eLIFE elifesciences.org

RESEARCH ARTICLE

Figures and figure supplements

Molecular basis of wax-based color change and UV reflection in dragonflies

Ryo Futahashi *et al*

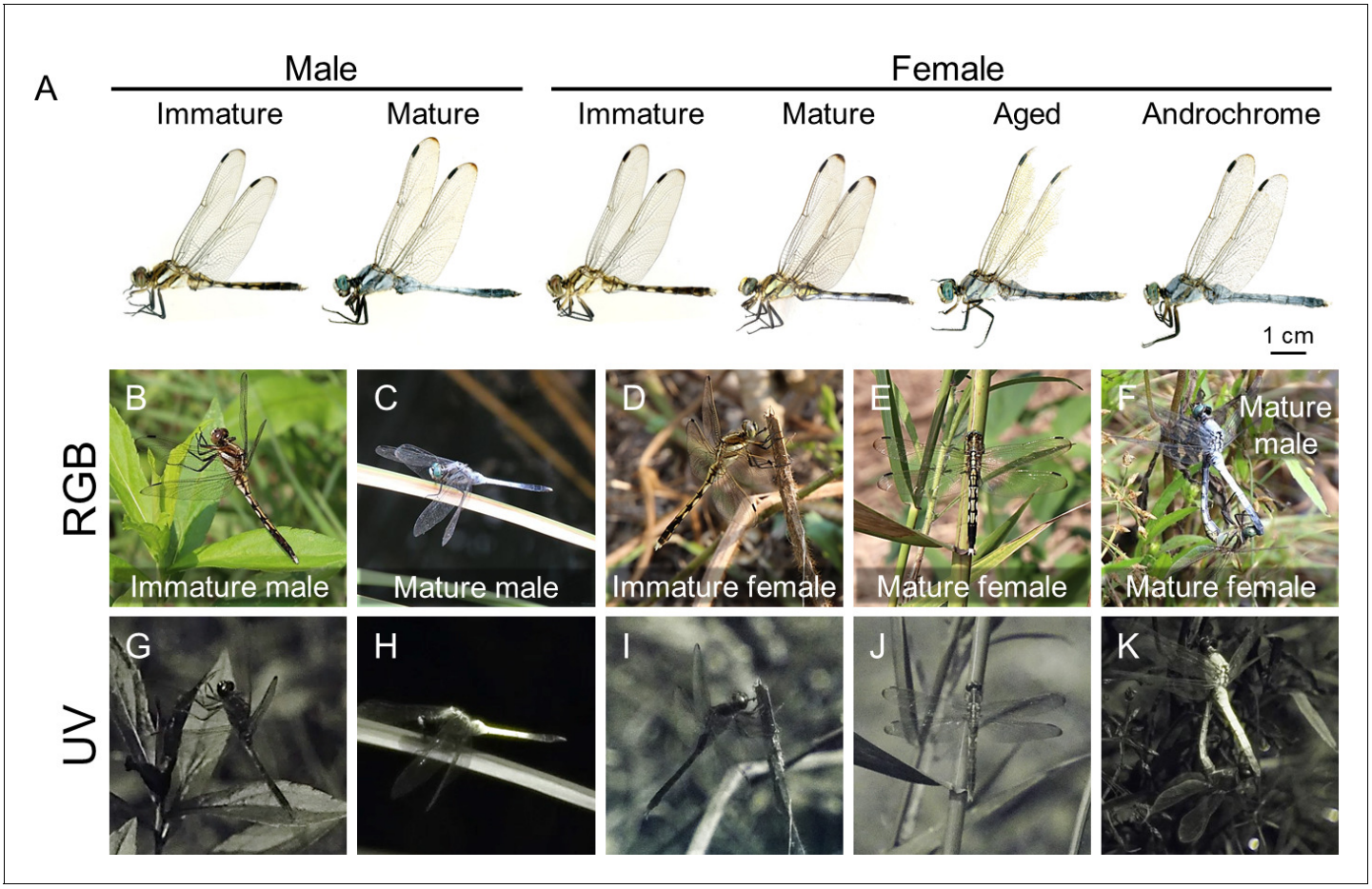


Figure 1. Stage- and sex-dependent adult color change in *O. albistylum* as visualized in red, green, blue (RGB) and ultraviolet (UV) light. (**A**) Adult males and females of *O. albistylum*. (**B, G**) Immature male. (**C, H**) Mature male. (**D, I**) Immature female. (**E, J**) Mature female. (**F, K**) Mating pair. Images photographed normally (**B–F**) or through a UV filter (**G–K**) in the field.

DOI: https://doi.org/10.7554/eLife.43045.003

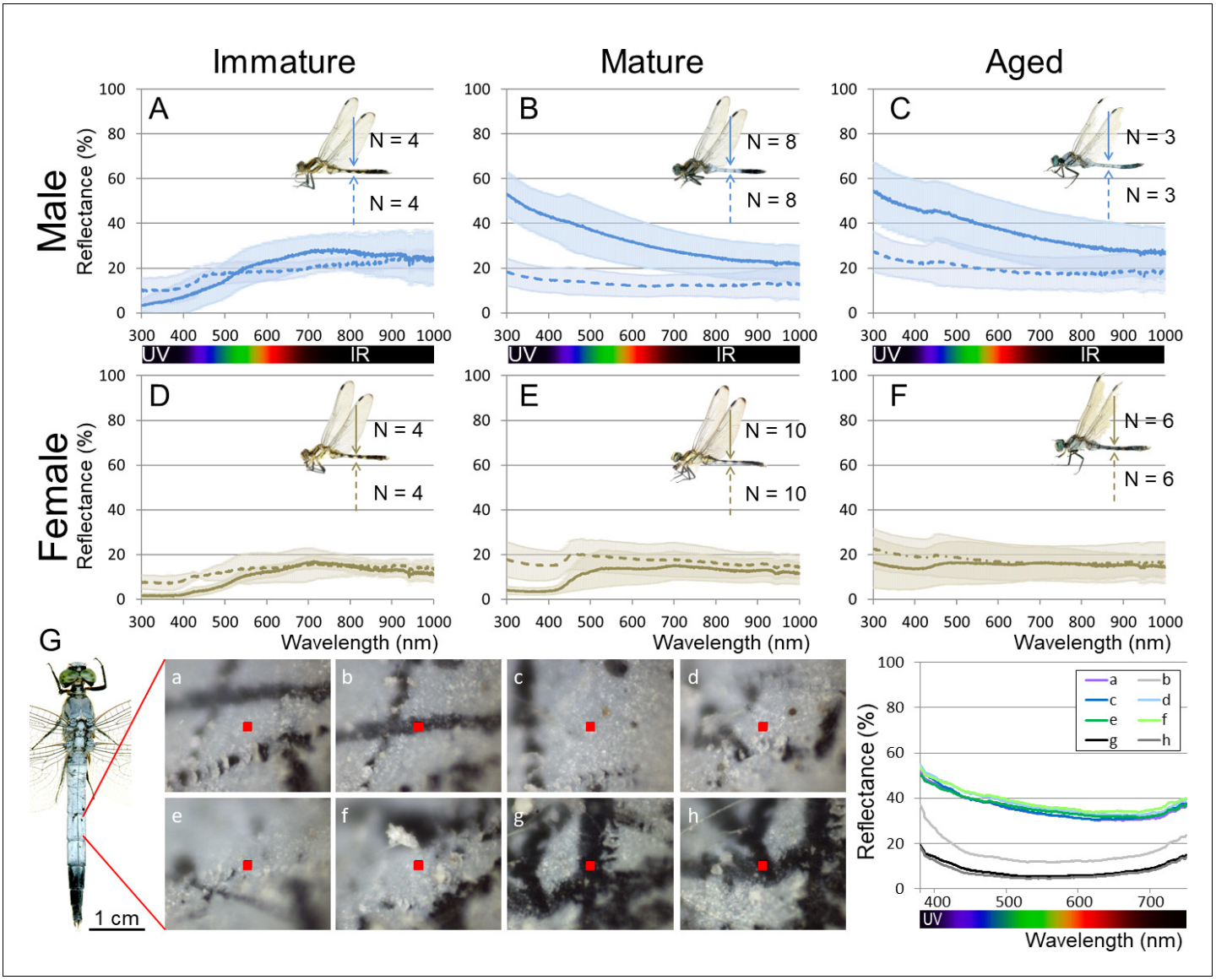


Figure 2. Reflectance of the adult body surface at the 5th abdominal segment of *O. albistylum*. (**A–F**) Spectrometry of a round area (6 mm in diameter) in males (**A–C**) and females (**D–F**). (**A, D**) Immature individuals. (**B, E**) Mature individuals. (**C, F**) Aged individuals. Solid and dotted lines indicate averaged UV reflectance on the dorsal and ventral sides of the abdomen, respectively. The standard deviation is shaded. (**G**) Micro-spectrometry of the 5th abdominal segment of a mature male. UV reflectance was measured in eight micro-areas (10 μm x 10 μm each) depicted as red squares in the photos. In the photos, white areas are covered with secreted wax whereas black areas are without wax, presumably because of accidental scratches and cracks on the adult body surface.

