

Supplementary Figures

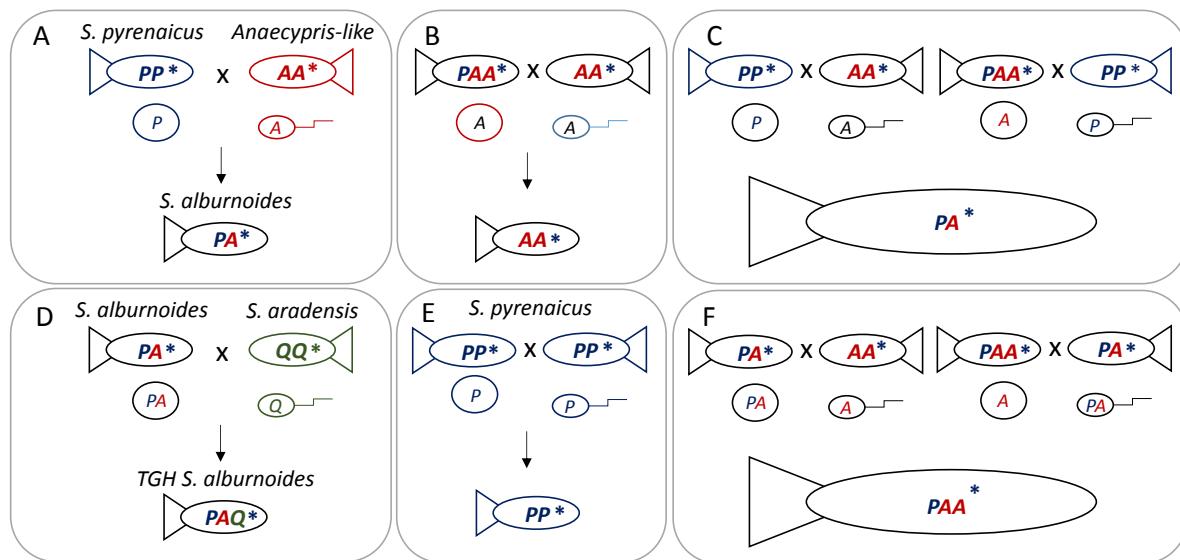


Fig. S1: Simplified overview of the *S. alburnoides* reproductive complex. A) Initial hybridization at the origin of the complex. B) *Anaecypris-like* nuclear genomotype reconstitution within the complex. C) Main crosses leading to diploid hybrid *S. alburnoides*. D) Cross leading to the artificial production of THG *S. alburnoides*. E) Independent maintenance of *S. pyrenaicus* species. F) Main crosses leading to triploid hybrid *S. alburnoides*. C and F exemplify the shift between ploidy levels and genomic compositions in each generation, and how the same genomotype can result from distinct crosses. Asterisk represents mitochondrial genotype: blue from *S. pyrenaicus*, red from *A. hispanica*-like and green from *S. aradensis*. This figure covers only the genomotypes involved in this study.

