

Table S1. Genes differentially expressed between *S. invicta* males of different *Gp-9* genotypes.

sample type	gene ID	description	expression ratio ¹	p-value
haploid male pupae (<i>B</i> vs. <i>b</i>)	SI.MKN.02136	hypothetical protein SINV_13217	0.28	0.001
	SI.MKN.03300	E3 ubiquitin-protein ligase RFWD2	4.88	0.001
	SI.MKN.02125	hypothetical protein SINV_01823	0.20	<0.001
	SI.MKN.00089	putative deoxyribonuclease TATDN1	0.21	0.002
	SI.MKN.04391	ectopic P granules protein 5 homolog	0.08	<0.001
	SI.MKN.02136	hypothetical protein SINV_13217	0.28	0.008
	SI.MKN.02206	trypsin inhibitor	0.06	0.008
1-day-old diploid males (<i>BB</i> vs. <i>Bb</i>)	SI.MKN.03934	hypothetical protein SINV_07124	0.23	0.001
11-day-old diploid males (<i>BB</i> vs. <i>Bb</i>)	SI.MKN.04583	piggyBac transposon	0.06	0.001
	SI.MKN.04055	cytoplasmic trna 2-thiolation protein 1-like	0.18	0.007

¹ Expression ratio for the first **genotype** relative to the second **genotype**

Table S3. Number of multiple-clone genes that showed expression differences between clones.

developmental stages	comparison between	No. of differentially expressed genes	No. of differentially expressed genes with multiple-clones	No. of genes where there were differences in the direction of expression between clones¹
pupae	dM-Qn	57	46	1 (2.2%)
	dM-hM	3048	1828	77 (4.2%)
	hM-Qn	2955	1783	78 (4.4%)
1d	dM-Qn	2413	1538	67 (4.4%)
	dM-hM	358	226	0
	hM-Qn	2482	1567	68 (4.3%)
11d	dM-Qn	2257	1386	44 (3.2%)
	dM-hM	0	0	0
	hM-Qn	2159	1334	44 (3.3%)

¹ Genes were counted only when at least one clone was significantly more expressed in one direction and at least one clone significantly more in the other direction

Table S4. Primer sequences used for qRT-PCR verification of alternative splicing and expression of the *S. invicta dsx* gene.

<i>dsx</i> target exon	forward primer	reverse primer
dsxExon4	GTGGAACATACTTGGAGGGAGATG	AATTCCTCGTCCAAGTGATAGGC
dsxExon5	GAAAACACTCCAGCCGGAAGATTA	GACATCTTCCTGTTGTTGAACTGG
dsxExon6	GCTTCAGAGGAGGCGCCTATTGG	AGGAAACGGAGATGTACTIONGACGC
dsxExon7	GAAACTCGCGTCGCCTCCGAAGTG	GCAGTGCTGGCCGTACACCTCGTG

Table S5. Results of Gene Ontology enrichment analysis for comparisons between *S. invicta* individuals of three different categories at three developmental timepoints (all FDR < 0.05). GO categories that are significantly over-represented in multiple comparisons for a given timepoint are highlighted in bold.

category	comparison ¹	direction of differential expression ²	GO category ³	GO identifier	GO term description	annotated	significant	expected	p-value	
pupae	dM-Qn	up	---	---	---	---	---	---	none significant	
		down	---	---	---	---	---	---	none significant	
	hM-dM	up	BP	---	---	---	---	---	---	none significant
			CC	---	---	---	---	---	---	none significant
		MF	GO:0051082	unfolded protein binding	39	25	12.64	0.068		
		down	BP	GO:0042254	ribosome biogenesis	131	73	33.98	<0.001	
				GO:0006412	translation	233	103	60.44	<0.001	
			CC	GO:0005840	ribosome	206	97	53.81	<0.001	
				GO:0005811	lipid particle	70	34	18.29	0.013	
		GO:0022627	cytosolic small ribosomal subunit	13	10	3.4	0.043			
		GO:0005578	proteinaceous extracellular matrix	8	7	2.09	0.091			
		MF	GO:0003735	structural constituent of ribosome	111	73	27.61	<0.001		
	hM-Qn	up	BP	GO:0006457	protein folding	63	37	19.98	0.030	
			CC	---	---	---	---	---	none significant	
MF		GO:0051082	unfolded protein binding	39	26	12.19	0.008			
down		BP	GO:0042254	ribosome biogenesis	131	73	32.1	<0.001		
			GO:0006412	translation	233	107	57.1	<0.001		
		CC	GO:0005840	ribosome	206	99	50.92	<0.001		
			GO:0022627	cytosolic small ribosomal subunit	13	12	3.21	<0.001		
	GO:0005578	proteinaceous extracellular matrix	8	7	1.98	0.082				
	MF	GO:0003735	structural constituent of ribosome	111	76	26.16	<0.001			
1-day-old adults	dM-Qn	up	BP	GO:0015992	proton transport	71	50	15.69	<0.001	
				GO:0006120	mitochondrial electron transport, NADH to	22	21	4.86	<0.001	
				GO:0006744	ubiquinone biosynthetic process	24	22	5.3	<0.001	
				GO:0042254	ribosome biogenesis	131	59	28.94	<0.001	
				GO:0006412	translation	214	82	47.28	<0.001	
				GO:0006814	sodium ion transport	32	21	7.07	<0.001	
				GO:0015986	ATP synthesis coupled proton transport	18	12	3.98	0.036	
				GO:0005747	mitochondrial respiratory chain complex I	12	11	2.74	<0.001	
				GO:0005840	ribosome	206	84	47.04	<0.001	
				GO:0005739	mitochondrion	216	100	49.33	0.005	
				GO:0022625	cytosolic large ribosomal subunit	7	7	1.6	0.006	
		GO:0005743	mitochondrial inner membrane	71	44	16.21	0.012			
		GO:0070469	respiratory chain	36	29	8.22	0.013			
		GO:0000276	mitochondrial proton-transporting ATP synthase	6	6	1.37	0.014			
		MF	GO:0008137	NADH dehydrogenase (ubiquinone) activity	22	21	4.73	<0.001		
			GO:0003735	structural constituent of ribosome	111	58	23.87	<0.001		
		down	---	---	---	---	---	---	none significant	
	hM-dM	up	---	---	---	---	---	---	---	none significant
		down	---	---	---	---	---	---	---	none significant
	hM-Qn	up	BP	GO:0015992	proton transport	71	46	17.34	<0.001	
				GO:0006120	mitochondrial electron transport, NADH to	22	17	5.37	<0.001	
				GO:0006744	ubiquinone biosynthetic process	24	16	5.86	0.013	
			GO:0042254	ribosome biogenesis	131	54	31.99	0.013		
		MF	GO:0008137	NADH dehydrogenase (ubiquinone) activity	22	18	5.13	<0.001		
			GO:0003735	structural constituent of ribosome	111	52	25.89	<0.001		
down		---	---	---	---	---	---	---	none significant	
11-day-old adults	dM-Qn	up	BP	GO:0015992	proton transport	71	53	14.2	<0.001	
				GO:0006120	mitochondrial electron transport, NADH to	22	20	4.4	<0.001	
				GO:0006744	ubiquinone biosynthetic process	24	19	4.8	<0.001	
				GO:0006814	sodium ion transport	32	21	6.4	<0.001	
				GO:0015986	ATP synthesis coupled proton transport	18	15	3.6	<0.001	
				GO:0005747	mitochondrial respiratory chain complex I	12	11	2.52	<0.001	
				GO:0005739	mitochondrion	216	104	45.38	<0.001	
				GO:0005743	mitochondrial inner membrane	71	44	14.92	0.005	
				GO:0070469	respiratory chain	36	30	7.56	0.012	
				GO:0000276	mitochondrial proton-transporting ATP synthase	6	6	1.26	0.012	
				MF	GO:0008137	NADH dehydrogenase (ubiquinone) activity	22	19	4.35	<0.001
		GO:0004129	cytochrome-c oxidase activity	16	11	3.16	0.021			
		GO:0046933	hydrogen ion transporting ATP synthase activity,	12	9	2.37	0.024			
		GO:0046961	proton-transporting ATPase activity, rotational	10	8	1.98	0.024			
		GO:0008121	ubiquinol-cytochrome-c reductase activity	8	7	1.58	0.024			
		GO:0003735	structural constituent of ribosome	111	38	21.93	0.049			
	down	---	---	---	---	---	---	---	none significant	
hM-dM	up	---	---	---	---	---	---	---	none significant	
	down	---	---	---	---	---	---	---	none significant	
hM-Qn	up	BP	GO:0015992	proton transport	71	48	13.68	<0.001		
			GO:0006120	mitochondrial electron transport, NADH to	22	20	4.24	<0.001		
			GO:0006744	ubiquinone biosynthetic process	24	20	4.62	<0.001		
			GO:0006814	sodium ion transport	32	20	6.16	<0.001		
			GO:0042254	ribosome biogenesis	131	44	25.24	0.040		
			CC	GO:0005747	mitochondrial respiratory chain complex I	12	10	2.46	0.004	
			GO:0005739	mitochondrion	216	96	44.27	0.013		
			GO:0070469	respiratory chain	36	28	7.38	0.013		
			GO:0000276	mitochondrial proton-transporting ATP synthase	6	6	1.23	0.013		
			GO:0005763	mitochondrial small ribosomal subunit	10	8	2.05	0.013		
			GO:0005743	mitochondrial inner membrane	71	41	14.55	0.019		
	MF	GO:0008137	NADH dehydrogenase (ubiquinone) activity	22	19	4.2	<0.001			
		GO:0003735	structural constituent of ribosome	111	44	21.21	<0.001			
		GO:0008121	ubiquinol-cytochrome-c reductase activity	8	7	1.53	0.031			
	down	---	---	---	---	---	---	---	none significant	