DOI: https://doi.org/10.7554/eLife.43045.004

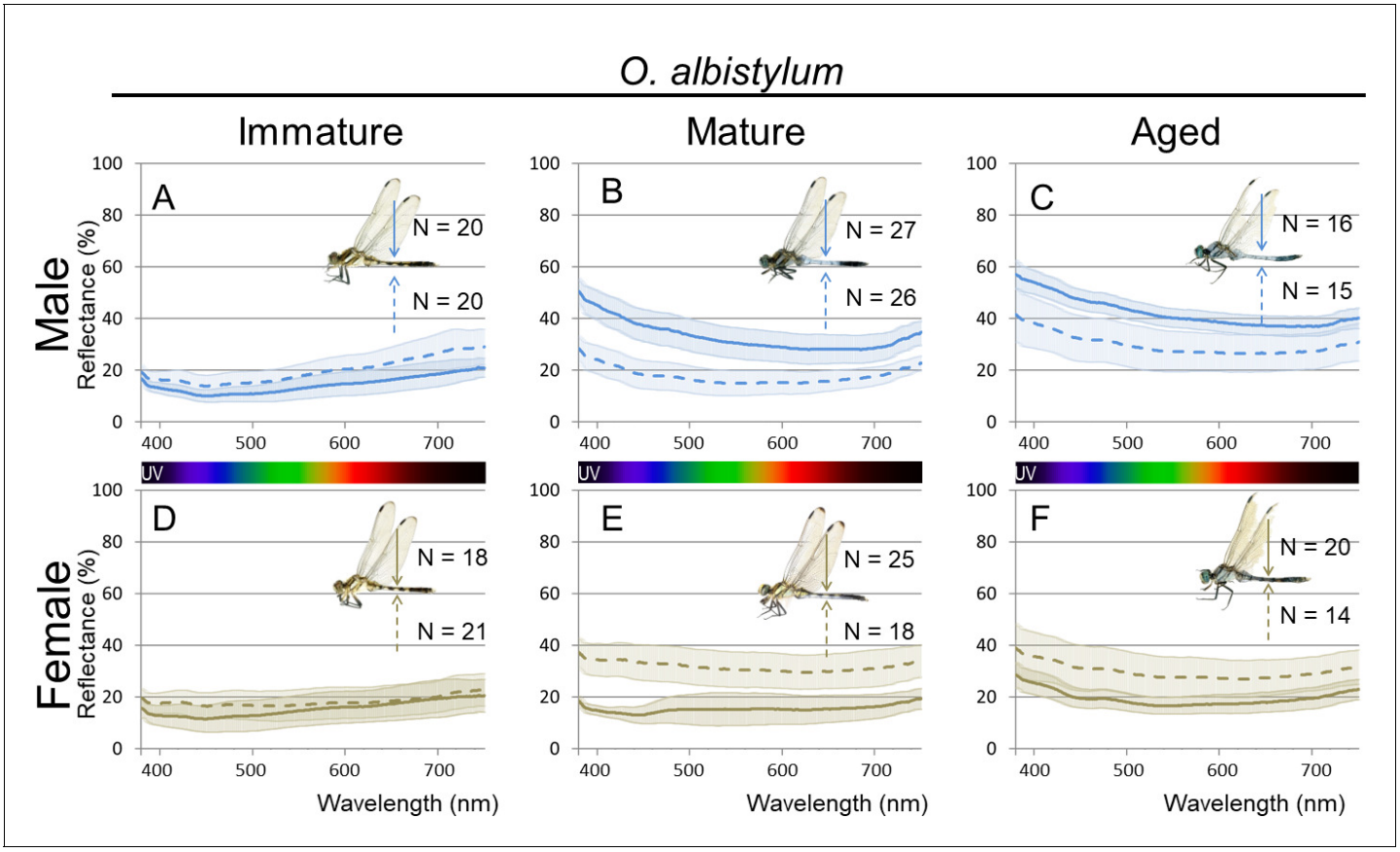


Figure 2—figure supplement 1. Micro-spectrometry (of 10 × 10 µm micro-areas) of *O. albistylum*. (**A–C**) Male. (**D–F**) Female. (**A, D**) Immature individuals. (**B, E**) Mature individuals. (**C, F**) Aged individuals. Three to eight micro-areas were measured for each individual.

DOI: https://doi.org/10.7554/eLife.43045.005

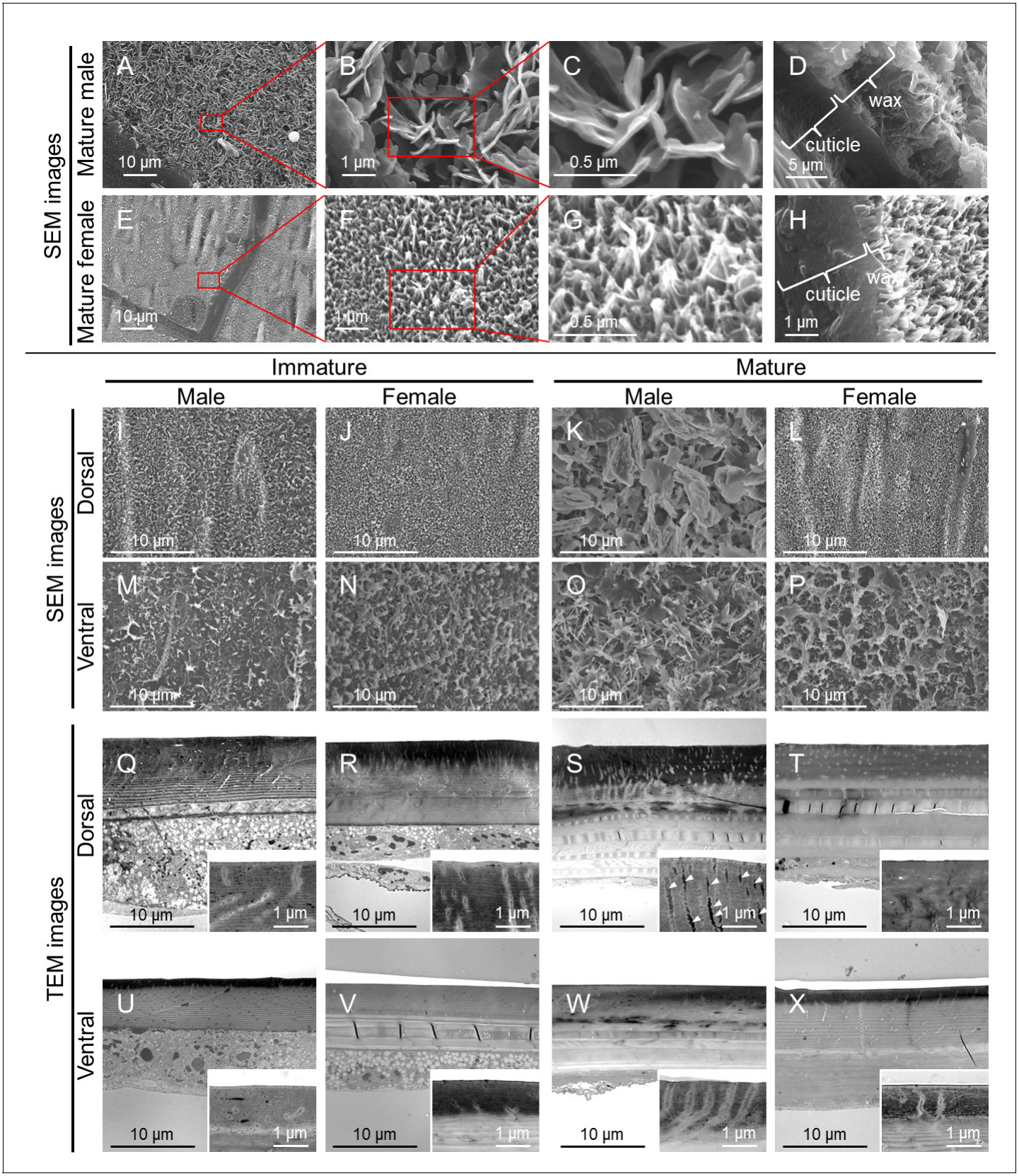


Figure 3. Fine structure of the adult body surface at the 5th abdominal segment of *O. albistylum*. (**A–P**) Scanning electron microscopic (SEM) images of the body surface. (**Q–X**) Transmission electron microscopic (TEM) images of the sectioned cuticle. It should be noted here that the surface wax was

