Genetic Architecture of Population Differences in the Sequential Hermaphroditism of *Kryptolebias marmoratus*

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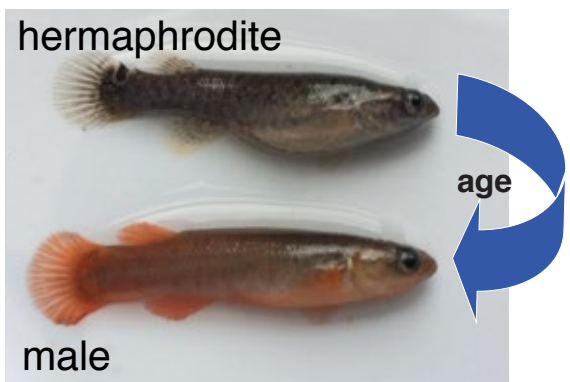
Abstract
The mangrove killifish, *Kryptolebias marmoratus*, possesses two unusual sex-related attributes. One is its self-fertile hermaphrodite sex, which is assumed at sexual maturity by the vast majority of fish. The other is sequential hermaphroditism, in which the initial hermaphrodite sex can transition into a dedicated male. These males lose all ovarian tissue, proliferate large testes, and assume typical male behavior and pigmentation. As a result, limited outcrossing does occur in both natural and laboratory conditions [1, 2]. Interestingly, within an effectively isogenic line individuals may or may not change sex, and lines can differ greatly in their tendency to undergo sex change when grown in a common environment [3]. For example, in the Floridian strain SOB10 about 4% of individuals become males, while the Belize Twin Cays strain BWS29 changes roughly 70% of the time. In addition, BWS29 males often develop without passing through an obvious fertile hermaphrodite phase, indicating some are so-called primary males. To investigate the genetic architecture of this difference, we are taking a hybrid genetics approach. Through pair-wise crosses between BWS29 and SOB10 lines and microsatellite genotyping [1] of offspring we identified five F1 hybrids. All of these remained hermaphrodites into their third year, indicating that early, frequent sex change is fully recessive to the SOB10 condition of rare/slow change. F2 offspring were collected, and a number have shown early sex change. This suggesting that variants in a small number of loci (potentially as few as one) may govern the propensity and timing of sex change in *K. marmoratus*.



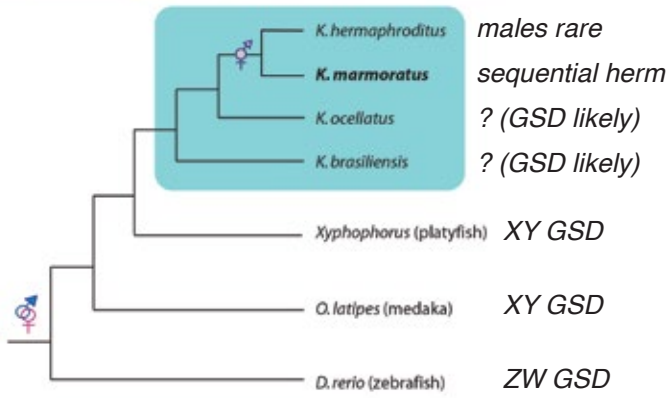
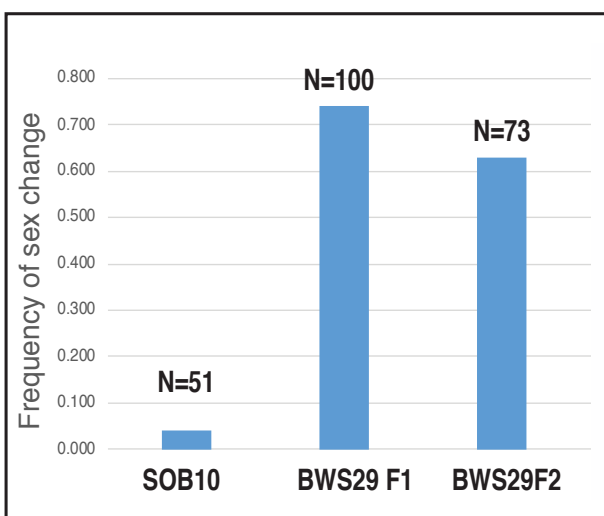


Figure 1. *K. marmoratus* is a sequential and simultaneous hermaphrodite (left) that evolved from gonochoristic (i.e. male-female) ancestors in coastal South America. Its closest relatives (right) likely have genetic sex determination (see poster 1175B by John Ficklin).

