

Figure S1. Procedure for data collection and analysis of the osmotic response of silkworm eggs. (A) A Schematic presentation of the procedure for imaging area changes in eggs during exposure to 1 M sucrose solution. (B) Photograph of the dechorionated eggs placed and clamped to the dish with tiny nuts. (C) An egg analyzed by ImageJ software; the red line indicates the area defined by related points around the membrane contour of the embryo. Scale bar: 500 μm .

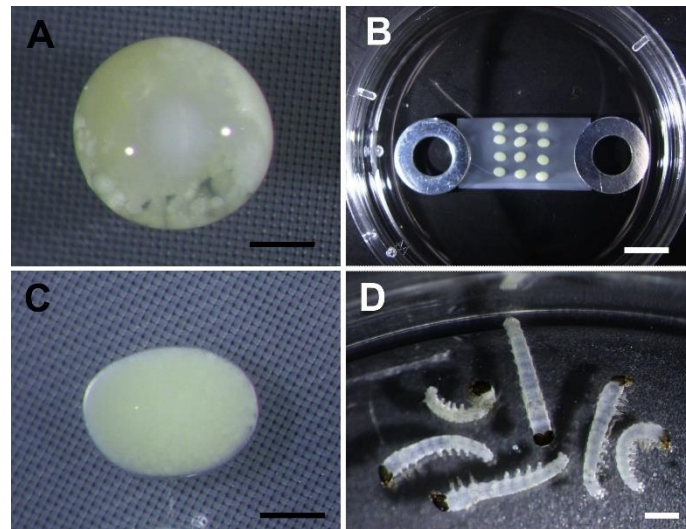


Figure S2. Images of permeabilized early embryonic stages of the pnd-w1 strain with a modified culture method using liquid paraffin. (A) An egg with an amorphous shape with the dry-moist culture method. (B) Eggs with the culture method using liquid paraffin. (C) An egg with a normal shape in the culture method using liquid paraffin. (D) Embryos developed following to the serosa ingestion in the modified culture method. Scale bar: 500 μm .

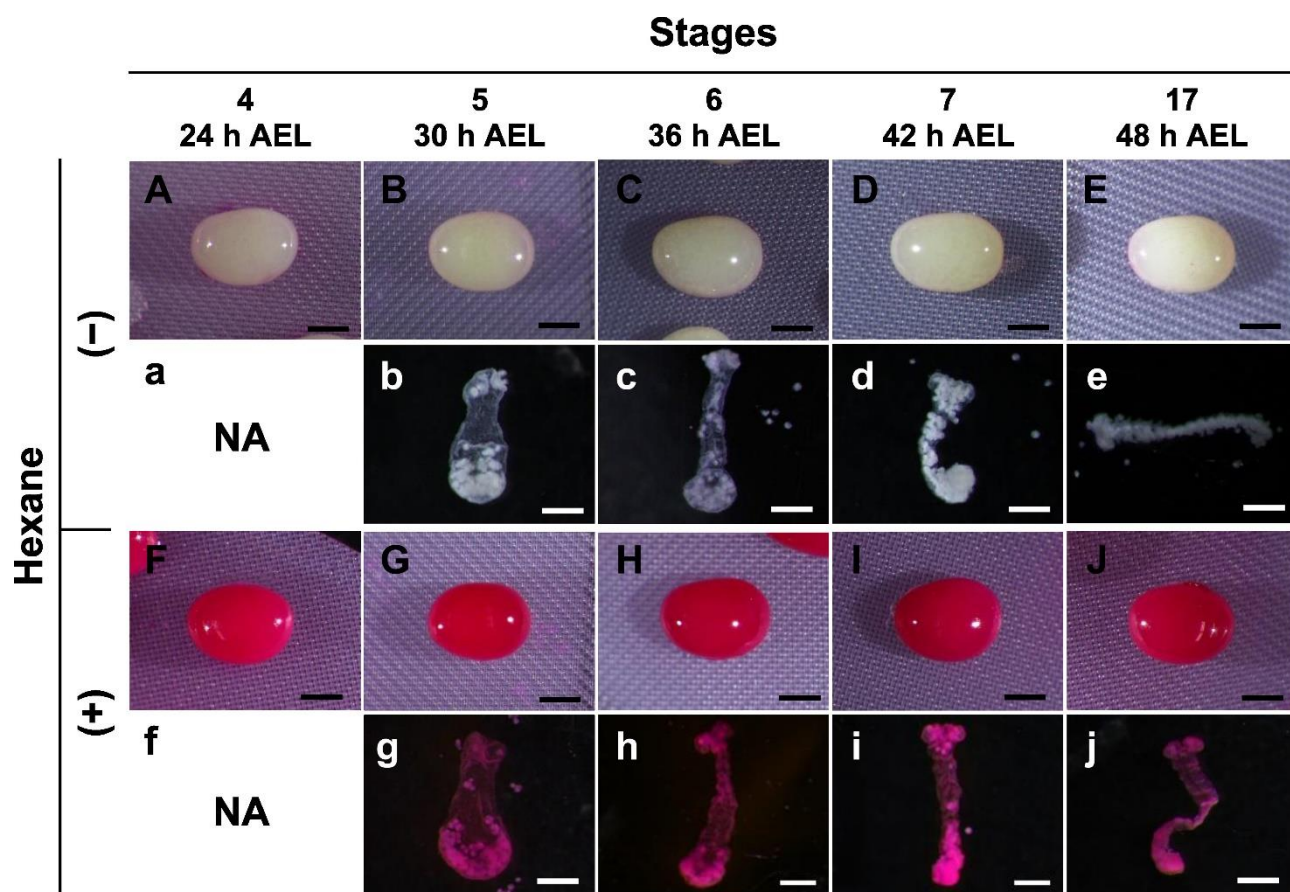


Figure S3. Images of permeabilized embryos in the early stages of the pnd-w1 strain. The dechorionated eggs were subjected to a permeabilization treatment with hexane for 30 s, immersed in 0.1% rhodamine B for 10 min, and then rinsed with PBS. (A-E) Eggs without hexane treatment. (a-e) Naked embryos derived from B-E eggs. (F-J) Eggs with hexane treatment. (f-j) Naked embryos derived from F-J eggs. Permeabilized embryos were stained red. NA, not available. Scale bar: 500 μ m.

Table S1. Egg hatchability of fertile moths derived from permeabilized embryos of pnd-w1 and w1 strains.

Strains	Stage	No. of moths	No. of eggs laid ^a	No. of hatched embryos ^b	Hatchability (%) ^c
pnd-w1	Early 1-Stage 25	21	338.9 ± 23.0	278.3 ± 25.2	81.9 ± 4.1
w1	Early 1-Stage 25	20	379.4 ± 50.3	294.5 ± 37.1	79.9 ± 4.3

^a Mean number of eggs laid ± SE

^b Mean number of hatched embryos ± SE

^c Percentage of hatched eggs to number of eggs laid ± SE