

# Supporting Information

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## SI Materials and Methods

**DNA Extraction.** DNA was extracted from intracapsular algae, embryos, and adult salamander tissues with the DNeasy Blood & Tissue Extraction Kit (Qiagen). For intracapsular algae, individual fertilization capsules containing Stage-35 embryos and algae were removed from the jelly coat, rinsed in sterile PBS, and algae were aspirated with a sterile syringe and stored in ethanol. Duplicate series of Stage-9, -12, -17, -26, and -37 embryos ( $n = 5$  per stage) were removed from their egg capsules, rinsed through five changes of sterile PBS (50 mL total), and stored in ethanol. Six adult salamanders (three female, three male) were anesthetized in buffered MS-222, rinsed in sterile PBS, and 25- to 50-mg portions of their reproductive tissues were dissected using sterile techniques. Six regions of the female reproductive tract were isolated: the posterior ovary (furthest from the cloaca), medial ovary, anterior ovary (adjacent to the infundibulum), anterior oviduct (pars recta, including portion of the infundibulum), medial oviduct (middle portion of pars convoluta), and posterior oviduct (uterine portion of the pars convoluta adjacent to cloaca). The male regions included portions of the anterior and posterior testes, the anterior end of the Müllerian duct, and a portion of the posterior Wolffian duct (which included the ends of several collecting ducts) and the posterior collapsed Müllerian ducts (1).

*Oophila* 16S (GenBank accession no. HM590633) and 18S (accession no. HM590634) rRNA genes were amplified by PCR with previously described primers (2, 3). Eight clones (pGEM-T Easy Vector; Promega) from the 16S rDNA amplifications and 19 from the 18S rDNA amplifications were sequenced on a Beckman Coulter CEQ8000. The taxonomic identity of nonalgal sequences were determined through BLAST (National Center for Biotechnology Information).

*Oophila*-specific 18S rDNA internal primers were used to screen for algal symbionts within adult tissues and during specific embryonic stages (forward: 5'-TCGGATCGTCTCGGTTTC-3'; reverse: 5'-CAGTGTGGCCACCGCTCG-3'). Nested PCR was used for adult tissue-specific 18S rDNA amplification, using 350–1,000 ng starting template DNA. Internal *Oophila*-specific 18S rDNA primers were used with 1  $\mu$ L of the outside-primer PCR product as template. Amphibian-specific histone-4 primers were used as a positive control on all tissues (4). Amplified 18S rDNA PCR products were verified by sequencing.

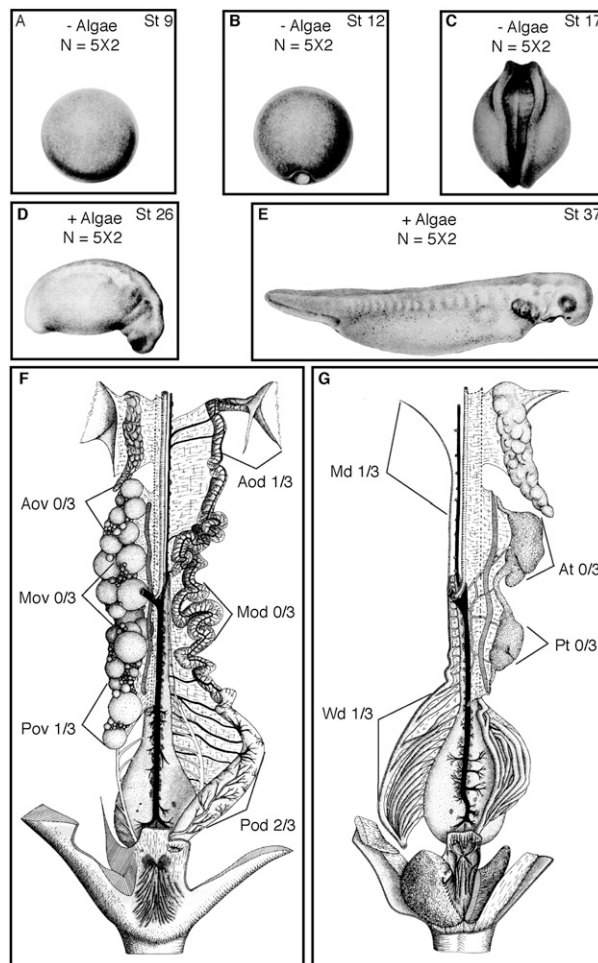
**Phylogenetic Analysis.** *Oophila*-specific 16S and 18S rDNA sequences were manually aligned with orthologous plant and algal sequences obtained from GenBank using MacClade (ver. 4.08). Ambiguous positions were trimmed, leaving 1,714 bp of 18S rDNA and 1,362 bp of the 16S rDNA in the alignment. Maximum likelihood (ML) analysis was based on the GTRMIX model of the RAXML package (ver. 7.04). Bootstrap analyses were obtained using ML (RAXML) and log determinant (Log-Det, PAUP\*) distance methods with 1,000 replicates each.