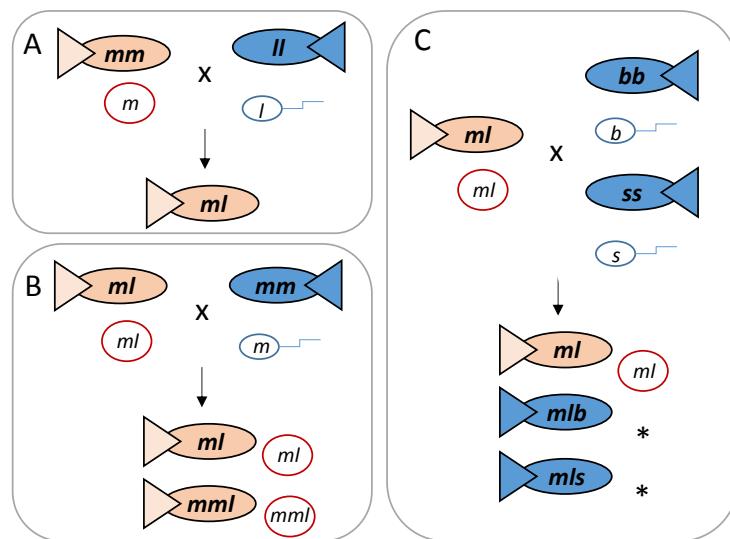


Fig. S2: Simplified overview of the *Poecilia formosa* complex. A) Initial hybridization in the origin of the complex. B) Gynogenetic diploid hybrid reproduction and paternal introgression leading to the occurrence of triploid hybrids. C) Crosses leading to the artificial production of THG *P. formosa*. Pink fish are females, blue fish are males. Asterisk represents sterility. This figure covers only the genotypes involved in this study.

Supplementary Information

Table S1. List of specific *Poecilia* strains used in this work. All are stocks in the aquarium of the Biocenter at the University of Würzburg, Germany.

Species	Genotype	Strain
<i>Poecilia mexicana limantouri</i>	<i>mm</i>	WLC1353
<i>Poecilia latipinna</i>	<i>ll</i>	WLC1368
<i>Black molly</i>	<i>bb</i>	WLC1351
<i>Poecilia salvatoris</i>	<i>ss</i>	WLC1330
<i>Poecilia formosa</i> 2n	<i>ml</i>	IV5
<i>Poecilia formosa</i> 3n	<i>mml</i>	WLC1055
<i>Poecilia formosa</i> 3n (TGH with <i>b</i>)	<i>mlb</i>	1588
		3331
		3830
		4069
		4335
		4664
		4665
<i>Poecilia formosa</i> 3n (TGH with <i>s</i>)	<i>mls</i>	1588
		1612
		3331
		4663
		4664

Table S2. Primer sequences and references* for each target gene for both *Squalius* and *Poecilia*.

	Gene	Primer	Sequence	Reference
<i>Squalius</i>	<i>actb</i> *	β-ACTIN-F1	5'-CAACGGCTCCGGCATGTG-3'	Pala <i>et al.</i> , 2008
		β-ACTIN-R1	5'-TGCCAGGGTACATGGTGG-3'	Pala <i>et al.</i> , 2008
	<i>rpl8</i> *	Rpl8 forward	5'-CTCCGTCTCAAAGCCCATGT-3'	Pala <i>et al.</i> , 2008
		Rpl8 reverse	5'-TGTTCCCTCGCAGTCTGCCAG-3'	Pala <i>et al.</i> , 2008
<i>Poecilia</i>	<i>Gapdh</i> *	GAPDH-F1	5'-ATCAGGCATAATGTTAAAGTTGG-3'	Pala <i>et al.</i> , 2008
		GAPDH-Ri	5'-GGCTGGGATAATGTTCTGAC-3'	Matos <i>et. al.</i> , 2010
	<i>actb</i> **	β-ACTIN-F1	5'-CAACGGCTCCGGCATGTG-3'	Pala <i>et al.</i> , 2008
		β-ACTIN-R1	5'-TGCCAGGGTACATGGTGG-3'	Pala <i>et al.</i> , 2008
		Actin F pro	5'-CCTTAAAGGCCCTGCCTACCT-3'	—
		Actin R pro	5'-AAGGGAAGGGATTGCTATGG-3'	—
<i>Poecilia</i>	<i>rpl8</i> **	mRPL8F1	5'-ACGGAGTTAGTCACGAT-3'	—
		mRPL8R1	5'-CTTCTCCTGGACGGTCTTG-3'	—
	<i>rpl8</i> ***	Rpl8 F pro	5'-CTGTTCCAYCCCCAGAACGT-3'	—
		Rpl8 R pro	5'-ACGATGCCCTTGATGTAGCC-3'	—
<i>Poecilia</i>	<i>Gapdh</i> **	3gapdhF	5'-GTGACCCGWGCTGTTTC-3'	—
		3gapdhR	5'-AGGTCACABACACGGTTGCT-3'	—
	<i>Gapdh</i> ***	Gapdh F pro	5'-CATTTCGRTTTGTGGTTG-3'	—
		Gapdh R pro	5'-CCTCACATCKTGGTCTGAAA-3'	—