¹dM = diploid male, hM = haploid male, Qn = queen.

²Direction of expression difference (up- or down-regulated) for the first category in the pairwise listing relative to the second category.

³Abbreviations for GO categories are as follows: BP = Biological process, CC = Cellular component, MF = Molecular function.

Table S6. Expression level (RPKM, Reads Per Kilobase per Million mapped reads with standard deviations) for each exon of the *dsx* gene in adult queens and adult haploid males of *S. invicta* based on unpublished RNAseq data.

exon	mean RPKM	
	males	queens
Exon1	16.39 (+/- 1.85)	27.16 (+/- 5.71)
Exon2	40.59 (+/- 2.04)	36.45 (+/-12.97)
Exon3	67.04 (+/-14.82)	63.87 (+/-33.15)
Exon4	29.65 (+/- 8.58)	22.81 (+/-10.17)
Exon5	2.15 (+/- 1.39)	13.91 (+/- 3.30)
Exon6	18.47 (+/- 5.44)	2.13 (+/- 1.48)
Exon7	105.78 (+/- 6.26)	6.64 (+/- 2.45)

Table S7. Expression of 12 genes implicated in sperm production in *S. invicta* pupae. Expression ratios and *p*-values in bold denote ratios that are significantly different from one ($p < 0.01$).

No.	gene ID	description	spot ID	expression ratio ¹			<i>p</i> -value		
				dM/Qn	hM/dM	hM/Qn	Qn-dM	dM-hM	hM-Qn
1	SI.MKN.02316	testis-specific serine threonine-protein kinase-like	SiJWE11BAA_pcr_2	1.0	33.3	31.8	0.931	<0.001	<0.001
2	SI.MKN.03583	testis-specific serine threonine-protein kinase 1-like	SiJWD07BCZ_pcr_1	1.0	22.6	23.2	0.961	<0.001	<0.001
3	SI.MKN.03943	testis skeletal muscle dual specificity phosphatase	SiJWC02ABH_pcr_2	1.0	1.5	1.5	0.980	0.002	0.003
4	SI.MKN.02503	sperm flagellar protein 1	SiJWG03ADE_pcr_1	1.2	11.0	13.5	0.510	<0.001	<0.001
5	SI.MKN.05070	outer dense fiber protein 3	SiJWE08BDF_pcr_1	1.1	4.2	4.5	0.762	<0.001	<0.001
6	SI.MKN.00746	sperm surface protein sp17	SiJWC07BAD_pcr_1	0.9	1.5	1.3	0.407	<0.001	0.008
7	SI.MKN.00816	motile sperm domain containing 1	SiJWC02ADS_pcr_1	1.0	1.5	1.6	0.754	<0.001	<0.001
			SiJWE11ABC_pcr_2	1.2	1.4	1.7	0.237	0.001	<0.001
			SiJWE05ACI_pcr_2	1.2	1.3	1.5	0.262	0.005	<0.001
8	SI.MKN.00821	spermatogenesis-associated protein 17	SiJWE10ACS_pcr_2	1.5	2.5	3.7	0.053	<0.001	<0.001
			SiJWE10ACS_pcr_1	1.4	3.0	4.2	0.228	<0.001	<0.001
9	SI.MKN.02084	spermatogenesis-associated protein 20	SiJWC10ACY_pcr_1	1.1	2.6	2.8	0.692	<0.001	<0.001
			SiJWC07ADY_pcr_newprimers	1.0	1.3	1.3	0.992	<0.001	<0.001
			SiJWC07ABG_pcr_2	1.0	1.3	1.3	0.991	<0.001	<0.001
10	SI.MKN.05000	nuclear autoantigenic sperm protein	SiJWC03ACE_pcr_2	1.2	2.9	3.6	0.562	<0.001	<0.001
11	SI.MKN.03704	peroxisomal n-acetyl-spermine spermidine oxidase-like	SiJWF04BAE_pcr_1	1.1	2.5	2.7	0.779	<0.001	<0.001
12	SI.MKN.03504	accessory gland protein	SiJWC09CAL_pcr_1	1.1	1.9	2.1	0.762	<0.001	<0.001
			SiJWA07AAX_pcr_2	1.0	1.7	1.8	0.846	<0.001	<0.001
			SiJWC12ACX_pcr_1	1.0	1.6	1.6	0.984	<0.001	<0.001
			SiJWA07AAX_pcr_1	1.0	1.8	1.8	0.917	<0.001	<0.001