Figure 3 continued on next page

Figure 3 continued

dissolved and removed during the processing of the sample for TEM observation. (**A–H**) Dorsal side of mature male (**A–D**) and mature female (**E–H**). Panels B and F are magnified images of panels A and E, as indicated by red rectangles. Likewise, panels C and G are magnified images of panels B and F. (**D, H**) Cross-sectioned images of cuticle and surface wax. (**I–L and Q–T**) Dorsal side. (**M–P and U–X**) Ventral side. (**I, M, Q, U**) Immature male. (**J, N, R, V**) Immature female. (**K, O, S, W**) Mature male. (**L, P, T, X**) Mature female.

DOI: https://doi.org/10.7554/eLife.43045.014

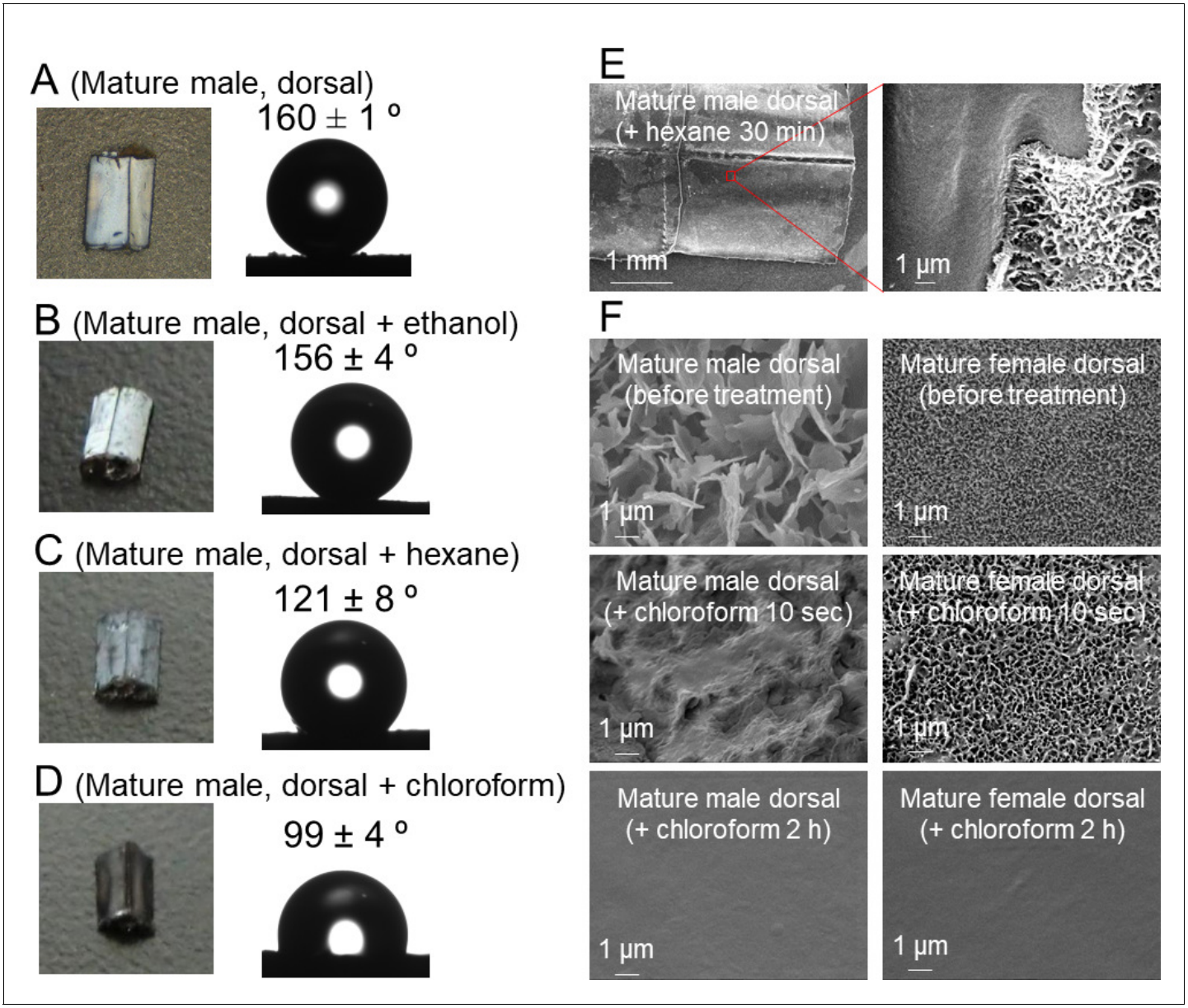


Figure 4. Solubility and wettability of the abdominal wax of *O. albistylum*. (**A–D**) Dorsal side of the 5th abdominal segment of mature males. (**A**) No treatment. (**B**) After ethanol treatment. (**C**) After hexane treatment. (**D**) After chloroform treatment. (**E**) Scanning electron microscope images of the dorsal surface of mature male 30 min after hexane treatment. (**F**) Scanning electron microscope images of dorsal side of a mature male (left) or a mature female (right) after chloroform treatment.

DOI: https://doi.org/10.7554/eLife.43045.016

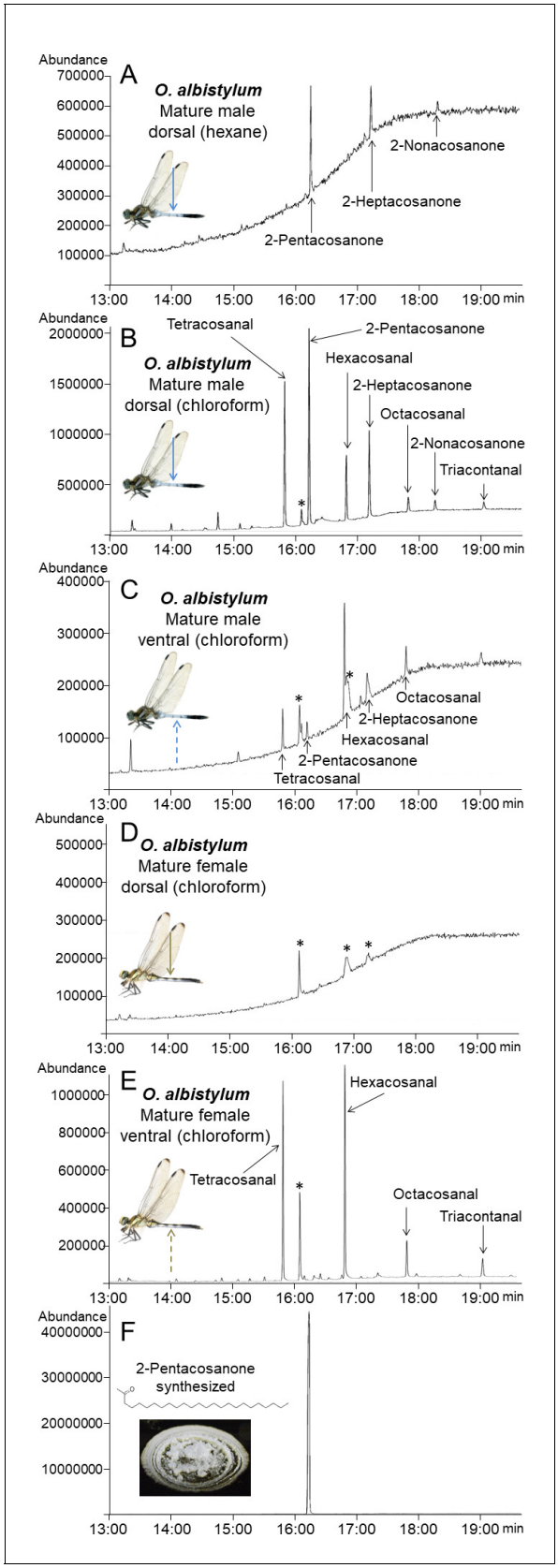


Figure 5. Identification and chemical synthesis of surface wax of *O. albistylum*. (**A**) Chromatogram of hexane extract from the dorsal abdomen of a mature male. (**B**) Chromatogram of chloroform extract from the dorsal abdomen of a mature male. (**C**) Chromatogram of chloroform extract from the

Figure 5 continued on next page

Figure 5 continued

ventral abdomen of a mature male. (**D**) Chromatogram of chloroform extract from the dorsal abdomen of a mature female. (**E**) Chromatogram of chloroform extract from the ventral abdomen of a mature female. (**F**) Chromatogram of chemically synthesized 2-pentacosanone. Asterisks indicate nonspecific peaks that are also detected with the solvent only. The Y-axis shows the abundance of total ion current.

