Supplementary Materials for Sourakov, A. & L. T. Shirai. 2020. Pharmacological and surgical experiments on wing pattern development of Lepidoptera, with the focus on eyespots of saturniid moths. *Tropical Lepidoptera Research*, 30(1): 4-19. DOI: 10.5281/zenodo.3765145

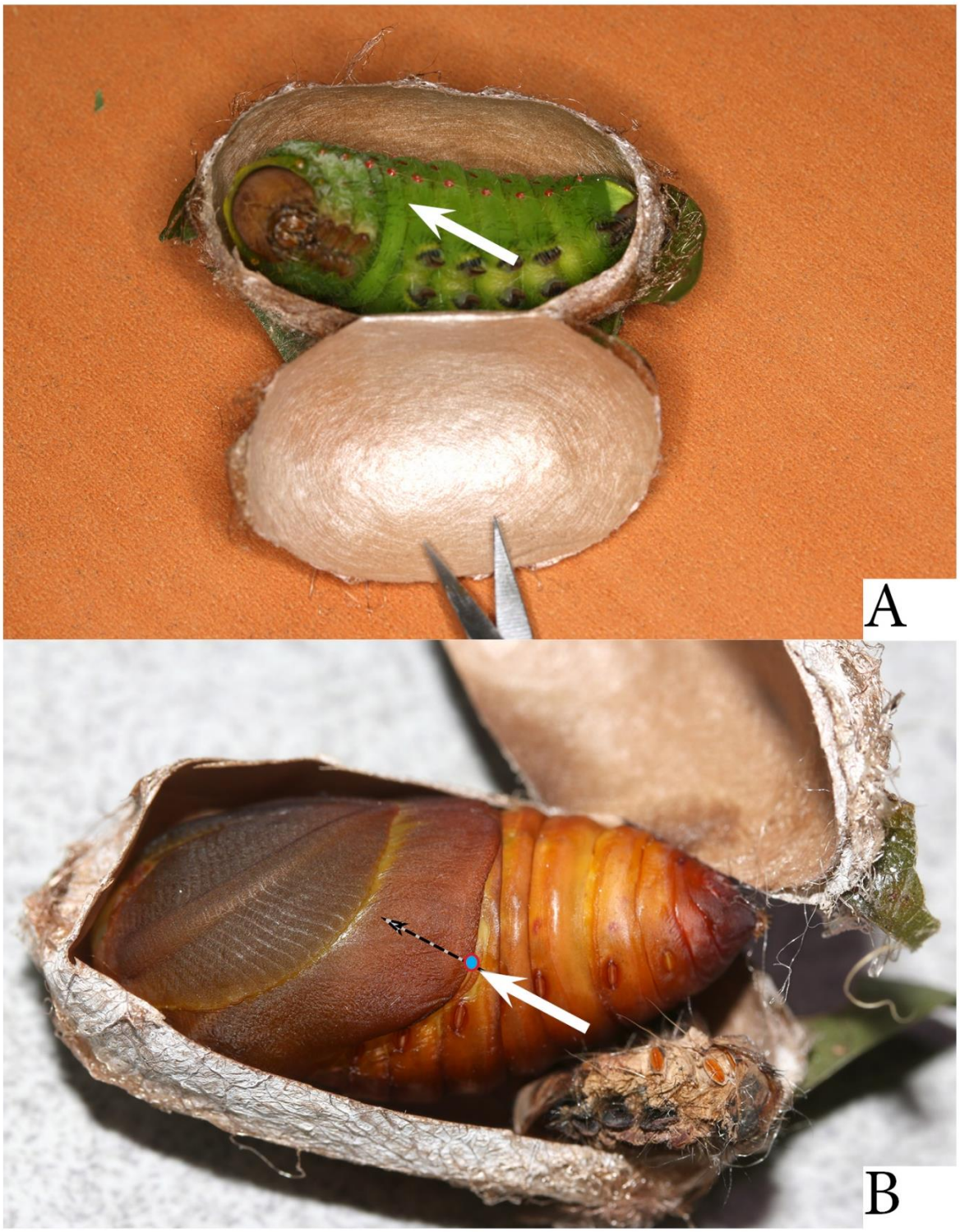

Figure S1. *Antheraea polyphemus* prepupa inside a cocoon 3-4 days following cocoon spinning (**A**), and a pupa (**B**). Arrow indicates the direction of needle during heparin injection, which was made into the HW compartment at the point indicated by the red-and-blue dot. To avoid heparin discharge as a result of bleeding, injections were made slowly and far (>2mm) from the wing margin.