¹ Expression ratio for the first category relative to the second category, dM = diploid male, hM = haploid male, Qn = queen.

Table S8. Expression of 49 genes implicated in pheromone production/perception in *S. invicta* pupae. Expression ratios and *p*-values in bold denote ratios that are significantly different from one ($p < 0.01$).

No.	gene ID	description	spot ID	expression ratio ¹			<i>p</i> -value		
				dM/Qn	hM/dM	hM/Qn	Qn-dM	dM-hM	hM-Qn
1	SI.MKN.00418	brain chitinase and chia; probable chitinase 3-like	SiJWA11CAR_pcr_1	0.69	0.39	0.27	0.129	< 0.001	< 0.001
			SiJWB08BBP_pcr_1	0.83	0.47	0.39	0.475	< 0.001	< 0.001
			SiJWC06ACG_pcr_2	0.81	0.66	0.54	0.366	0.003	< 0.001
			SiJWE09CAL_pcr_1	0.75	0.40	0.30	0.350	< 0.001	< 0.001
			SiJWF09BBO_pcr_1	0.62	0.39	0.24	0.129	< 0.001	< 0.001
2	SI.MKN.01261	chitinase	SiJWC02BDN_pcr_1	0.89	0.48	0.43	0.857	0.009	0.003
3	SI.MKN.01705	chitin deacetylase 4	SiJWD02ABJ_pcr_2	0.86	0.75	0.65	0.488	0.021	< 0.001
			SiJWH08CAK_pcr_1	1.03	0.49	0.50	0.967	0.001	0.002
			SiJWH12ADT_pcr_1	0.83	0.74	0.62	0.485	0.038	0.001
4	SI.MKN.03920	chito-oligosaccharidolytic beta-n-acetylglucosaminidase	SiJWH07ACS_pcr_1	0.82	2.36	1.94	0.454	< 0.001	< 0.001
			SiJWH07ACS_pcr_2	1.24	3.46	4.28	0.597	0.002	< 0.001
5	SI.MKN.01859	cuticle protein	SiJWD06BDX_pcr_1	1.11	0.71	0.78	0.646	0.002	0.033
			SiJWG02ABJ_pcr_2	0.92	0.38	0.35	0.800	< 0.001	< 0.001
6	SI.MKN.02972	cuticular protein hypothetical 28 precursor	SiJWB05ACB_pcr_2	1.22	0.57	0.69	0.545	0.002	0.041
			SiJWC02BAO_pcr_1	1.41	0.47	0.67	0.305	< 0.001	0.048
			SiJWC06ACT_pcr_2	1.31	0.50	0.66	0.455	0.001	0.044
			SiJWG04BAJ_pcr_2	1.23	0.45	0.55	0.577	< 0.001	0.003
			SiJWG10ABN_pcr_2	1.12	0.53	0.59	0.768	0.001	0.006
			SiJWH09ABN_pcr_2	1.27	0.48	0.61	0.545	< 0.001	0.021
7	SI.MKN.03418	larval cuticle protein a2b	SiJWD08ADG_pcr_1	1.03	1.81	1.86	0.953	< 0.001	< 0.001
8	SI.MKN.00858	chondroitin sulfate synthase 2-like	SiJWH06ABU_pcr_2	0.97	1.55	1.50	0.915	< 0.001	< 0.001
9	SI.MKN.01933 ²	endocuticle structural glycoprotein bd-1	SiJWB08BCB_pcr_1	0.35	0.43	0.15	0.008	0.002	< 0.001
			SiJWB08ACP_pcr_2	0.42	0.62	0.26	0.009	0.035	< 0.001
			SiJWG09CAN_pcr_1	0.44	0.48	0.21	0.057	0.012	< 0.001
10	SI.MKN.00488	acyl-delta desaturase	SiJWE01ADR_pcr_1	1.28	1.33	1.71	0.306	0.048	< 0.001
			SiJWG02CAJ_pcr_1	1.62	2.07	3.36	0.144	< 0.001	< 0.001
11	SI.MKN.03643	elongation of very long chain fatty acids protein 4	SiJWC04ABJ_pcr_2	0.82	0.76	0.63	0.162	0.003	< 0.001
12	SI.MKN.04235	elongation of very long chain fatty acids protein aael008004-like	SiJWE01BCA_pcr_1	0.92	3.88	3.57	0.748	< 0.001	< 0.001
			SiJWF05BCY_pcr_1	0.99	3.50	3.45	0.976	< 0.001	< 0.001
			SiJWF06CAU_pcr_1	1.02	4.73	4.81	0.978	< 0.001	< 0.001
			SiJWG09ACJ_pcr_2	1.04	4.03	4.21	0.905	< 0.001	< 0.001
			SiJWH07BCW_pcr_1	0.84	3.37	2.82	0.583	< 0.001	< 0.001

¹ Expression ratio for the first category relative to the second category, dM = diploid male, hM = haploid male, Qn = queen.

² Genes that were both ploidy- and sex-specific.

³ Possible alternatively spliced variants.

