Flexible Reproductive Mode and Offspring Development in Unisexual (All Female) *Ambystoma* Complex Salamanders

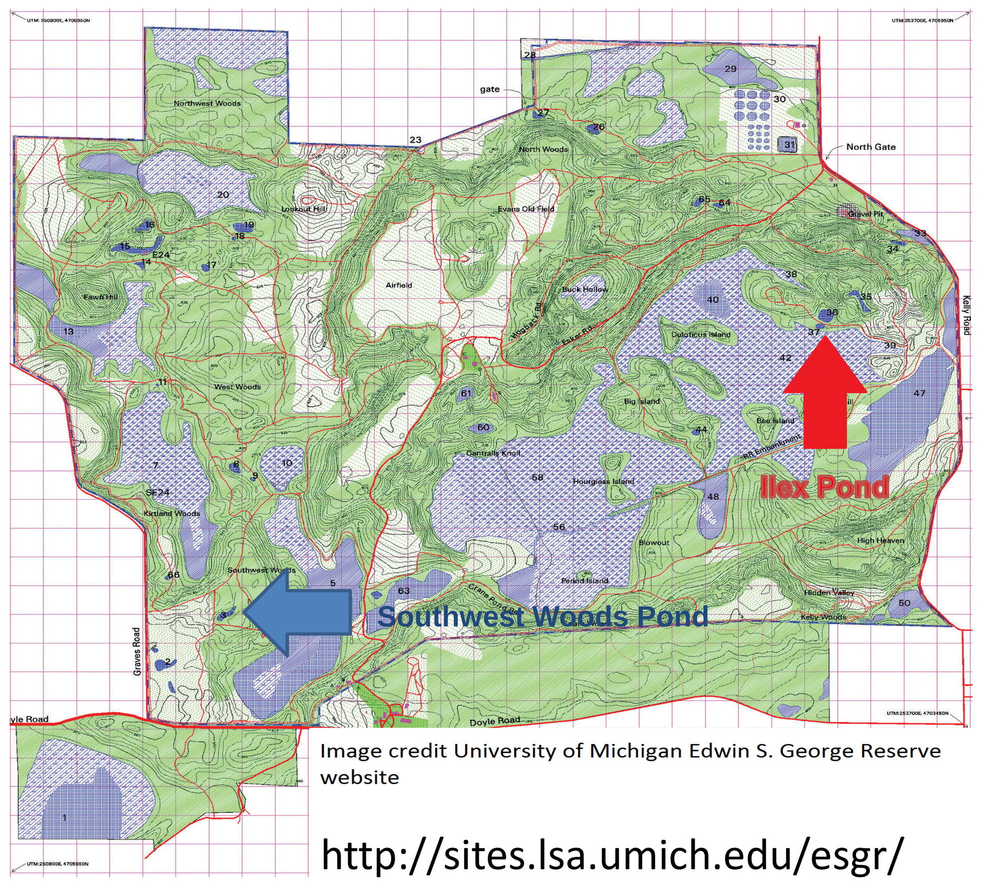
Marisa C. Hildebrandt and Katherine Greenwald
Department of Biology, Eastern Michigan University, 441 Mark Jefferson, Ypsilanti MI 48197

Background

- Unisexual *Ambystoma* salamanders have a unique mode of reproduction known as kleptogenesis in which they "steal" sperm from males of other species (Bogart et al. 2007)
- Offspring can be produced either asexually, or by replacing one of the maternal genomes with the paternal one, or by adding the paternal genome to the full maternal complement
- Variation in ploidy, the number of genomes, occurs because of this addition
- The unisexual complex is over 5 million years old (Bi and Bogart, 2010)
- In this experiment, we examine reproductive mode (asexual or sexual) and resulting ploidy and egg development

Methods

- At our study site, several ponds are encircled by drift fence and pitfall trap arrays where we capture salamanders before they enter the pond



http://sites.lsa.umich.edu/esgr/

This experiment utilized salamanders from the Edwin S. George Reserve in Pinckney, MI.

Further research was carried out in the Greenwald Laboratory at Eastern Michigan University.

