

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 clones ¹ |
|----------------------|--------------------|---------------------------------------|--|--|
| 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 | AATTCCCTCGTCCAAGTGATAGGC |
| dsxExon5 | GAAAACACTCCAGCCGGAAGATTA | GACATCTCCTGTTGTTGAACCTGG |
| dsxExon6 | GCTTCAGAGGAGGCGCCTATTGG | AGGAAACGGAGATGTACTTGACGC |
| dsxExon7 | GAAACTCGCGTGCCTCCGAAGTG | 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 GO:0006412 GO:0005840 GO:0005811 GO:0022627 GO:0005578 GO:0003735 | ribosome biogenesis translation ribosome lipid particle cytosolic small ribosomal subunit proteinaceous extracellular matrix structural constituent of ribosome | 131 233 206 70 13 8 111 | 73 103 97 34 10 7 73 | 33.98 60.44 53.81 18.29 3.4 2.09 27.61 | <0.001 <0.001 <0.001 0.013 0.043 0.091 <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 GO:0006412 GO:0005840 GO:0022627 GO:0005578 GO:0003735 | ribosome biogenesis translation ribosome cytosolic small ribosomal subunit proteinaceous extracellular matrix structural constituent of ribosome | 131 233 206 13 8 111 | 73 107 99 12 7 76 | 32.1 57.1 50.92 3.21 1.98 26.16 | <0.001 <0.001 <0.001 <0.001 0.082 <0.001 |
| 1-day-old adults | dM-Qn | up | BP | GO:0015992 GO:0006120 GO:0006744 GO:0042254 GO:0006412 GO:0006814 GO:0015986 GO:0005747 GO:0005840 GO:0005739 GO:0022625 GO:0005743 GO:0070469 GO:0000276 GO:0008137 GO:0003735 | proton transport mitochondrial electron transport, NADH to ubiquinone biosynthetic process ubiquinone biosynthetic process ribosome biogenesis translation sodium ion transport ATP synthesis coupled proton transport mitochondrial respiratory chain complex I ribosome mitochondrion cytosolic large ribosomal subunit mitochondrial inner membrane respiratory chain mitochondrial proton-translocating ATP synthase NADH dehydrogenase (ubiquinone) activity structural constituent of ribosome | 71 22 24 131 214 32 18 12 206 216 7 71 36 6 22 111 | 50 21 22 59 82 21 12 11 84 100 7 44 29 6 21 58 | 15.69 4.86 5.3 28.94 47.28 7.07 3.98 2.74 47.04 49.33 1.6 16.21 8.22 1.37 4.73 23.87 | <0.001 <0.001 <0.001 <0.001 <0.001 0.036 <0.001 0.012 0.013 <0.001 0.006 0.012 0.013 <0.001 <0.001 |
| | | down | --- | --- | --- | --- | --- | --- | none significant |
| | hM-dM | up | --- | --- | --- | --- | --- | --- | none significant |
| | | down | --- | --- | --- | --- | --- | --- | none significant |
| | hM-Qn | up | BP | GO:0015992 GO:0006120 GO:0006744 GO:0042254 GO:0008137 GO:0003735 | proton transport mitochondrial electron transport, NADH to ubiquinone biosynthetic process ubiquinone biosynthetic process ribosome biogenesis NADH dehydrogenase (ubiquinone) activity structural constituent of ribosome | 71 22 24 131 22 111 | 46 17 16 54 18 52 | 17.34 5.37 5.86 31.99 5.13 25.89 | <0.001 <0.001 0.013 0.013 <0.001 <0.001 |
| | | down | --- | --- | --- | --- | --- | --- | none significant |