Table S8. (Continued)

No.	gene ID	description	spot ID	expression ratio ¹			p-value			
				dM/Qn	hM/dM	hM/Qn	Qn-dM	dM-hM	hM-Qn	
13	SI.MKN.00534	fatty acid binding protein	SiJWA10BCT_pcr_1	0.92	0.54	0.50	0.665	< 0.001	< 0.001	
			SiJWB01BCJ_pcr_1	0.96	0.49	0.47	0.846	< 0.001	< 0.001	
			SiJWB12BAJ_pcr_2	0.99	0.47	0.47	0.961	< 0.001	< 0.001	
			SiJWF05BDY_pcr_1	0.91	0.61	0.55	0.592	< 0.001	< 0.001	
			SiJWH04BBF_pcr_1	0.97	0.49	0.47	0.897	< 0.001	< 0.001	
14	SI.MKN.04259	fatty acid synthase	SiJWC07ADW_pcr_1	0.86	1.77	1.51	0.275	< 0.001	< 0.001	
15	SI.MKN.00197 ²	fatty acid synthase	SiJWF04ADN_pcr_1	0.66	0.70	0.46	0.006	0.001	< 0.001	
			SiJWC09ADF_pcr_1	0.61	0.72	0.44	0.109	0.099	< 0.001	
16	SI.MKN.03327	fatty acid synthase	SiJWA03BBE_pcr_1	1.27	0.72	0.91	0.064	< 0.001	0.302	
			SiJWA10BAE_pcr_1	0.95	2.50	2.37	0.803	< 0.001	< 0.001	
			SiJWH04BBA_pcr_1	1.00	1.75	1.75	0.985	< 0.001	< 0.001	
			SiJWG12BCN_pcr_1	0.72	0.51	0.37	0.098	< 0.001	< 0.001	
17	SI.MKN.00095	fatty acyl-reductase 1	SiJWC07ABC_pcr_2	1.26	0.44	0.56	0.560	< 0.001	0.009	
18	SI.MKN.03476	fatty acyl-reductase cg5065-like	SiJWA12ADF_pcr_1	0.97	2.61	2.54	0.930	< 0.001	< 0.001	
19	SI.MKN.01301 ³	fatty-acid amide hydrolase 2 (possible transcript1)	SiJWE10BBR_pcr_1	0.96	0.65	0.62	0.832	< 0.001	< 0.001	
			fatty-acid amide hydrolase 2 (possible transcript2)	SiJWF02AAG_pcr_2	0.95	0.72	0.69	0.800	< 0.001	< 0.001
			fatty-acid amide hydrolase 2 (possible transcript2)	SiJWA07AAE_pcr_2	0.92	0.64	0.59	0.703	< 0.001	< 0.001
20	SI.MKN.00856	long-chain-fatty-acid coa ligase	SiJWD03ABH_pcr_2	0.73	0.97	0.71	0.087	0.832	0.004	
			SiJWG12ADU_pcr_1	0.93	0.45	0.42	0.746	< 0.001	< 0.001	
			SiJWD07ACW_pcr_2	0.90	0.58	0.52	0.817	0.009	0.002	
21	SI.MKN.02780 ³	long-chain-fatty-acid ligase 3 (possible transcript1)	SiJWD11BAL_pcr_2	1.34	1.61	2.16	0.243	0.003	< 0.001	
			long-chain-fatty-acid ligase 3 (possible transcript2)	SiJWH06ABK_pcr_2	1.37	1.91	2.61	0.257	< 0.001	< 0.001
			long-chain-fatty-acid ligase 3 (possible transcript2)	SiJWA10ABR_pcr_2	1.08	0.41	0.44	0.824	< 0.001	< 0.001
22	SI.MKN.01188	cytochrome p450	SiJWH06ABH_pcr_2	0.99	0.63	0.62	0.981	< 0.001	< 0.001	
			SiJWC03BCF_pcr_1	0.99	1.30	1.29	0.970	0.006	0.008	
23	SI.MKN.04072	cytochrome p450	SiJWF12BCR_pcr_1	0.89	2.34	2.09	0.771	< 0.001	< 0.001	
24	SI.MKN.04463	cytochrome P450	SiJWB04BCA_pcr_1	1.01	0.44	0.44	0.982	< 0.001	< 0.001	
25	SI.MKN.04572	cytochrome P450	SiJWD04ABB_pcr_2	0.63	0.52	0.33	0.104	< 0.001	< 0.001	
26	SI.MKN.00079	cytochrome p450 4c1	SiJWE01BEA_pcr_2	0.64	0.86	0.55	0.063	0.369	< 0.001	
			SiJWH09ACV_pcr_2	1.12	1.75	1.96	0.703	< 0.001	< 0.001	
27	SI.MKN.00426	cytochrome p450 4c1	SiJWC07BBR_pcr_1	1.44	0.57	0.82	0.119	< 0.001	0.178	
			SiJWE11CAR_pcr_1	0.88	0.54	0.48	0.655	< 0.001	< 0.001	

¹ Expression ratio for the first category relative to the second category, dM = diploid male, hM = haploid male, Qn = queen.

² Genes that were both ploidy- and sex-specific.

³ Possible alternatively spliced variants.

Table S8. (Continued)