- Captured salamanders were separated into male-female pairs in Rubbermaid totes, with a handful of leaf litter as substrate for egg laying
- Females were from Ilex pond and males were from either Ilex or Southwest Woods pond to see if they prefer local or foreign males for genome addition
- Clutches laid were collected and placed in 95% ethanol with a subset allowed to develop

Hypothesis 1: Tetraploid offspring will be larger and grow faster, but have more issues with development compared to triploid offspring

- Extractions were done using InstaGene matrix
- We used PCR followed by gel electrophoresis to see if the extractions worked, and microsatellite analysis to determine the genomes that were present for each egg (Ramsden, et al. 2006)
- This method of identifying biotype has been used extensively by the Greenwald lab (Teltser and Greenwald, 2015)

Hypothesis 2: Incorporation of paternal genome will be more frequent for inter-pond pairs where female is not locally adapted versus intra-pond pairs where the female is locally adapted.

Results

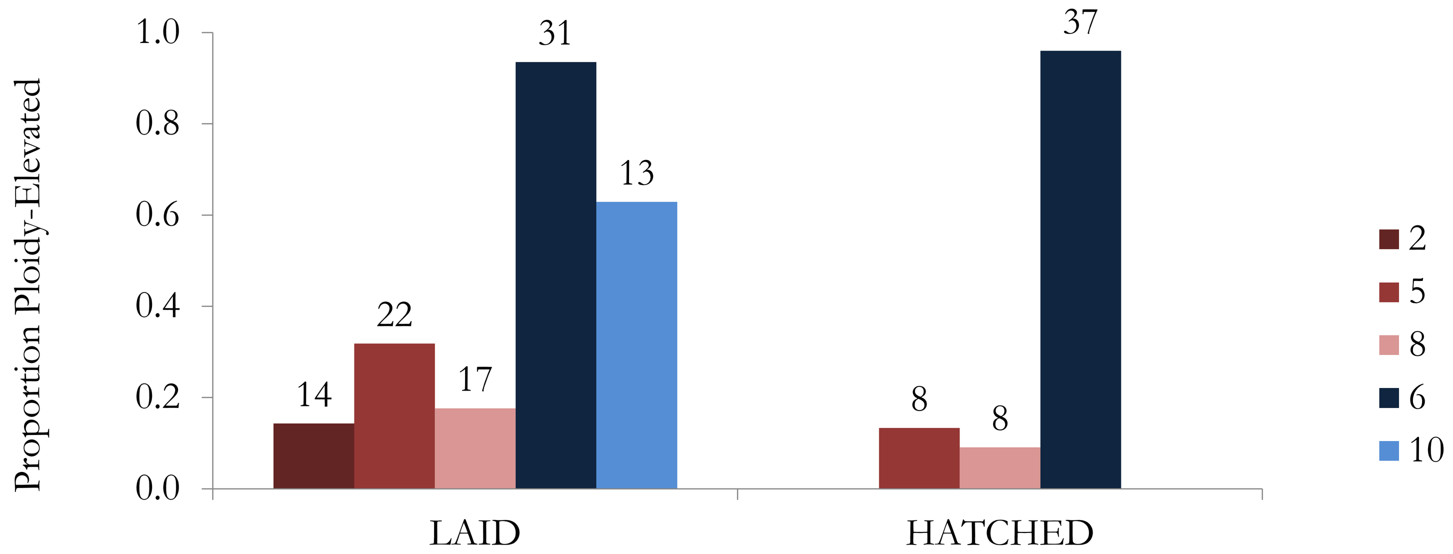
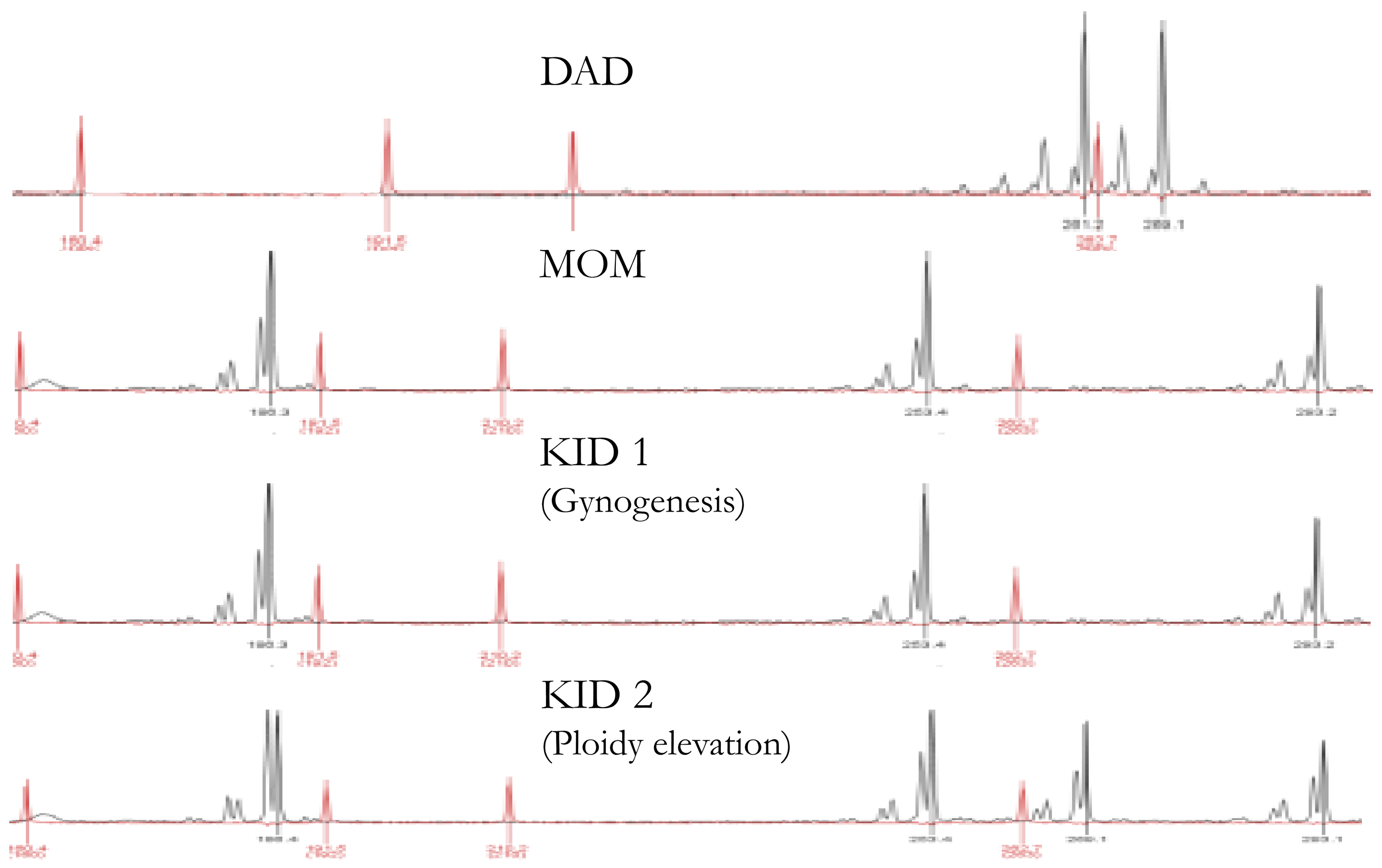


