indicative of their performance and could be

helpful in future studies. It could be beneficial to study how the early social environment might alter the hormone levels of individuals and whether these changes alter the long-term response of individuals to stress and their behavioral strategies.

Another possible mechanism underlying these patterns is that the catch-up growth which the small partners underwent had a developmental cost. Compensatory growth may have negative effects on later cognition and other aspects of performance. For example, in zebra finches, although the final body size in adult birds was not affected by food deprivation due to compensatory growth, there were long-term negative consequences on learning (Fisher et al. 2006). Similarly, compensatory growth has adverse effects on song learning and spatial learning, as well as adult physiology and reproduction (Hsu et al. 2017). In future studies, it could be worthwhile to distinguish between early dominance rank and compensatory growth as the primary cause of the boldness and foraging performance differences between individuals.

Regardless of the mechanism underlying these eventual differences, what caused individuals to diverge in body size initially? In other words, how could one individual become the dominant individual when they were genetically identical? It is important to consider inherent boldness in individuals since inherently bolder individuals may have become larger by out-competing the other individual. Although these partners of fish are genetically identical, boldness may have arisen from aspects that were not considered. For example, individuals' hatching time may have had consequences. The time of hatching has been observed to have effects on the larval size of pollock (Porter and Bailey 2007). Early hatching larvae were smaller

and had weaker escape responses to predators. In the future, tracking egg development and hatching time may provide insight into differences between individuals.

Overall, I observed that early experiences as a subordinate partner decreased exploration and performance on the foraging assay while early experiences as a dominant partner increased exploration and performance on the foraging assay. These patterns are not due to differences in genetics or maternal effects between partners because partners were paired within genotype and from the same parent, nor are they due to current differences in body size. My results suggest that the early social environment can have long-term consequences for behavior and how individuals might navigate foraging challenges. Future studies exploring the effects of compensatory growth and hormone levels would help us understand the mechanisms underlying these patterns.

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