No.	gene ID	description	spot ID	expression ratio ¹			<i>p</i> -value		
				dM/Qn	hM/dM	hM/Qn	Qn-dM	dM-hM	hM-Qn
29	SI.MKN.02054	cytochrome p450 4c1	SiJWE03ABR_per_2	0.78	0.63	0.49	0.407	0.011	< 0.001
			SiJWH11ACV_per_2	0.86	0.56	0.48	0.672	0.002	< 0.001
30	SI.MKN.04318	cytochrome p450 4c1	SiJWB02BBZ_per_1	0.84	0.59	0.49	0.546	0.002	< 0.001
31	SI.MKN.04334	cytochrome p450 4c1	SiJWH08BCX_per_1	1.00	0.69	0.69	0.996	0.007	0.008
32	SI.MKN.05047	cytochrome P450 4C1	SiJWD12BBE_per_1	0.96	1.47	1.41	0.896	0.002	0.005
33	SI.MKN.02795	cytochrome p450 4g15	SiJWE06ABI_per_2	1.08	5.61	6.05	0.730	< 0.001	< 0.001
			SiJWE09ACN_per_2	0.89	3.49	3.09	0.694	< 0.001	< 0.001
			SiJWF04BAD_per_1	0.90	4.33	3.88	0.759	< 0.001	< 0.001
			SiJWB05ADH_per_1	1.10	0.68	0.75	0.668	0.001	0.013
34	SI.MKN.00599	cytochrome p450 6k1-like	SiJWA10AAK_per_2	1.18	1.58	1.86	0.536	0.002	< 0.001
35	SI.MKN.04264	cytochrome p450 6k1-like	SiJWB02AAG_per_2	0.93	2.04	1.90	0.851	< 0.001	< 0.001
36	SI.MKN.00844	cytochrome p450 6a1; 6a2-like	SiJWA01CAH_per_1	0.93	3.61	3.37	0.744	< 0.001	< 0.001
			SiJWE05ADV_per_1	1.65	0.49	0.82	0.004	< 0.001	0.097
37	SI.MKN.01627	probable cytochrome p450 6a13	SiJWA11BCJ_per_1	0.96	1.65	1.59	0.936	0.003	0.006
38	SI.MKN.04965	cytochrome P450 9e2electron carrier activity	SiJWB01AAC_per_1	0.81	0.45	0.36	0.500	< 0.001	< 0.001
			SiJWB01AAC_per_2	0.81	0.54	0.44	0.430	< 0.001	< 0.001
39	SI.MKN.03889	nadph-cytochrome p450 reductase	SiJWC01ACM_per_2	1.01	0.54	0.54	0.982	< 0.001	< 0.001
			SiJWE03ABW_per_2	1.00	0.65	0.65	0.995	< 0.001	< 0.001
			SiJWG12CAX_per_1	1.04	0.60	0.63	0.847	< 0.001	< 0.001
			SiJWH02BAB_per_1	0.91	0.56	0.51	0.532	< 0.001	< 0.001
			SiJWH10CAX_per_1	1.04	0.51	0.53	0.859	< 0.001	< 0.001
			SiJWA03ABL_per_2	0.99	0.73	0.73	0.988	0.005	0.004
40	SI.MKN.04671	odorant binding protein SiOBP1	SiJWA08ABR_per_2	0.93	0.41	0.38	0.858	< 0.001	< 0.001
			SiJWD04CAE_per_1	0.98	0.47	0.46	0.952	< 0.001	< 0.001
			SiJWD11BBJ_per_1	1.08	0.38	0.41	0.883	< 0.001	< 0.001
			SiJWF11BCW_per_1	0.92	0.44	0.40	0.699	< 0.001	< 0.001
42	SI.MKN.00700	odorant binding protein SiOBP13	SiJWA03ACS_per_1	1.01	0.69	0.70	0.965	< 0.001	< 0.001
43	SI.MKN.05034	odorant binding protein SiOBP10	SiJWD07ABA_per_2	1.18	0.47	0.55	0.364	< 0.001	< 0.001
44	SI.MKN.04549	chemosensory protein	SiJWF11BCE_per_1	0.92	0.58	0.53	0.778	< 0.001	< 0.001
45	SI.MKN.04697	chemosensory protein SiCSP2	SiJWA03BCG_per_1	0.94	0.61	0.58	0.888	0.007	0.003
46	SI.MKN.00127	sol s 2 allergen	SiJWD02BAY_per_1	1.02	1.42	1.45	0.952	0.005	0.003
47	SI.MKN.00134	protein g12; contains allergen domain	SiJWF02ABD_per_2	1.01	3.06	3.09	0.976	< 0.001	< 0.001
48	SI.MKN.04507	obstructor C1 (peritrophin), chitin binding protein	SiJWH04BCY_per_1	0.95	0.31	0.29	0.936	< 0.001	< 0.001
49	SI.MKN.04650	chemosensory protein SiCSP6	SiJWH07ABA_per_2	1.07	2.72	2.90	0.836	< 0.001	< 0.001

¹ Expression ratio for the first category relative to the second category, dM = diploid male, hM = haploid male, Qn = queen.

² Genes that were both ploidy- and sex-specific.

³ Possible alternatively spliced variants.

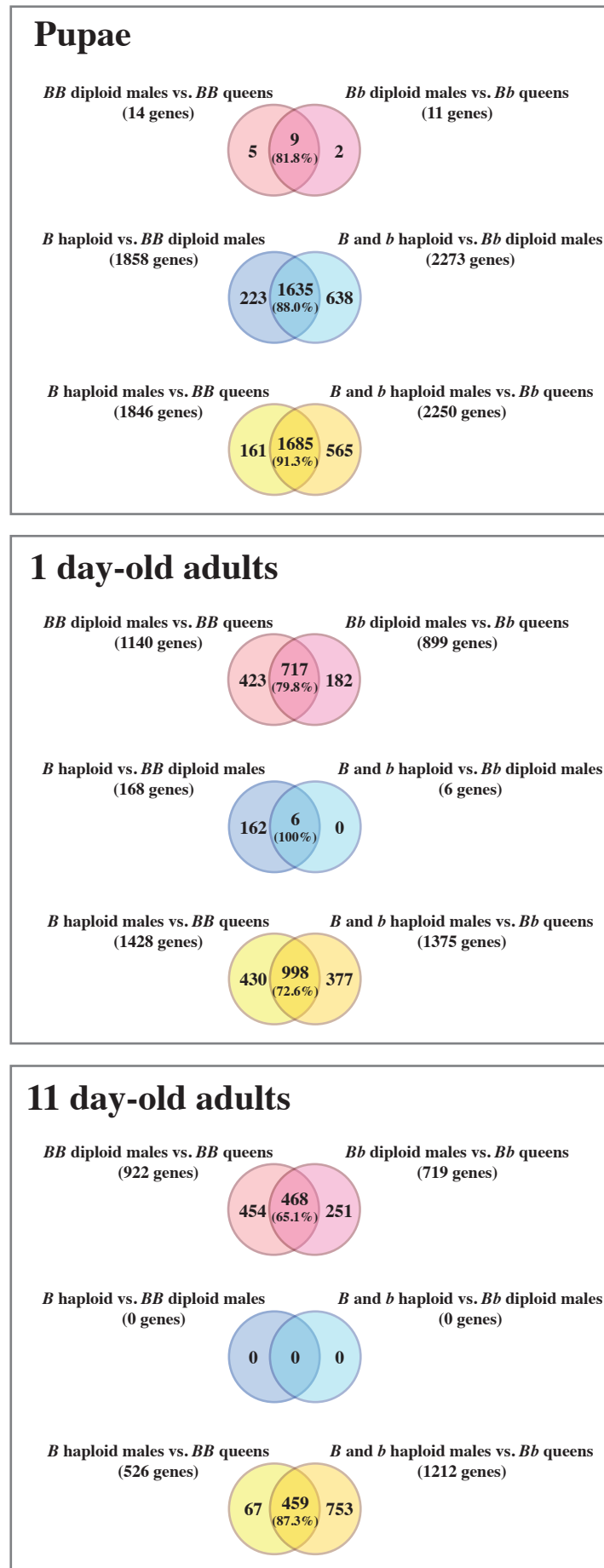


Figure S1. Venn diagram showing the numbers of genes differentially expressed between *S. invicta* individuals of different categories with different *Gp-9* genotypes. Because haploid males can have either the *B* or *b* genotype, we compared both types of males to *Bb* diploid individuals.