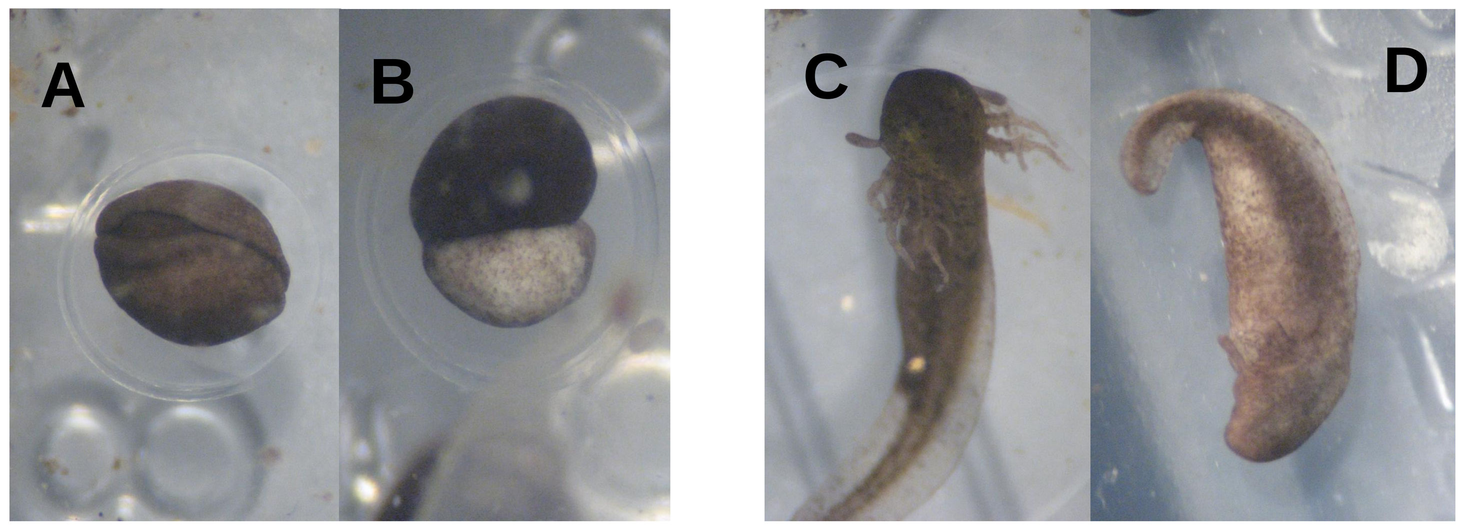
Figure 1. Proportion of ploidy-elevated (tetraploid) eggs that were laid and that hatched, color-coded by clutch.