*PCR conditions as described in reference

PCR conditions: pre-heating at 95°C for 3 min, 30 cycles at 95°C for 30 s, 60°C (*gapdh*)/58°C (*rpl8* and *β-actin*) for 30 s and 72°C for 1 min and a final extension at 72°C for 10 min.*PCR conditions: pre-heating at 95°C for 3 min, 30 cycles at 95°C for 30 s, 60°C (*gapdh*)/62°C (*rpl8* and *β-actin*) for 30 s and 72°C for 1,30 min and a final extension at 72°C for 13 min.

Table S3. Relative comparison (ratio) between the mean observed methylation value and an expected methylation level in case of additivity (obs/ exp) for a hybrid situation.

	Liver			Muscle		
	Obs* 5mC (ng)	Exp**5mC (ng)	obs/exp	Obs* 5mC (ng)	Exp**5mC (ng)	obs/exp
<i>S. alburnoides</i>	<i>aa</i>	2,91	-	-	4,78	-
	<i>pp</i>	1,78	-	-	3,13	-
	<i>qq</i>	1,71	-	-	3,62	-
	<i>pa</i>	1,97	2,34	0,84	3,36	3,95
	<i>paa</i>	2,88	3,80	0,76	4,29	6,34
	<i>pqa</i>	3,17	3,20	0,99	4,69	5,76
<i>P. formosa</i>	<i>mm</i>	1,07	-	-	0,61	-
	<i>ll</i>	1,18	-	-	0,77	-
	<i>bb</i>	1,16	-	-	0,77	-
	<i>ss</i>	1,01	-	-	0,69	-
	<i>ml</i>	1,65	1,13	1,46	1,17	0,69
	<i>mml</i>	1,95	1,66	1,17	1,32	1,00
	<i>mlb</i>	0,98	1,71	0,58	0,81	1,08
	<i>mls</i>	1,03	1,63	0,63	0,78	1,04

* mean observed methylation value ** expected methylation calculated from the mean methylation level obtained for each parental diploid genomotype (*pp*, *aa* and *qq*) considering that *p*, *a* and *q* genomic contributions in the hybrids are methylated at the same level as in the non-hybrid situation.

Table S4. Identity matrix between all the parental genomes of *P. formosa* for each target gene from Bioedit.

	Seq <i>actb</i> ->	<i>mm</i>	<i>ll</i>	<i>bb</i>	<i>ss</i>
<i>actb</i>	<i>mm</i>		0.983	0.991	0.976
	<i>ll</i>	0.983		0.978	0.977
	<i>bb</i>	0.991	0.978		0.974
	<i>ss</i>	0.976	0.977	0.974	

	Seq <i>gapdh</i> ->	<i>mm</i>	<i>ll</i>	<i>bb</i>	<i>ss</i>
<i>gapdh</i>	<i>mm</i>		0.988	0.961	0.958
	<i>ll</i>	0.988		0.958	0.956
	<i>bb</i>	0.961	0.958		0.927
	<i>ss</i>	0.958	0.956	0.927	

	Seq <i>rpl8</i> ->	<i>mm</i>	<i>ll</i>	<i>bb</i>	<i>ss</i>
<i>rpl8</i>	<i>mm</i>		0.97	0.987	0.98
	<i>ll</i>	0.97		0.967	0.966
	<i>bb</i>	0.987	0.967		0.972
	<i>ss</i>	0.98	0.966	0.972	

Table S5. Number of CpG sites per 1Kb within the *mm*, *ll*, *bb* and *ss* sequences, obtained with the Sequence Manipulation Suite.

	mm	ll	bb	ss	
# CpG sites per 1Kb	<i>rpl8</i>	27	27	29	29
	<i>gapdh</i>	25	25	23	19
	<i>actb</i>	19	19	19	18