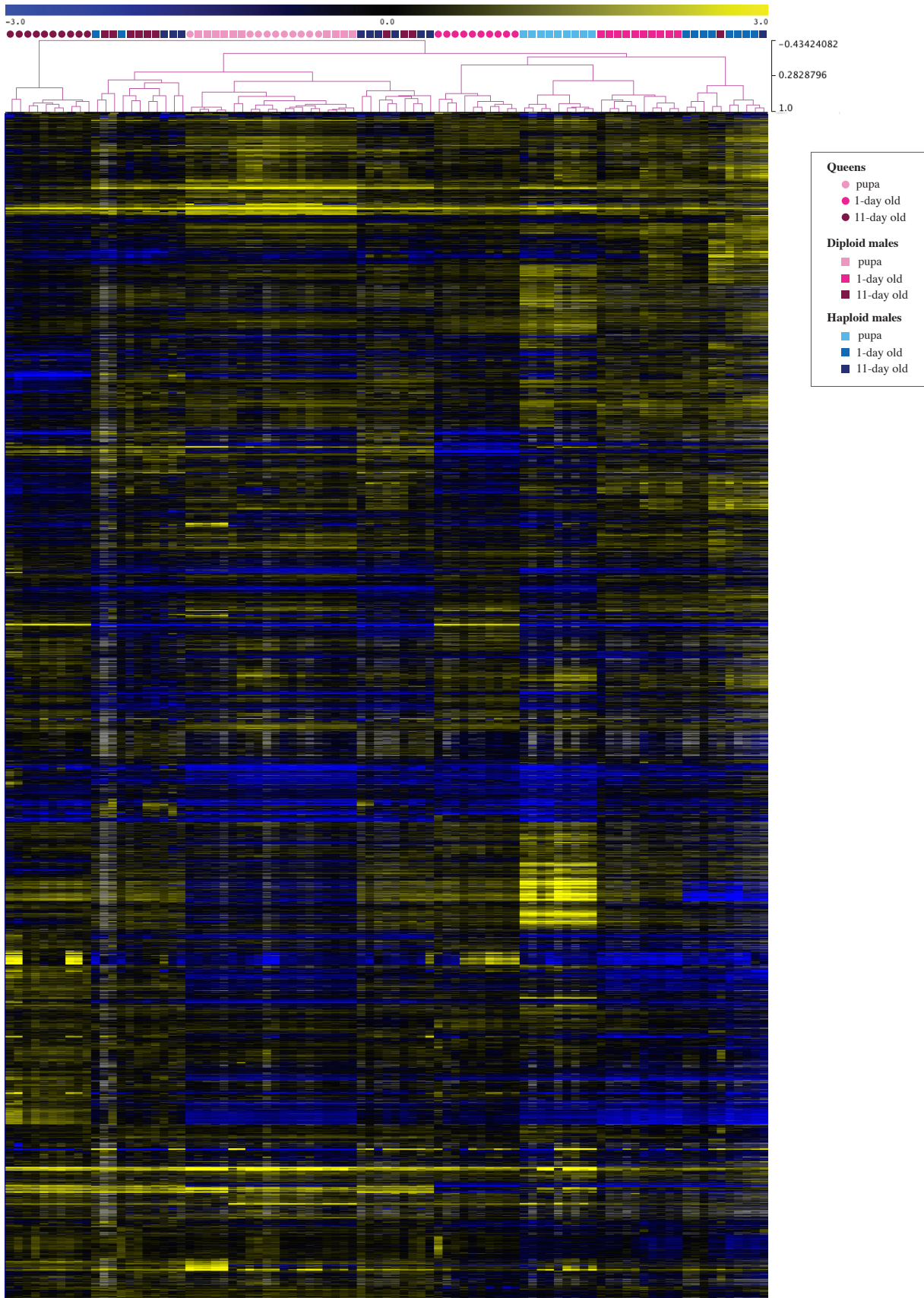


Figure S2 Hierarchical clustering of gene expression profiles of individual queens, diploid males and haploid males at three developmental timepoints in *S. invicta*. Colors in the heat map represent relative levels of expression: blue=highly expressed, yellow=lowly expressed, gray = NA (data not available). Numbers for heat map are log₂ transformed relative expression levels. Scale bar on the right of the cluster represents an index of similarity between samples.