Table 1. Clutch number, pair type (intra- or interpond), and the number of eggs laid and number of eggs immediately preserved. We raised eggs from a subset of clutches ("Raised" column); for each of these we report the number and percentage successfully hatched.

Clutch	Type	Laid	Preserved	Raised	Hatched	Pct
2	ILEX-SWW	26	26			
5	ILEX-SWW	77	37	40	23	0.58
8	ILEX-SWW	74	33	41	26	0.63
6	ILEX-ILEX	157	75	82	46	0.56
9	ILEX-ILEX	82	34	48	40	0.83
10	ILEX-ILEX	42	42			

Figure 2. Chromatographs illustrating the microsatellite loci used for genotyping to determine parentage and ploidy elevation.





Normal versus abnormal development:
These photographs represent (A) normal formation of the neural cleft and (C) normal gill, balancer, and spinal formation. Contrast these with photos showing (B) exoneurulation and (D) curved spine with other defects.

Discussion

- Tetraploidy is very common at the egg and larval stages but decreases with age
- Tetraploids developed faster and larger but had more developmental issues
- Females placed with males from foreign ponds did not incorporate the male genomes at the higher rate we were anticipating, perhaps due the females lacking cues that they had immigrated into a new pond

Acknowledgments

We thank Dr. Bob Winning for help with the microscopy and hatching, and the University of Michigan for access to the ESGR, and Dr. Mike Benard for drift fence installation.

References: Bi K, Bogart JP. (2010) Time and time again: unisexual salamanders (genus *Ambystoma*) are the oldest unisexual vertebrates. BMC Evol Biol. Aug 3;10:238. doi: 10.1186/1471-2148-10-238. Bogart JP, Bi K, Fu JZ, Noble DWA, Niedzwiecki J (2007) Unisexual salamanders (genus *Ambystoma*) present a new reproductive mode for eukaryotes. Genome, 50, 119–136. Harrison R. 1969. Harrison stages and description of the normal development of the spotted salamanders, *Ambystoma punctatum* (Linn.).*in* R. Harrison, editor. Organization and Development of the Embryo. Yale University Press, New Haven, CT. Ramsden C., K. Beriault, and J. P. Bogart. 2006. A nonlethal method of identification of *Ambystoma laterale*, *Ambystoma jeffersonianum* and sympatric unisexuals. Molecular Ecology Notes **6**:261-264. Teltser, C and KR Greenwald. 2015. Survivorship of ploidy-variable unisexual *Ambystoma* salamanders across developmental stages. *Herpetologica* 71(2): 81-87.