DOI: https://doi.org/10.7554/eLife.43045.017

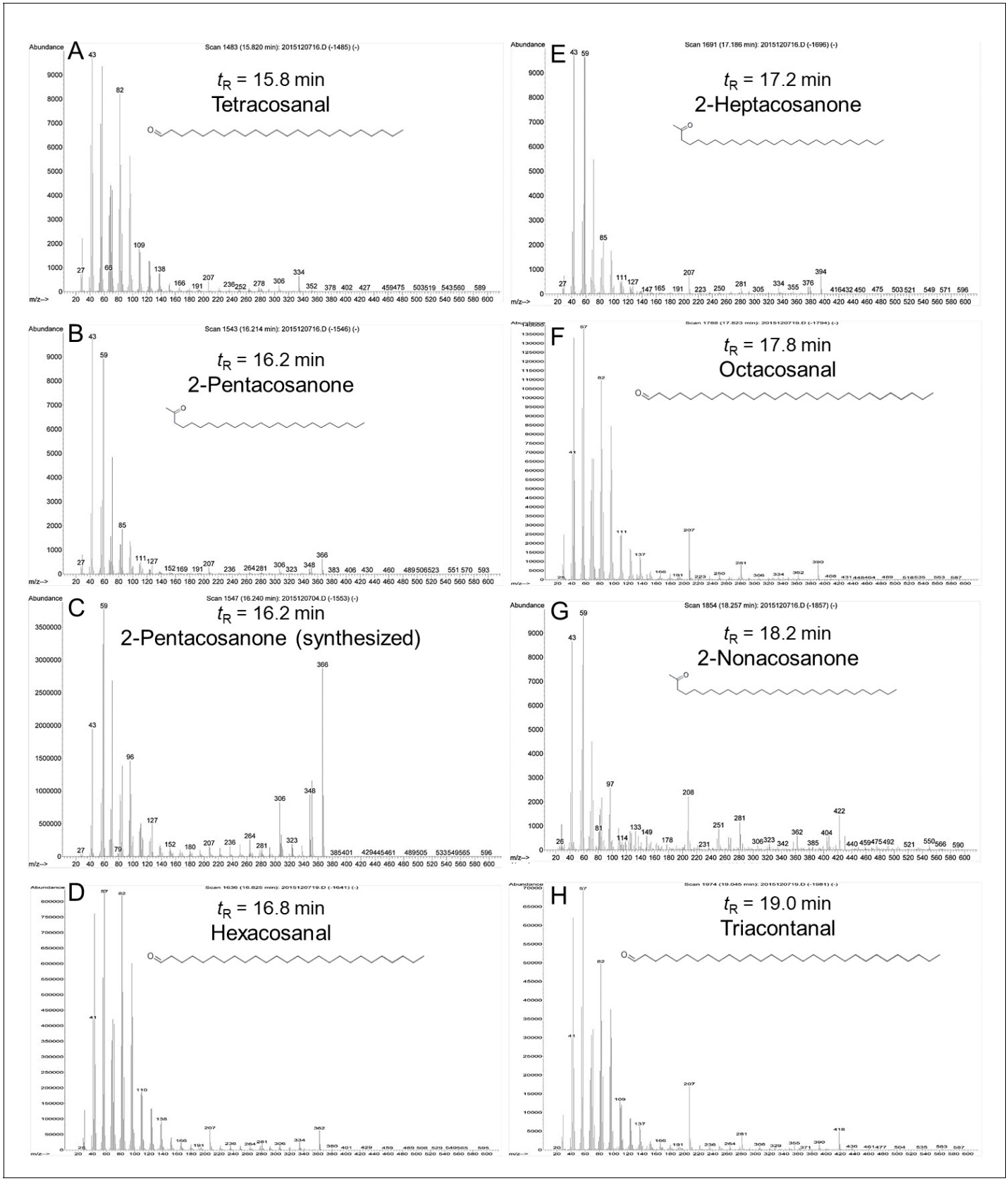


Figure 5—figure supplement 1. Mass spectra and extracted ion chromatogram of dragonfly wax (A, B, D–H) and of synthetic 2-pentacosanone (C).
DOI: https://doi.org/10.7554/eLife.43045.018

