Evolutionary Changes in Left-Right Visceral Asymmetry in *Astyanax* Cavefish

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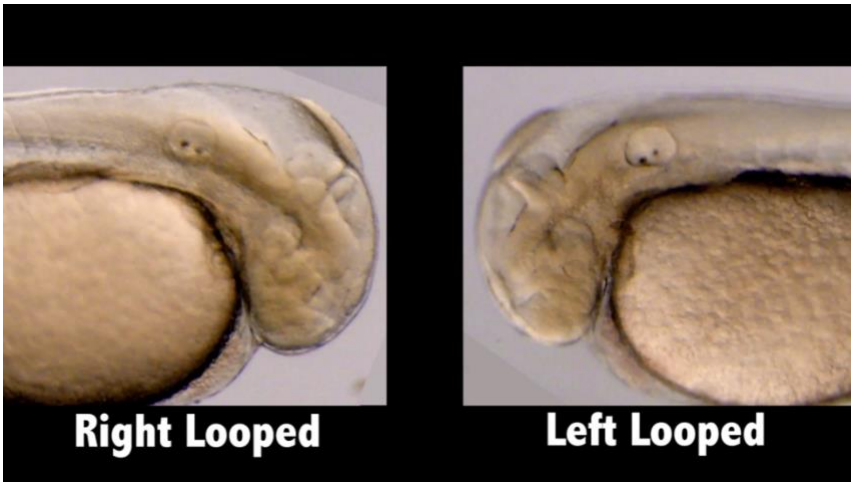
Supplementary Files

Supplementary Movie.

Video showing beating of right (D) lopped and left (L) looped hearts in Pachón cavefish at 3 days post-fertilization.



Supplementary Figures and Legends.

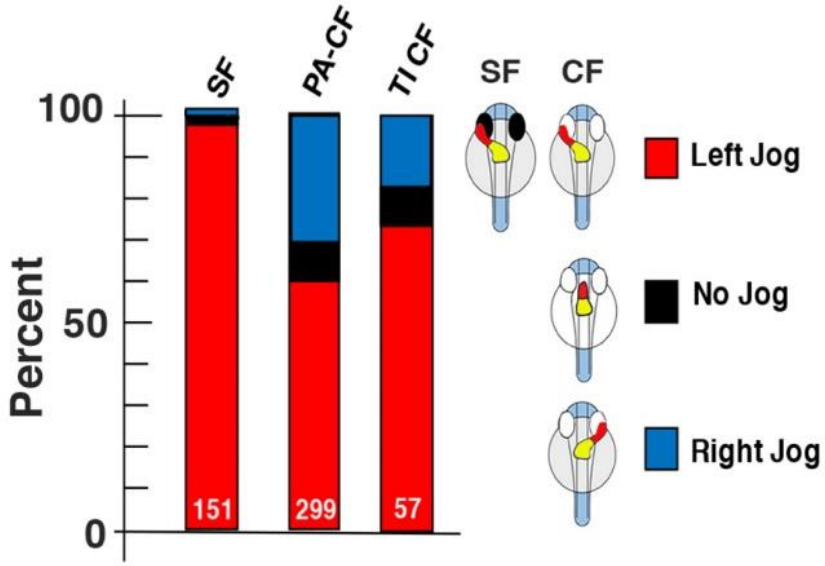


Figure S1. Bar graph showing the proportion of left jogged, non-jogged, and right jogged cardiac tubes in surface fish (SF), Pachón cavefish (PA-CF), and Tinaja cavefish (TI-CF) at 1.5 dpf. N is shown at the bottom of each bar.