Figure S2. Results of the pilot study on *Antheraea polyphemus*: (**A**) Parental female; (**B**) F-1 (female 1) resulting from a prepupa injected ca. 5 ul of 18.5% (ca. 1 mg*) heparin at 50 hBP; (**C**) F-1 (female 2) resulting from a pupa injected ca. 10 ul of 18.5% (ca. 2 mg*) heparin at 5 hAP; (**i**) dorsal, (**ii**) ventral. *the measurements and amounts are approximate as measurements/injections were made with a hypodermic syringe.

Figure S3. *Automeris io* transformed by heparin. (**A**) ca. 5 ul of 30% (ca. 2 mg) heparin as prepupa (superficially) within 1 dBP; (**B**) 4 ul of 27% (1.5 mg) heparin as pupa 10 hAP; (**C**) ca. 5 ul of 30% heparin as pupa (R wing compartment) 5-6 hAP; (**D**) 4 ul of 27% (1.5 mg) heparin as pupa (R wing compartment) 5 hAP; (**E**) 7 ul of 18% (1.5 mg) heparin as pupa (L wing compartment) 10-12 hAP; (**F**) ca. 5 ul of 29% (ca. 2 mg) heparin as pupa (L wing compartment) 12 hAP; (**G**) 4 ul of 43% (2.8 mg) heparin as prepupa (superficially, L side) 75 hBP; (**H**) 2 ul of 3% (0.06 mg) heparin as pupa (deep into abdomen) 1.5 hAP; (**I**) 1 ul of 3% (0.03 mg) heparin as pupa (deep into abdomen) ca. 8 hAP; (**J**) 1 ul of 3% (0.03 mg) heparin as pupa (deep into abdomen) ca. 8 hAP; (**K**) 4 ul of 16% (0.8 mg) heparin as prepupa 63 hBP. Except for individuals in Figs. H, I, J, all other may have experienced some heparin expulsion due to "bleeding." Hence, 0.03-0.1mg for males and twice as much for females would likely produce an observable effect on wing pattern without being detrimental to survival.

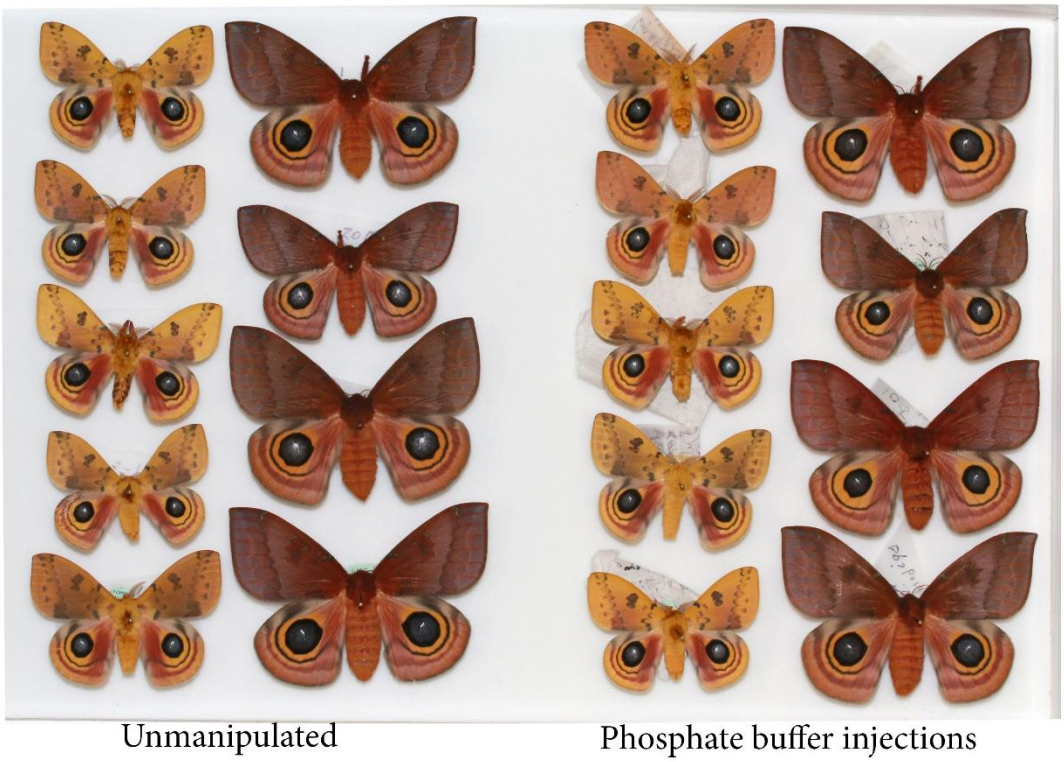

Figure S4. *Automeris io* from the same brood, unmanipulated (left) and injected with phosphate buffer solution as prepupae and early pupae (right). No phenotypic change similar to that resulting from injections of heparin was detected.