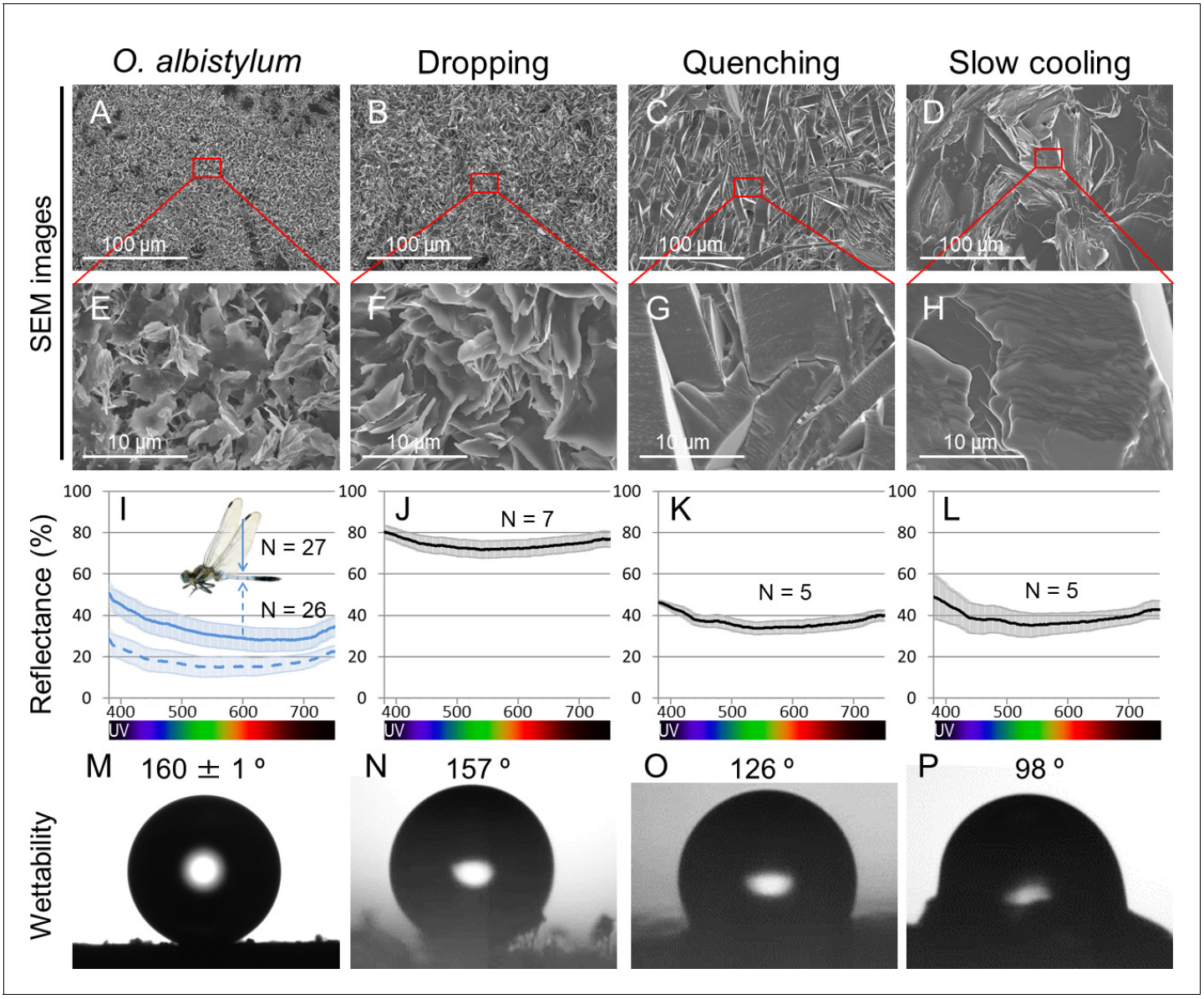


Figure 6. Comparison of surface fine structure, reflectance, and wettability between the dorsal abdomen of mature males of *O. albistylum* and synthetic 2- pentacosanone crystallized on glass plates using three different cooling processes. (**A–H**) Scanning electron microscope images. Panels E, F, G and H are magnified images of panels A, B, C and D, respectively, as indicated by red rectangles. (**I–L**) Micro-spectrometry from a 10 µm x 10 µm area. (**M–P**) Wettability measured with a 1 nL water droplet. (**A, E, I, M**) The dorsal abdomen of mature males of *O. albistylum*. (**B, F, J, N**) Synthetic wax crystallized by the dropping method. (**C, G, K, O**) Synthetic wax crystallized by the quenching method. (**D, H, L, P**) Synthetic wax crystallized by the slow-cooling method.

DOI: https://doi.org/10.7554/eLife.43045.019

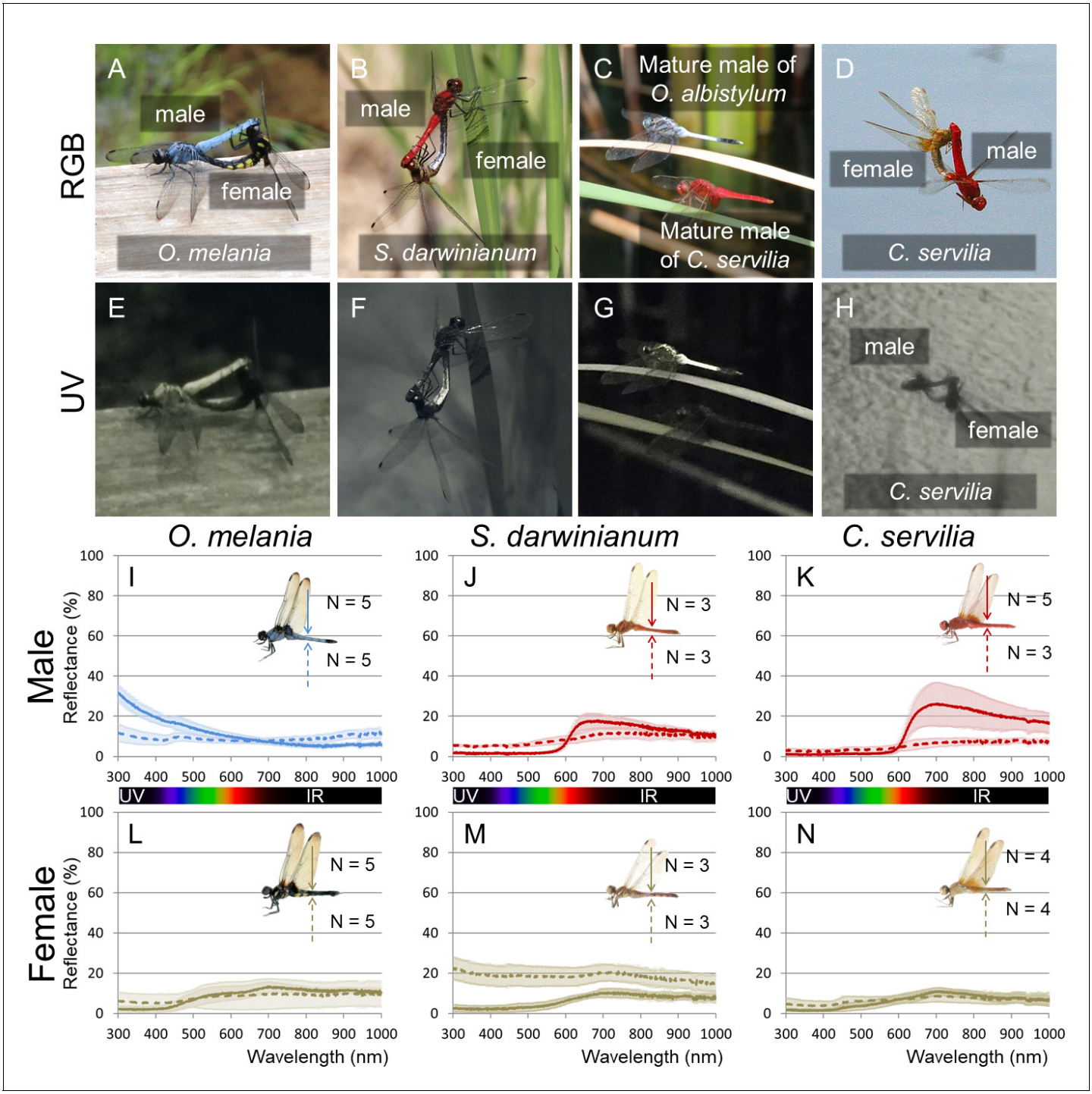


Figure 7. UV reflection patterns in *O. melania*, *S. darwinianum* and *C. servilia*. (**A, E**) Mating pair of *O. melania*. (**B, F**) Mating pair of *S. darwinianum*. (**C, G**) Mature male of *C. servilia* with mature male of *O. albistylum*. (**D, H**) Mating pair of *C. servilia*. Each image photographed normally (RGB) (**A–D**) or through a UV filter (**E–H**) in the field. (**I–N**) Spectrometry of a round area (6 mm in diameter) on the 5th abdominal segment of *O. melania* (**I, L**), *S. darwinianum* (**J, M**), and *C. servilia* (**K, N**). (**I–K**) Male. (**L–N**) Female. Solid and dotted lines indicate averaged UV reflectance on the dorsal and ventral sides of the abdomen, respectively. The standard deviation is shaded.