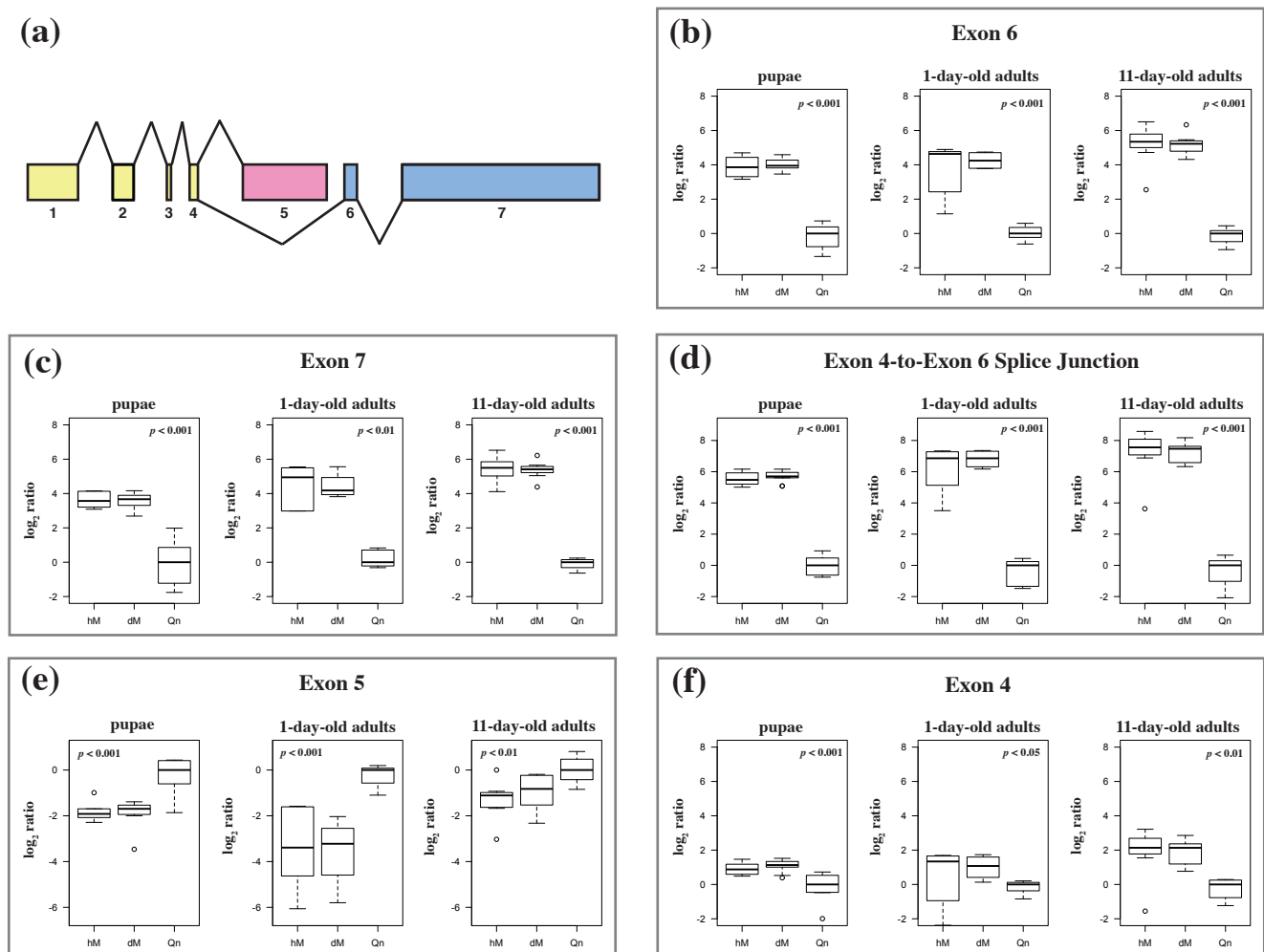


Figure S3. Alternative splicing of the *S. invicta dsx* gene in pupae. (a) Diagram depicting alternative splicing pattern of *dsx* (black, pattern for both sexes; gray, queen pattern; vertical stripe, male pattern). (b-f) Box-and-whisker plots of \log_2 transformed relative expression levels based on qRT-PCR analysis: the top and bottom of the box are the first and third quartiles, respectively; the horizontal bar within the box is the median, whiskers extend from the box to the most extreme value within $1.5\times$ of the interquartile range of the box; data beyond the whiskers are outliers plotted as points. Abbreviations for categories of individuals: hM = haploid males, dM = diploid males, Qn = queens. *P*-values indicate significance of variation in expression levels among individuals of different categories at each timepoint (ANOVA). (b) Exon 6, (c) Exon 7, (d) direct Exon 4-to-Exon 6 splice junction, (e) Exon 5, and (f) Exon 4.

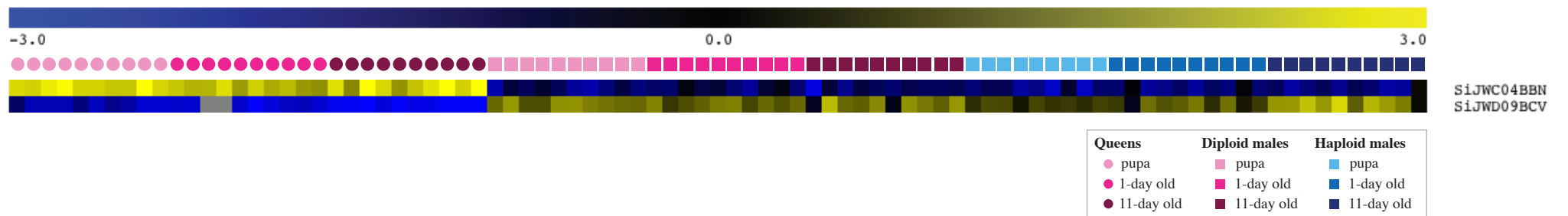


Figure S4. Hierarchical clustering of the two *dsx* microarray spots at three developmental timepoints in *S. invicta*. Colors in the heat maps represent relative levels of expression: blue=highly expressed, yellow=lowly expressed, gray = NA (data not available). Numbers are \log_2 transformed relative expression levels.

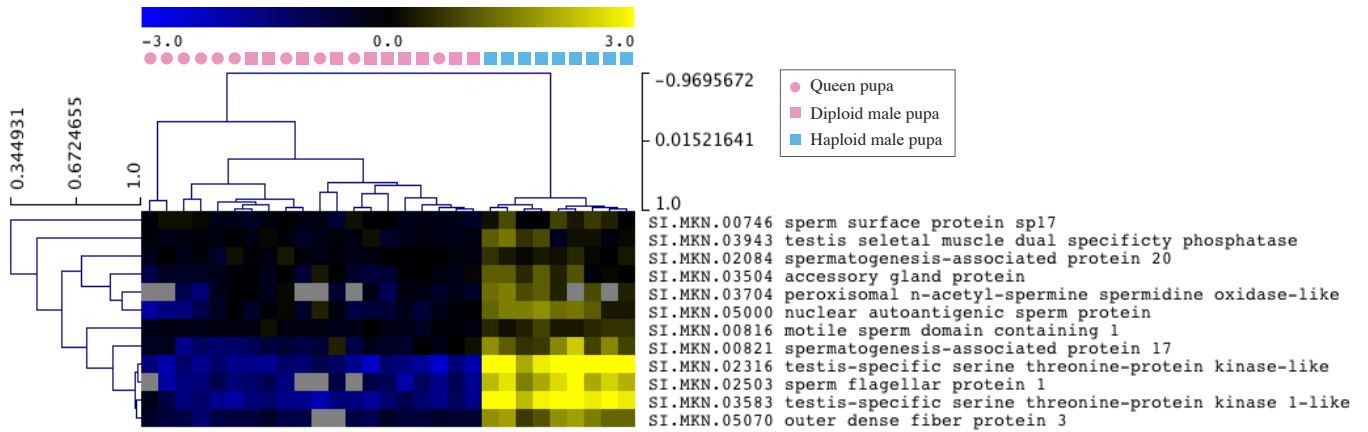


Figure S5. Hierarchical clustering of genes implicated in sperm production in *S. invicta* pupae. Each row in the heat map represents a gene and each column represents an individual sample. Colors in the heat maps represent relative levels of expression: blue = highly expressed, yellow = lowly expressed, gray = NA. Numbers are \log_2 transformed relative expression levels. Scale bars of the dendrograms represent an index of similarity between samples and between genes.