Figure S5. *Antheraea polyphemus* from the same brood, unmanipulated (bottom) and injected with 5 ul of distilled water (top) early pupae. No phenotypic change similar to that resulting from injections of heparin was detected.

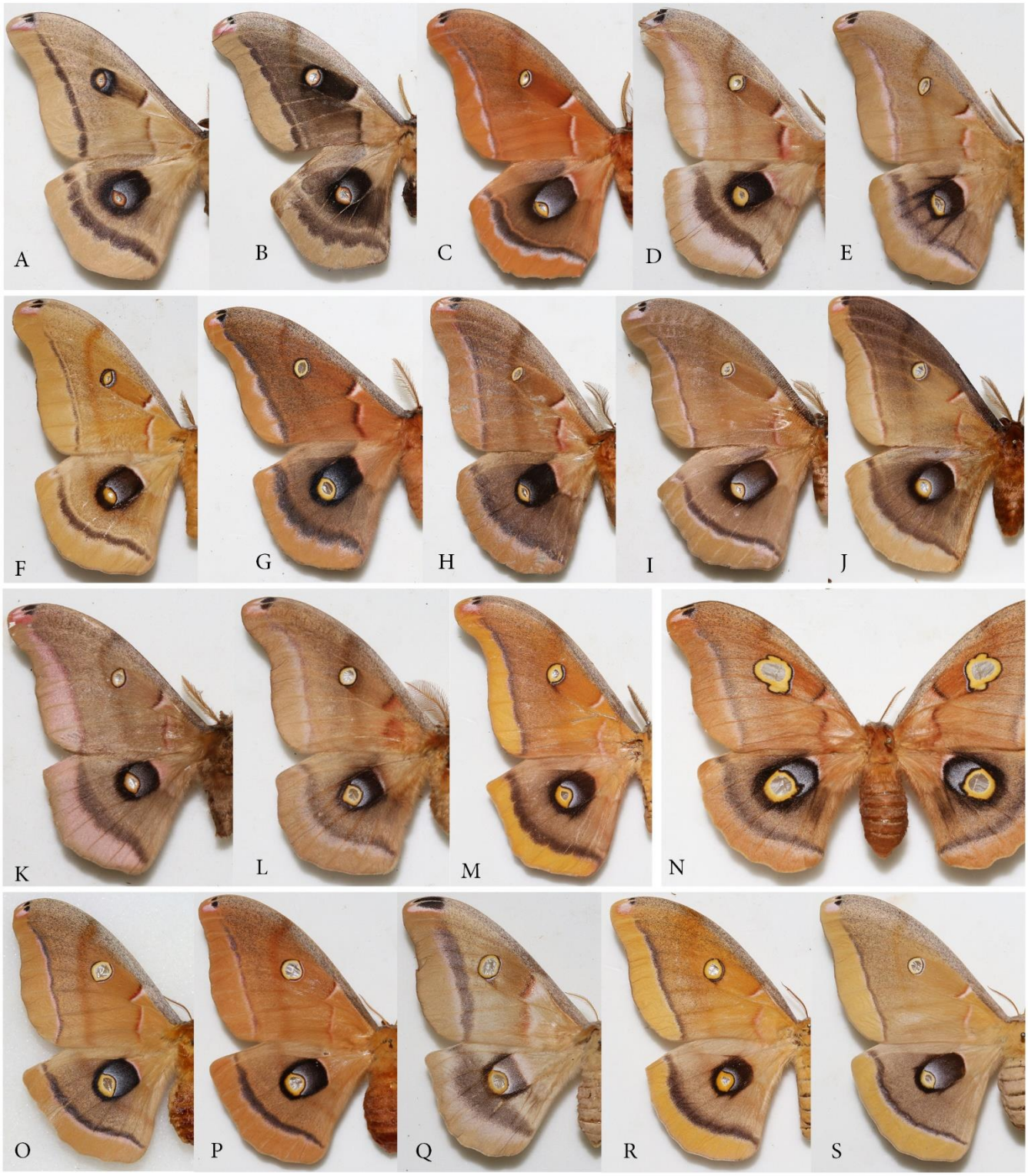

Figure S6. Variety of wild *Antheraea* phenotypes from the MGCL collection, including a number of rare aberrations: (**A-B**) ***A. oculea***: (**A**) Arizona, Aug 1999 (ex ova); (**B**) Pima Co, Arizona, 7 Aug, 2005; (**C-S**) ***A. polyphemus*****:** (**C**) Florida; (**D**) Indiana, 1 June 1918; (**E**) Illinois, 12June 1929; (**F**) Salt Lake Co., Utah, 5 Sept 1987 (ex ova); (**G**) Saddlestring, Wyoming, 22 May 1979. (ex larva col. Aug 1978); (**H**) Lake Junalusca, North Carolina, 6 July 1961; (**I**) Florida, 9 October 1967; (**J**) Alameda Co, California, 30 May 1962; (**K**) Clackamas Co., Oregon, 10 August 1984; (**L**) Hamond, Indiana, 28 May 1932; (**M**) Chimalapa, Oaxaca, Mexico, 7 October 1957; (**N**) Quebec, June 2005 (ex ova); (**O**) Alameda Co., California 30 May 1957; (**P**) Alameda Co., California 6 May 1962; (**Q**) Chicago, Illinois, 3 June 1921; (**R**) Chimalapa, Oaxaca, Mexico, September 1964; (**S**) Alachua Co., Florida, 25 December 1972 (ex larva collected 12 September 1972).