DOI: https://doi.org/10.7554/eLife.43045.021

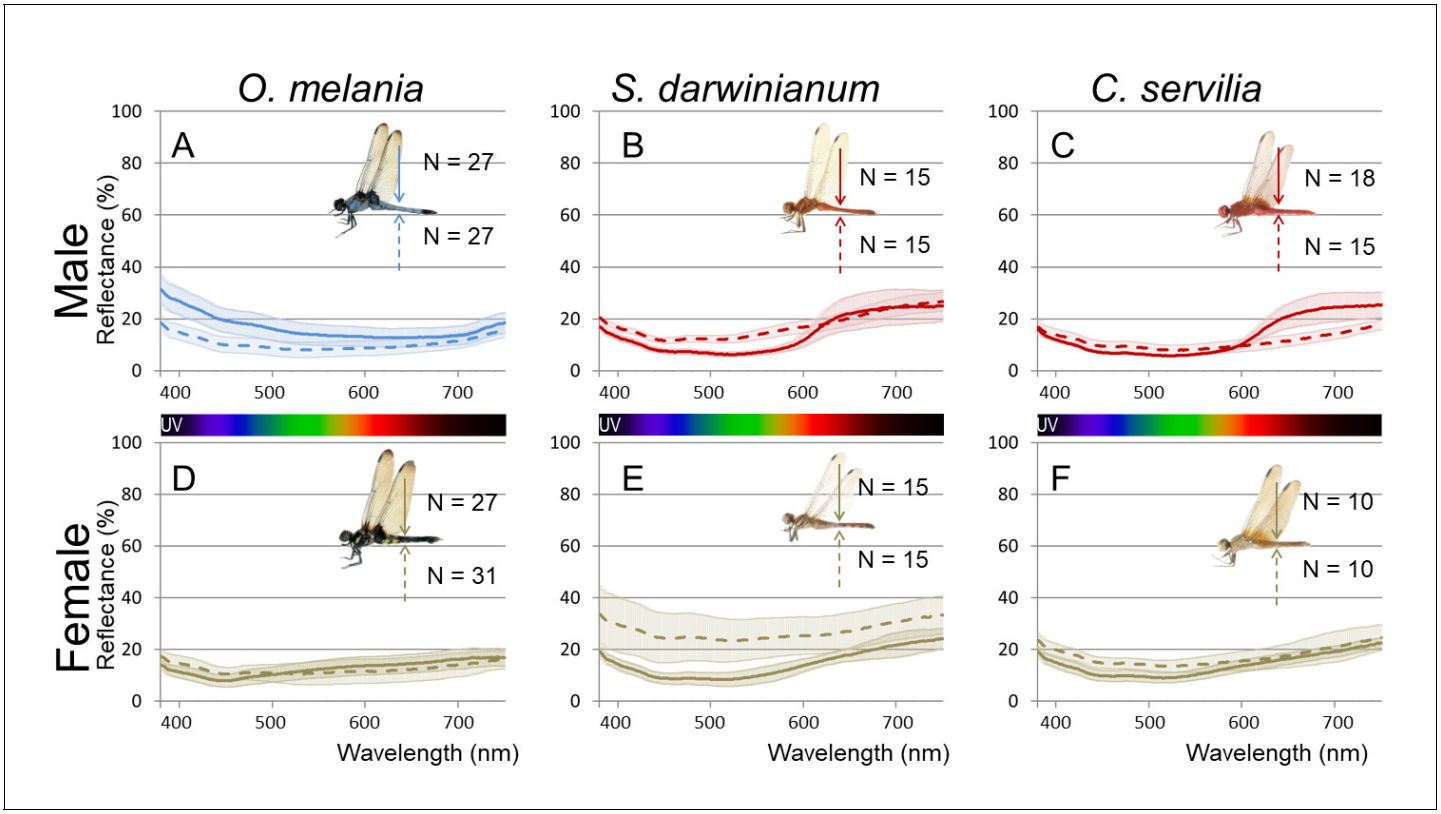
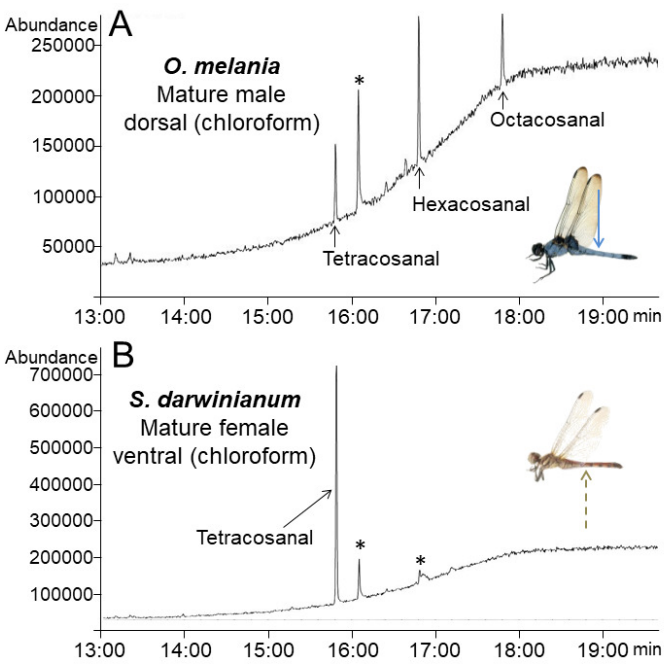


Figure 7—figure supplement 1. Micro-spectrometry (of a 10 × 10 µm micro-area) of *O. melania* (**A, D**), *S. darwinianum* (**B, E**), and *C. servilia* (**C, F**). (**A–C**) Male. (**D–F**) Female. Five to ten micro-areas were measured for each individual.

DOI: https://doi.org/10.7554/eLife.43045.022



C

	Retention Time	15.8	16.2	16.8	17.2	17.8	18.2	19.0
	Molecular weight	334 352	366	362 380	394	390 408	422	418 436
	Candidate	Tetracosanal	2-Pentacosanone	Hexacosanal	2-Heptacosanone	Octacosanal	2-Nonacosanone	Triacontanal
Dorsal	*O. albistylum* male	+++	+++	++	+++	+	+	+
Dorsal	*O. albistylum* female							
Dorsal	*O. melania* male	++		++		++		
Dorsal	*O. melania* female							
Dorsal	*S. darwinianum* male							
Dorsal	*S. darwinianum* female							
Ventral	*O. albistylum* male	+	+	++	+	+		
Ventral	*O. albistylum* female	+++		+++		+		+
Ventral	*O. melania* male					+		
Ventral	*O. melania* female							
Ventral	*S. darwinianum* male							
Ventral	*S. darwinianum* female	+++						

Figure 8. Comparison of wax components on the dorsal and ventral abdomen of *O. albistylum*, *O. melania* and *S. darwinianum*. (**A**) Chromatogram of chloroform extract from the dorsal abdomen of a mature male of *O. melania*. (**B**) Chromatogram of chloroform extract from the ventral abdomen of a mature female of *S. darwinianum*. Asterisks indicate nonspecific peaks also detected with the solvent only. (**C**) Summary of wax components detected from *O. albistylum*, *O. melania* and *S. darwinianum*. Relative amount was judged from the abundance of total ion current. +++, high amount; ++, moderate amount; +, small amount.