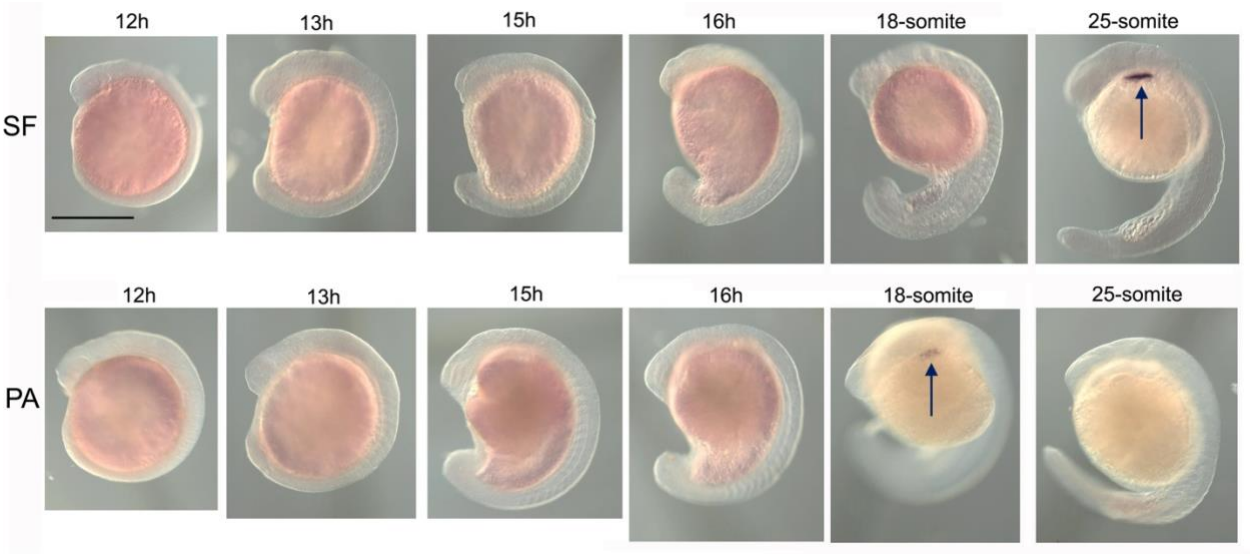


Figure S2. Determination of *lft2* gene expression in left lateral plate mesoderm (LM) of surface fish and cavefish (F 61 PA-CF) by *in situ* hybridization between the 12 hour (h) and 25-somite stages. The *lft2* gene is expressed strongly in the anterior left LPM at the

25-somite stage in surface fish embryos (arrow) but weakly in cavefish embryos at the 18-somite stage (arrow). Scale bar is 200 µm; magnification is the same in all frames.

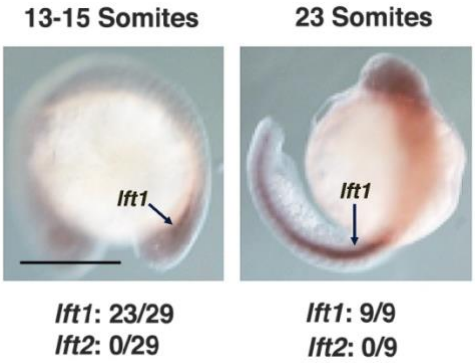


Figure S3. Double *in situ* hybridization with *lft1* and *lft2* probes. Stages shown at top of each frame. Number of *lft1* or *lft2* stained individuals per total number shown on bottom of frames. Scale bar is 200 µm; magnification is the same in all frames.