**In Vivo Time-Lapse Microscopy.** Freshly collected egg masses were placed in 9-L glass aquaria containing pond water, maintained at 23 °C, and illuminated with daylight-spectrum compact fluorescent lighting to promote algal growth and for imaging. Embryo development in the egg capsules and jelly coat was imaged in seven egg masses over consecutive seasons from 2008 and 2009. Algal blooms were observed at the same developmental stage in four of the egg masses. The other three did not produce visible algal blooms and succumbed to fungal infection. Time-lapse sequences were captured with a Nikon D200 with 105-mm macro lens. Images were batch processed by auto leveling in Adobe Photoshop and compiled into movies with QuickTime Pro.

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2. Reysenbach A, Pace N (1995) Reliable amplification of hyperthermophilic archaeal 16S rRNA genes by the polymerase chain reaction. *Archaea: A Laboratory Manual, Volume 1. Thermophiles: Molecular Biology and Genetics*, ed Robb F (Cold Spring Harbor Lab Press, Cold Spring Harbor, New York), pp 101–105.

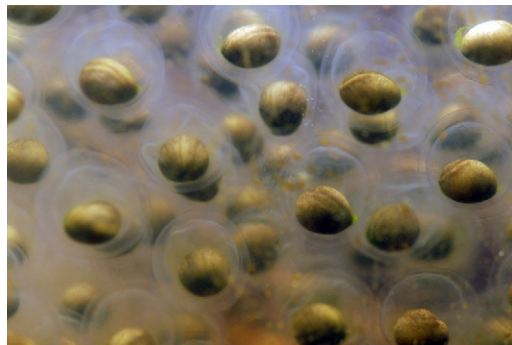
3. Kim E, Simpson AG, Graham LE (2006) Evolutionary relationships of apusomonads inferred from taxon-rich analyses of 6 nuclear encoded genes. *Mol Biol Evol* 23: 2455–2466.
4. Ding X, Hausen P, Steinbeisser H (1998) Pre-MBT patterning of early gene regulation in *Xenopus*: The role of the cortical rotation and mesoderm induction. *Mech Dev* 70: 15–24.





**Fig. S2.** Stage- and tissue-specific amplification of *Oophila* sp. 18S rDNA. (A–E) *Oophila* sp. 18S rDNA was not detected from embryonic Stages 9–17 but was amplified from Stages 26 and 37. DNA samples were pooled from five individuals with two replicates. (F and G) *Oophila*-specific 18S rDNA was amplified from regions of the ovaries, oviducts, Wolffian ducts, and Müllerian ducts in different adult salamanders. Three sample replicates were used for female (F) and male (G) tissue-specific amplifications. The ovaries and testes are not shown on the left sides of F and G, respectively, to reveal underlying ducts. AOD, anterior oviduct; AOV, anterior ovary; AT, anterior testis; MD, Müllerian duct; MOD, medial oviduct; MOV, medial ovary; POD, posterior oviduct; POV, posterior ovary; PT, posterior testis; WD, Wolffian duct. A–E reprinted with permission from ref. 1 (Copyright 1969, Yale University Press). F and G reprinted with permission from ref. 2, Society for the Study of Amphibians and Reptiles.

- Harrison R (1969) Harrison stages and description of normal development of the spotted salamander, *Ambystoma punctatum* (Linn). *Organization and Development of the Embryo*, ed Wilens S (Yale Univ Press, New Haven, CT), pp 44–66.
- Francis E (1934) *The Anatomy of the Salamander* (Clarendon Press, Oxford).



**Movie S1.** Time-lapse movie of developing *Ambystoma maculatum* embryos during initial algal invasion. Rapid algal proliferation can be seen occurring near the blastopore in several of the eggs that are in the frame. This sequence from 2009 represents 16.6 h of development imaged at 1-min intervals.

[Movie S1](#)