DOI: https://doi.org/10.7554/eLife.43045.029

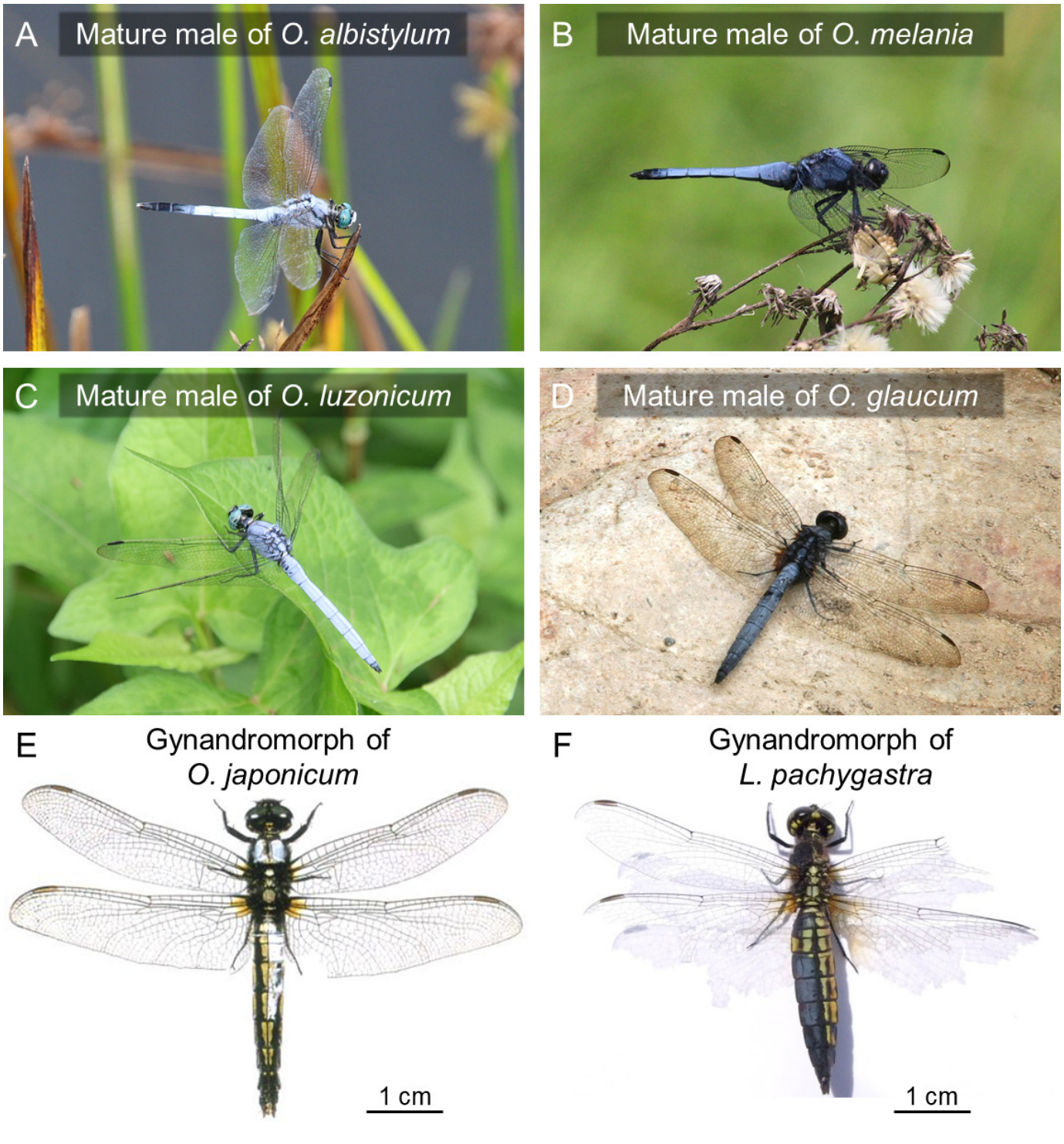


Figure 9. Surface wax of *Orthetrum* species and allied dragonflies. (**A–D**) Mature males of *O. albistylum* (**A**), *O. melania* (**B**), *O. luzonicum* (**C**) and *O. glaucum* (**D**). (**E–F**) Gynandromorphic individuals of *O. japonimum* (**E**) and *Lyriothemis pachygastra* (**F**). Photos courtesy of Mitsutoshi Sugimura (**E**) and Makoto Machida (**F**).

DOI: https://doi.org/10.7554/eLife.43045.031

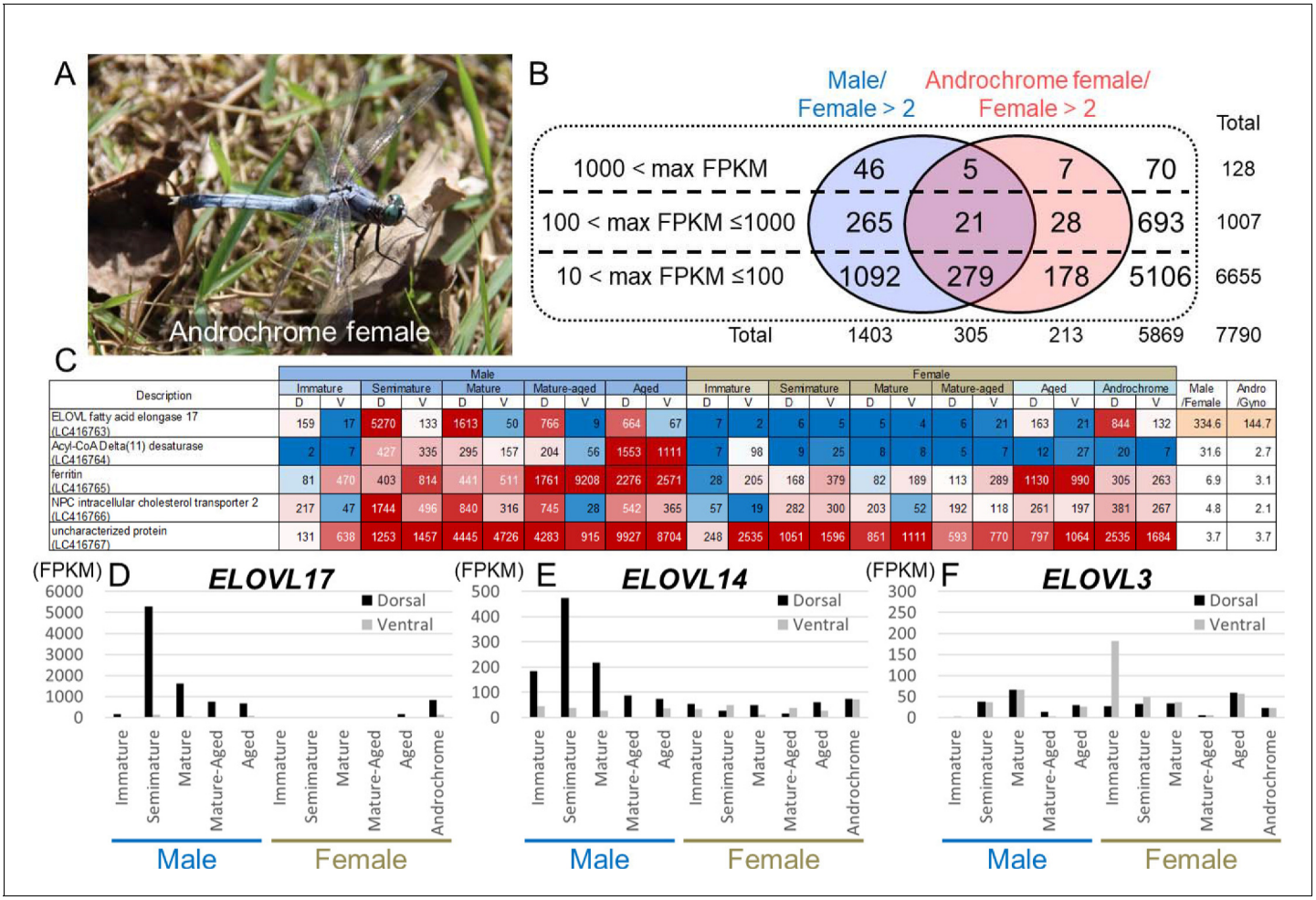


Figure 10. Genes associated with UV reflective wax. (**A**) An androchrome female used for transcriptome analysis. (**B**) The number of genes that were upregulated in the dorsal abdominal epidermis of males and/or an androchrome female compared to normal females. (**C**) The list of genes upregulated in the dorsal abdominal epidermis of both males and an androchrome female (max FPKM >1000). Gene expression levels are displayed as a heat map. The numbers indicate FPKM values. Red and blue fills indicate high and low expression levels, respectively. D and V indicate the dorsal and ventral abdominal regions, respectively. (**D–F**) Expression level of three elongation of very long-chain fatty acids (ELOVL) genes in the dorsal and ventral parts of the epidermis of *O. albistylum*.