What cue triggers sex change?

What genes are required for regulating sex change?

Is there natural variation that could answer the above?



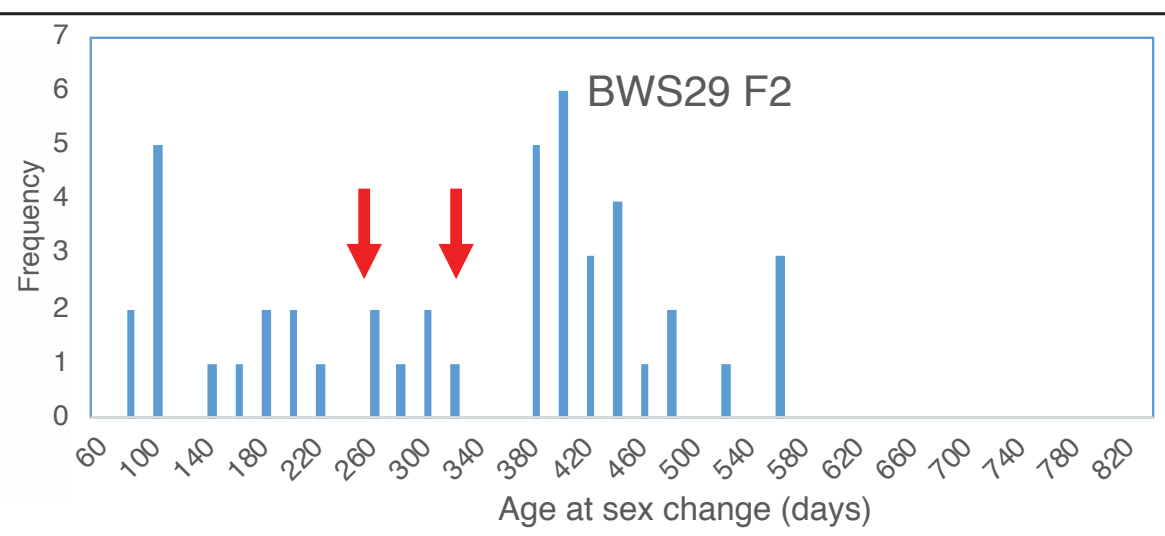


Figure 2. Left: In their first two years, *K. marmoratus* SOB10 hermaphrodites from Florida change sex only rarely, while both F1 and F2 descendants of wild-caught Twin Cays, Belize founders (BWS29) change sex roughly two-thirds of the time. **Right:** BWS29 males develop at a wide range of ages, with those under 180 days being male at first sexual maturity. Rare SOB10 males are indicated with red arrows.

Hybrid analysis: SOB10 x BWS29 cross

F1 fish	age	current sex
373	1172	hermaphrodite
399	1062	hermaphrodite
309	1111	hermaphrodite
396	1028	male (change @ 974d)
294	1091	hermaphrodite

F2 family	males/total*
373	5/26
399	0/11
309	0/20

* Scored at at least 240 d

Hybrids identified using two different microsatellite assays via fin clips.
Vertebrate animal work under UMD IACUC Protocol R-AUG-18-49

- Early sex change is recessive.
- One F2 family (#373) produced ~1/4 males.
- Two other F2 families produced no males.
- #373 may be segregating a major effect allele, but if so it is unclear why other two F1s are not, given near-isogenic founder lines used in crosses.

Figure 3. F2 hybrid male #637, switched at 106 days.

Ongoing test of major effect locus hypothesis:
2/3 of F2 hermaphrodites from #373 family should carry BWS29 “fast change allele”, selfed F3 ~1/4 early males. ~100 F3 collected so far.

REFERENCES
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