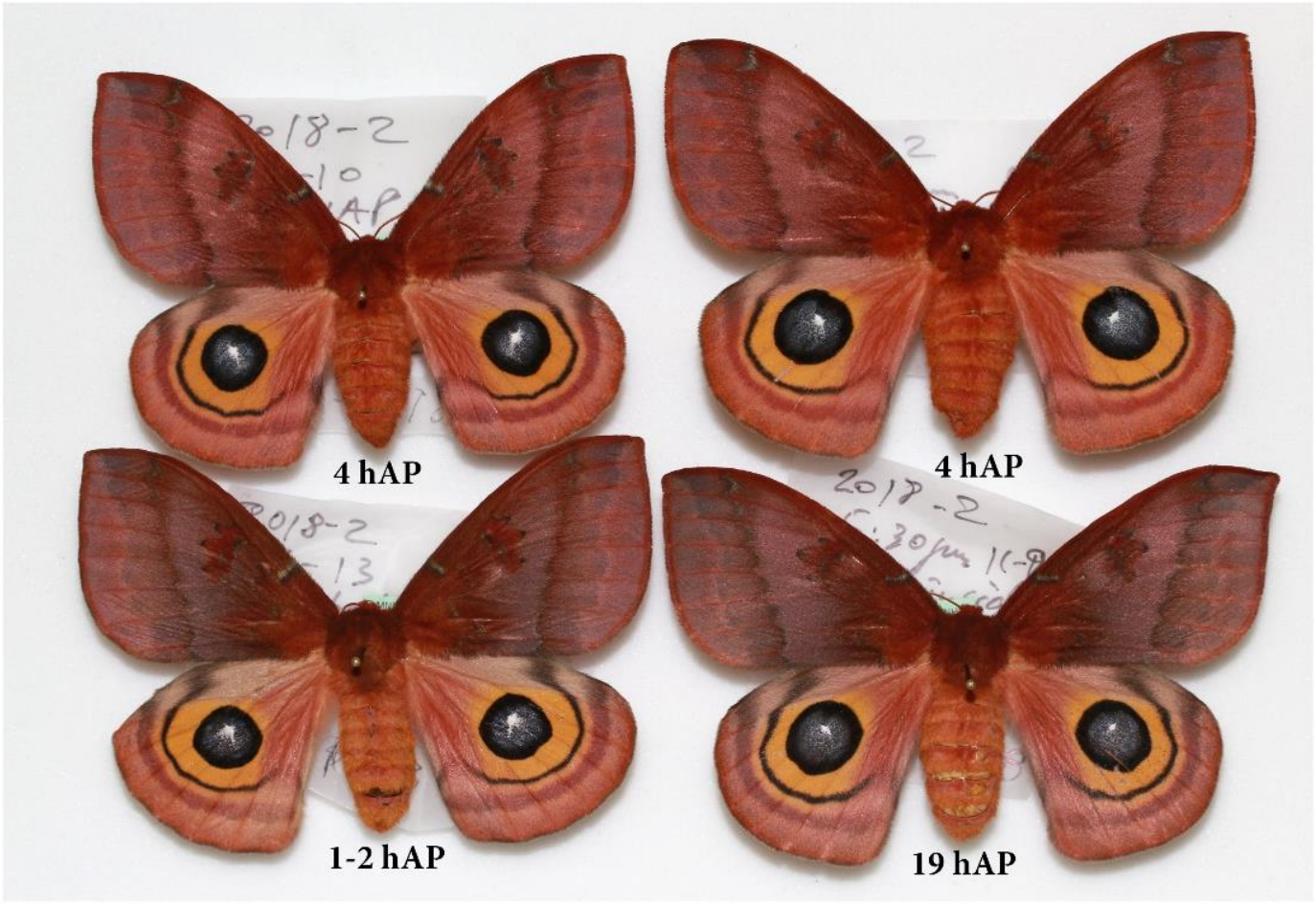

Figure S7. Mechanical injury to the forewing discal cell of pupae did not lead to wound-induced responses.

Table S1: Experimental brood of *Antheraea polyphemus* with heparin injections details and voucher specimen numbers (see corresponding images below).

Photo #	MGCL-voucher #	Individual was injected hours before (BP) or after (AP) pupation	Heparin volume	Heparin concentration %	Heparin quantity (mg)
IMG_7159-60	292161	9 hAP	5ul	5	0.25
IMG_7162-63	292162	5.5 hAP	5ul	3.7	0.2
IMG_7255_56	292111	1.5 hBP	5ul	3.7	0.2
IMG_7264-65	292108	5 hAP	5ul	13	0.75
IMG_7267-68	292107	3.25 hAP	5ul	3.7	0.2
IMG_7270-71	292106	4 hBP	5ul	15.7	1
IMG_7277-78	292104	2 hBP	5ul	15.7	1
IMG_7696-97	292236	9 hBP	5ul	3.7	0.2
IMG_7708, 7710	292239	2.5 hAP	5ul	3.7	0.2
IMG_7712-13	292240	44 hBP	5ul	3.7	0.2
IMG_7715-16	292241	9.5 hAP	5ul	5	0.25
IMG_7721-22	292243	5 hAP	5ul	15.7	1
IMG_7729-30	292245	6 hBP	5ul	13	0.75
IMG_7732-33	292246	5 hAP	5ul	5	0.25
IMG_7735-36	292247	5 hAP	5ul	3.7	0.2
IMG_7738-39	292248	24 hBP	5ul	5	
IMG_7741-42	292249	19 hBP	5ul	3.7	0.2
IMG_7750-51	292254	4 hAP	5ul	5	0.25
IMG_7751-52	292251	4 hAP	5ul	5	0.25