DOI: https://doi.org/10.7554/eLife.43045.032

	Immature		Semimature		Mature		Mature-aged		Aged		Immature		Semimature		Mature		Mature-aged		Aged		Androchrome		Description (Top hit)	E-value	Male /Female	Andro /Gyno	Max FPKM (Dorsal)
contig	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V					
c153509R	131	638	1253	1457	4445	4726	4283	915	9927	8704	248	2535	1051	1596	851	1111	593	770	797	1064	2535	1684	NA		3.7	3.7	9927
c147539R (ELOVL17)	159	17	5270	133	1613	50	766	9	664	67	7	2	6	5	5	4	6	21	163	21	844	132	elongation of very long chain fatty acids protein AAEL008004 [Camponotus floridanus]	3.00E-122	334.6	144.7	5270
c148747R	81	470	403	814	441	511	1761	9208	2276	2571	28	205	168	379	82	189	113	289	1130	990	305	253	ferritin [Ornithodoros parkeri]	1.00E-89	6.9	3.1	2276
c153703R	217	47	1744	496	840	316	745	28	542	365	57	19	282	300	203	52	192	118	261	197	381	267	NPC intracellular cholesterol transporter 2 [Agrilus planipennis]	2.00E-44	4.8	2.1	1744
c145398R	2	7	427	335	295	157	204	56	1553	1111	7	96	9	25	8	8	5	7	12	27	20	7	Acyl-CoA Delta(11) desaturase [Zootermopsis nevadensis]	0	31.6	2.7	1553
c143830R	15	8	110	12	689	19	89	6	47	93	15	12	12	6	9	7	8	18	57	18	56	38	Serine protease inhibitor 1/8 [Lygus hesperus]	4.00E-24	20.9	5.1	689
c142423R (ELOVL14)	185	44	473	37	217	26	87	3	73	36	54	33	27	49	49	11	16	38	60	27	73	73	elongation of very long chain fatty acids protein AAEL008004 [Camponotus floridanus]	2.00E-158	6.6	2.0	473
c145528R	52	52	445	79	238	54	149	15	258	166	36	65	63	71	76	21	26	47	78	88	200	126	mid1-interacting protein 1-like [Zootermopsis nevadensis]	3.00E-70	4.4	4.0	445
c139526R	1	0	91	144	33	54	97	47	54	211	1	1	11	1	45	27	15	4	370	467	37	59	CG9269 [Drosophila melanogaster]	5.00E-16	3.1	2.1	370
c148278R	18	9	284	139	174	64	58	4	111	70	12	16	33	20	52	10	9	11	31	15	53	21	acetyl-coenzyme A synthetase [Zootermopsis nevadensis]	0	5.0	2.0	284
c146555R	3	0	29	48	130	142	275	48	0	0	0	0	18	55	32	44	34	47	0	0	49	31	CG34166 [Drosophila melanogaster]	1.00E-13	5.3	2.4	275
c153289R	262	805	93	133	143	187	167	29	79	99	28	92	84	124	97	78	57	71	256	278	227	89	apolipoprotein D-like [Zootermopsis nevadensis]	4.00E-66	2.5	3.4	262
c151984R	1	3	32	33	114	51	31	4	248	179	1	2	6	5	11	14	5	5	8	9	41	14	trehalose-6-phosphate synthase 1 [Blattella germanica]	0	8.0	7.3	248
c149439R	0	0	205	20	0	0	24	1	4	17	11	1	1	0	0	7	5	20	0	0	22	42	tyrosine 3-monooxygenase [Zootermopsis nevadensis]	0	13.2	5.0	205
c149196R	33	27	48	51	109	114	142	107	108	90	27	51	1	0	23	15	72	101	60	51	197	213	40S ribosomal protein S15 [Blattella germanica]	2.00E-64	2.7	6.4	197
c150668R	3	14	55	76	51	37	192	43	142	97	5	38	21	34	14	14	8	10	27	37	25	11	mitochondrial basic amino acids transporter [Cryptotermes secundus]	8.00E-104	6.1	2.1	192
c146712R	71	30	157	166	20	15	70	55	14	0	2	1	8	6	12	3	3	8	31	33	14	49	cystathionine gamma-lyase [Crassostrea gigas]	0	13.1	2.3	157
c148028R	23	4	155	8	40	5	20	1	14	4	6	4	9	7	3	2	2	5	5	2	18	13	putative fatty acyl-CoA reductase [Cryptotermes secundus]	0	12.3	3.7	155
c154118R	10	8	48	98	141	122	55	16	108	88	3	8	4	0	49	62	16	29	38	52	61	39	icarapin-like [Orussus abietinus]	2.00E-18	3.5	3.3	141
c153348R	41	87	130	63	34	45	23	32	88	73	21	19	42	35	30	22	20	26	48	40	60	19	ATP-binding cassette sub-family G member 4 [Cryptotermes secundus]	0	2.0	2.1	130
c149130R	40	11	125	39	37	31	20	3	28	13	13	5	9	7	25	2	10	13	46	17	32	41	Serine protease inhibitor 88Ea [Blattella germanica]	5.00E-96	3.9	2.2	125
c150904R	60	262	63	68	120	125	101	25	80	78	16	75	61	96	46	51	40	48	93	96	91	29	hypothetical protein B7P43_G14218 [Cryptotermes secundus]	3.00E-58	2.1	2.2	120
c147603R	38	137	71	85	119	135	68	32	38	35	2	41	3	4	0	0	39	63	11	21	34	20	Regenectin [Periplaneta americana]	4.00E-51	6.7	3.1	119
c153322R	0	0	92	69	13	32	119	63	11	17	0	4	14	28	2	8	2	11	13	29	39	14	kynurenine/alpha-aminoadipate aminotransferase [Habropoda laboriosa]	2.00E-130	12.4	8.7	119
c148957R	1	6	49	56	72	48	95	19	118	88	0	7	4	3	23	32	13	17	21	19	29	13	NA		5.4	2.9	118
c148343R	1	6	27	36	54	46	41	10	117	62	1	7	5	3	2	2	4	2	36	52	28	14	chitotriosidase-1 [Megachile rotundata]	8.00E-26	10.1	9.2	117
c149270R	9	5	64	29	102	88	35	16	40	32	23	18	40	30	29	13	8	20	7	2	54	45	pyruvate kinase-like [Cryptotermes secundus]	0	2.1	2.2	102

Figure 10—figure supplement 1. List of genes that are upregulated in the dorsal abdominal epidermis of both males and an androchrome female (max FPKM >100). Gene expression levels are displayed as a heat map. The numbers indicate FPKM values. Red and blue fills indicate high and low expression levels, respectively. D and V indicate the dorsal and ventral abdominal regions, respectively. Andro and Gyno mean androchrome and normal (gynochrome) females, respectively.

DOI: https://doi.org/10.7554/eLife.43045.033

C

	Male										Female											
	Immature		Semimature		Mature		Mature-aged		Aged		Immature		Semimature		Mature		Mature-aged		Aged		Androchrome		Male /Female	Andro /Gyno
	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V		
ELOVL fatty acid elongase 1 (LC416747)	57	11	74	23	18	10	19	7	14	10	24	9	27	14	14	3	13	8	19	11	18	18	2.1	0.9
ELOVL fatty acid elongase 2 (LC416748)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
ELOVL fatty acid elongase 3 (LC416749)	0	3	38	36	66	67	14	3	30	25	27	182	32	49	33	37	5	5	60	56	23	23	1.2	0.9
ELOVL fatty acid elongase 4 (LC416750)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0	0.0
ELOVL fatty acid elongase 5 (LC416751)	2	0	0	0	0	0	0	0	0	0	1	2	0	1	0	0	0	0	0	0	0	0	1.7	0.2
ELOVL fatty acid elongase 6 (LC416752)	2	5	0	0	0	0	0	0	0	3	0	0	0	0	26	24	1	11	1	0	7	198	0.1	1.1
ELOVL fatty acid elongase 7 (LC416753)	2	10	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	6.7	0.0
ELOVL fatty acid elongase 8 (LC416754)	0	1	0	0	0	0	0	0	1	1	0	0	0	0	5	5	0	5	0	0	1	26	0.2	0.9
ELOVL fatty acid elongase 9 (LC416755)	672	111	49	17	41	16	102	6	27	28	57	15	37	14	44	16	45	33	76	30	65	17	4.7	1.4
ELOVL fatty acid elongase 10 (LC416756)	0	1	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	1	0.0	0.0
ELOVL fatty acid elongase 11 (LC416757)	28	5	6	0	5	1	10	0	2	2	23	3	4	2	0	0	2	1	1	1	0	0	1.7	0.0
ELOVL fatty acid elongase 12 (LC416758)	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	47.7	0.0
ELOVL fatty acid elongase 13 (LC416759)	4	28	51	7	23	6	11	5	10	7	5	3	1	1	9	5	2	7	2	3	38	16	5.0	8.5
ELOVL fatty acid elongase 14 (LC416760)	185	44	476	37	217	26	87	3	73	36	54	33	27	49	49	11	16	38	60	27	73	73	6.6	2.0
ELOVL fatty acid elongase 15 (LC416761)	98	42	14	8	11	7	8	1	1	4	80	29	7	7	39	10	25	25	7	5	2	21	0.9	0.1
ELOVL fatty acid elongase 16 (LC416762)	2	1	1	1	0	0	0	0	0	0	1	1	4	2	0	0	0	0	0	0	0	0	0.6	0.0
ELOVL fatty acid elongase 17 (LC416763)	159	17	5270	133	1613	50	766	9	664	67	7	2	6	5	5	4	6	21	163	21	844	132	334.6	144.7

Figure 11. Identification of 17 ELOVL genes in dragonflies. (**A**) Phylogenetic tree of ELOVL family genes produced on the basis of their amino-acid sequences. A maximum likelihood phylogeny is shown, but neighbor-joining and Bayesian phylogenies exhibit substantially the same topologies. Statistical supporting values are indicated for each node (shown as (bootstrap value of neighbor-joining)/(bootstrap value of maximum likelihood)/(posterior probability of Bayesian)). Asterisks indicate support values < 50%. Blue and gray shading indicates *O. albistylum* and *L. fulva* genes, respectively. Accession numbers or annotation identities are shown in parentheses. (**B**) Alignment of 17 ELOVL genes of *O. albistylum*. The conserved histidine motif is boxed. (**C**) Gene expression levels of 17 ELOVL genes in *O. albistylum*. The numbers indicate FPKM values. Red and blue shading indicates high and low expression levels, respectively. D and V indicate the dorsal and ventral abdominal regions, respectively.

DOI: https://doi.org/10.7554/eLife.43045.034