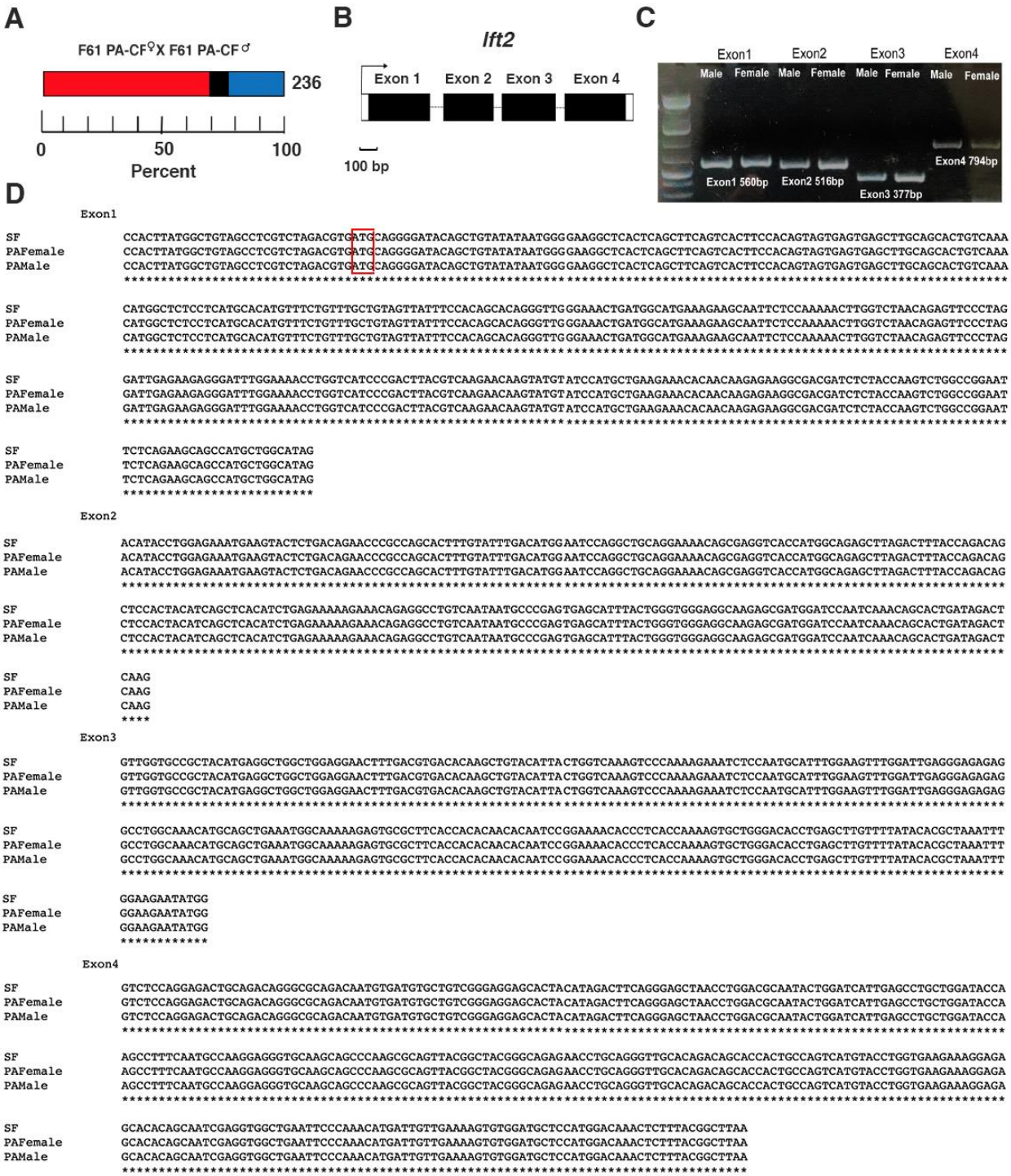


Figure S4. Survey of cavefish *Ift2* genomic DNA for coding region changes. A. Comparison of numbers of embryos with D-looping hearts, no looping hearts, and left looping hearts in the progeny of a single surface fish x surface fish and a cavefish x

cavefish (both F61 PA-CF) cross. B. Exon-intron organization of the *Astyanax lft2* gene. C. PCR amplification of 4 *lft2* exons from male and female F61 PA-CF individuals. Exons shown as red outlined bars; bars showing 5' UTR in exon 1 is not filled. Introns shown as lines. D. PCR amplification of *lft2* exons from male and female individuals used in the cavefish x cavefish cross in A. D. Alignment of *lft2* exon sequences obtained from the male and female individuals used in the cavefish x cavefish cross in A with the surface fish *lft2* exon sequence. Red box: ATG translation start site. Asterisks: identical nucleotides.

Supplementary Tables

Supplementary Table 1. Oligonucleotide primers used to amplify gene sequences for preparation of RNA probes for *in situ* hybridization.

Gene	Primers
spaw	Forward: TTTAACGTGACCGCTCTGCT Reverse: TGCATGTAGGCGTGATTGGT
lefty1	Forward: CAGGACCCCAGCGATAACTC Reverse: GCCGCACTTCTCCACTATCA
lefty2	Forward: GGCAAAAAGAGTGCGCTTCA Reverse: TGTCCATGGAGCATCCACAC
pitx2	Forward: CCCAAAATGGACGCAAAGGG Reverse: TATGGTGGCAATTGCAGGGT
cbsa	Forward: CGCATGCTCATCAGAGACGA Reverse: GGCAAAGTGATCCGTCTCCA

Supplementary Table 2. Oligonucleotide primers used in RT-PCR.

Gene name	Froward primers	Reverse primers
spaw	CGCTAAAGACTGTCATCAGGTTG	AACAACAGCCCGTTTGGTTG
pitx2	CTACACACCCCCTTAGCCAT	GTCTTTATCTGCGCACTCGG
lft1	GACCCCAGCGATAACTCACT	CTGCAGCACTGACCCTGA
lft2	GAATCAGTCTTCGCGTTATTTCC	GACGTAAGTCGGGATGACCA
ndr1	ACCCTAAGCGATACAATGCCT	AGCTTCAGAAGACTCTGCATGT
ndr2	CACGCCTACATGCAGAGTC	TCTCGCCGTTCTCGTAGTAG
gapdh	TCCTGAACTCAATGGCAAGC	TTCTCCAAGCGGACAGTCAA

Supplementary Table 3. Oligonucleotide primers used to amplify *Ift2* exons by PCR.

Ift2 Exon	Forward primers	Reverse primers
1	GCACAGTTTGGGCAACAGAG	AAAGCAGAGCCTTAACATACCT
2	GCATAGGTATGTTAAGGCTCTGC	CACGATGACAAAACTACCCCT
3	TCAGGGGTAGTTTTGTCATCGT	ACACACACCTCAACATTACCTCA
4	TGAGGTAATGTTGAGGTGTGTGT	AACTGTCGAGTGTTGCCGTA