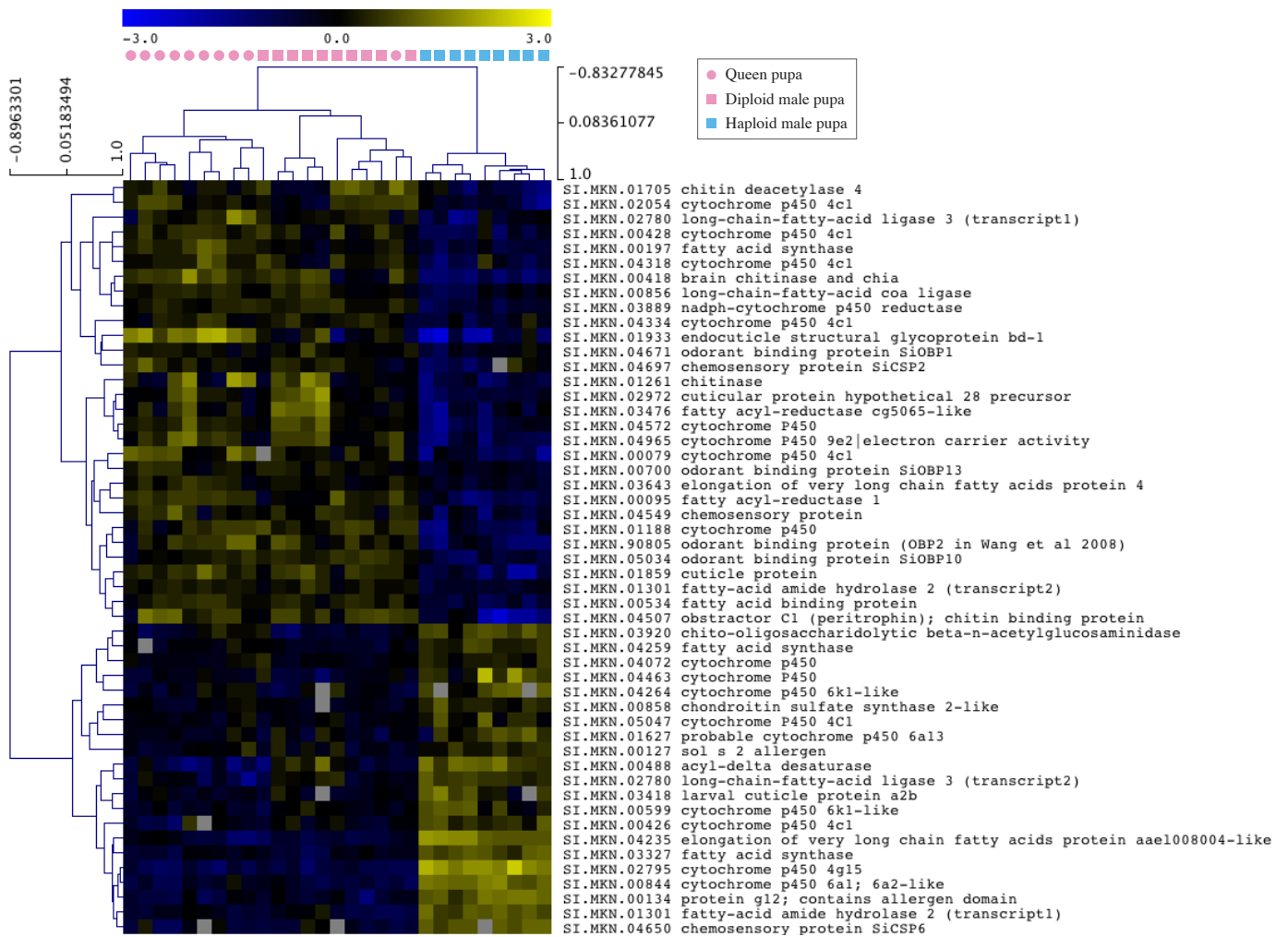


Figure S6. Hierarchical clustering of genes implicated in pheromone production/perception in *S. invicta* pupae. Each row in the heat map represents a gene and each column represents an individual sample. Colors in the heat maps represent relative levels of expression: blue = highly expressed, yellow = lowly expressed, gray = NA. Numbers are \log_2 transformed relative expression levels. Scale bars of the dendrograms represent indices of similarity between samples and between genes.

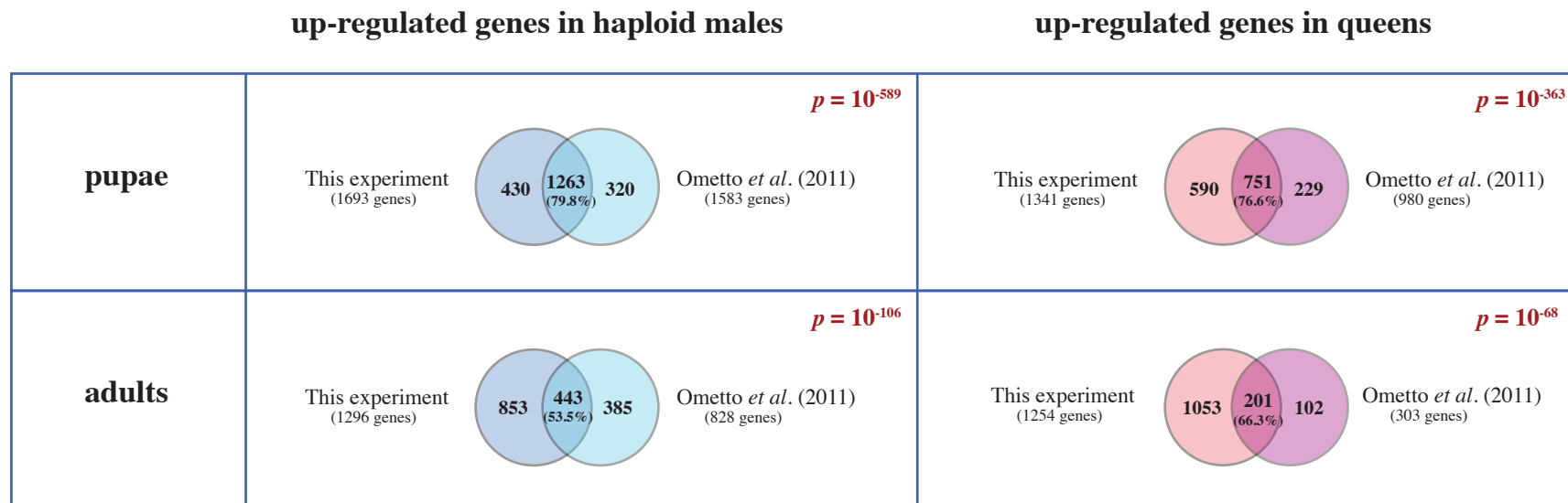


Figure S7. Venn diagram showing number of up-regulated genes in *S. invicta* haploid males and queens at three developmental time-points in this study and in Ometto *et al.* (2011). Percentages in the intersection areas are relative to Ometto *et al.* (2011). Hypergeometric tests (p -values given in each of the four panels) revealed good concordance between the two studies with genes being overexpressed in one study being greatly overrepresented among the genes differently expressed in the other study. Note that adults used for this comparison in our study were 1-day old, whereas in Ometto *et al.* (2011) they were 1h after eclosion.