IMG_7754-55	292252	9 hAP	5ul	3.1	0.15
IMG_7757-58	292253	13.5 hAP	5ul	13	0.75
IMG_7810-11	292262	?(7<16) hAP (soft pupa)	5ul	3.7	0.2
IMG_7816-17	292264	4 hAP	5ul	3.7	0.2
IMG_7822-23	292266	24 hAP	5ul	15.7	1
IMG_7827-28	292267	10.5 hAP	5ul	15.7	1
IMG_7864-65	292269	1 hBP	5ul	3.7	0.2
IMG_7867-68	292270	36 hBP	5ul	5	0.2

IMG_7873; 7880	292271	8 hAP	5ul	15.7	1
IMG_7884; 7890	292272	31 hBP	5ul	3.1	0.15
IMG_7893; 7896	292273	12 hBP	5ul	15.7	1
IMG_7917-18	292274	4 hBP	5ul	5	0.25
IMG_7252-53	292083	unmanipulated	n/a	n/a	Control
IMG_7258-59	292110	unmanipulated	n/a	n/a	
IMG_7261-62	292109	unmanipulated	n/a	n/a	
IMG_7274-75	292105	unmanipulated	n/a	n/a	
IMG_7699-7700	292237	unmanipulated	n/a	n/a	
IMG_7705-06	292238	unmanipulated	n/a	n/a	
IMG_7718-19	292242	unmanipulated	n/a	n/a	
IMG_7726-27	292244	unmanipulated	n/a	n/a	
IMG_7744-45	292250	unmanipulated	n/a	n/a	
IMG_7804-05	292260	unmanipulated	n/a	n/a	
IMG_7807-08	292261	unmanipulated	n/a	n/a	
IMG_7813-14	292263	unmanipulated	n/a	n/a	
IMG_7819-20	292265	unmanipulated	n/a	n/a	
IMG_7861-62	292268	unmanipulated	n/a	n/a	


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