| 11-day-old adults | dM-Qn | up | BP | GO:0015992 GO:0006120 GO:0006744 GO:0006814 GO:0015986 GO:0005747 GO:0005739 GO:0005743 GO:0070469 GO:0000276 GO:0008137 GO:0004129 GO:0046933 GO:0046961 GO:0008121 GO:0003735 | proton transport mitochondrial electron transport, NADH to ubiquinone biosynthetic process ubiquinone biosynthetic process sodium ion transport ATP synthesis coupled proton transport mitochondrial respiratory chain complex I mitochondrion mitochondrial inner membrane respiratory chain mitochondrial proton-translocating ATP synthase NADH dehydrogenase (ubiquinone) activity cytochrome-c oxidase activity hydrogen ion transporting ATP synthase activity, proton-translocating ATPase activity, rotational ubiquinol-cytochrome-c reductase activity structural constituent of ribosome | 71 22 24 32 18 12 216 71 36 6 22 16 12 10 8 8 111 | 53 20 19 21 15 11 104 44 30 6 19 11 9 8 7 38 | 14.2 4.4 4.8 6.4 3.6 2.52 45.38 14.92 7.56 1.26 4.35 3.16 2.37 1.98 1.58 21.93 | <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 0.005 0.012 0.012 <0.001 0.021 0.024 0.024 0.049 |
| | | down | --- | --- | --- | --- | --- | --- | none significant |
| | hM-dM | up | --- | --- | --- | --- | --- | --- | none significant |
| | | down | --- | --- | --- | --- | --- | --- | none significant |
| | hM-Qn | up | BP | GO:0015992 GO:0006120 GO:0006744 GO:0006814 GO:0042254 GO:0005747 GO:0005739 GO:0005743 GO:0070469 GO:0000276 GO:0008137 GO:0004129 GO:0046933 GO:0046961 GO:0008121 GO:0003735 | proton transport mitochondrial electron transport, NADH to ubiquinone biosynthetic process ubiquinone biosynthetic process sodium ion transport ribosome biogenesis mitochondrial respiratory chain complex I mitochondrion respiratory chain mitochondrial proton-translocating ATP synthase mitochondrial small ribosomal subunit NADH dehydrogenase (ubiquinone) activity ubiquinol-cytochrome-c reductase activity structural constituent of ribosome | 71 22 24 32 131 12 216 71 36 6 22 16 12 10 8 8 111 | 48 20 20 20 44 10 96 28 6 6 41 8 41 7 44 7 | 13.68 4.24 4.62 6.16 25.24 2.46 44.27 7.38 1.23 1.23 14.55 2.05 14.55 0.019 21.21 1.53 | <0.001 <0.001 <0.001 <0.001 0.040 0.004 0.013 0.013 0.013 0.013 0.019 0.013 0.019 0.021 <0.001 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 SiJWE11ABC_pcr_2 SiJWE05ACI_pcr_2 | 1.0 1.2 1.2 | 1.5 1.4 1.3 | 1.6 1.7 1.5 | 0.754 0.237 0.262 | <0.001 0.001 0.005 | <0.001 <0.001 <0.001 |
| 8 | SI.MKN.00821 | spermatogenesis-associated protein 17 | SiJWE10ACS_pcr_2 SiJWE10ACS_pcr_1 | 1.5 1.4 | 2.5 3.0 | 3.7 4.2 | 0.053 0.228 | <0.001 <0.001 | <0.001 <0.001 |
| 9 | SI.MKN.02084 | spermatogenesis-associated protein 20 | SiJWC10ACY_pcr_1 SiJWC07ADY_pcr_newprimers SiJWC07ABG_pcr_2 | 1.1 1.0 1.0 | 2.6 1.3 1.3 | 2.8 1.3 1.3 | 0.692 0.992 0.991 | <0.001 <0.001 <0.001 | <0.001 <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 | SiJWC09CAI_pcr_1 SiJWA07AAX_pcr_2 SiJWC12ACX_pcr_1 SiJWA07AAX_pcr_1 | 1.1 1.0 1.0 1.0 | 1.9 1.7 1.6 1.8 | 2.1 1.8 1.6 1.8 | 0.762 0.846 0.984 0.917 | <0.001 <0.001 <0.001 <0.001 | <0.001 <0.001 <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 |
| | | | 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 |
| 10 | SI.MKN.00488 | acyl-delta desaturase | SiJWC04ABJ_pcr_2 | 0.82 | 0.76 | 0.63 | 0.162 | 0.003 | < 0.001 |
| 11 | SI.MKN.03643 | elongation of very long chain fatty acids protein 4 | 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.00197² | 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 |
| 17 | SI.MKN.00095 | fatty acyl-reductase 1 | SiJWG12BCN_pcr_1 | 0.72 | 0.51 | 0.37 | 0.098 | < 0.001 | < 0.001 |
| 18 | SI.MKN.03476 | fatty acyl-reductase cg5065-like | SiJWC07ABC_pcr_2 | 1.26 | 0.44 | 0.56 | 0.560 | < 0.001 | 0.009 |
| 19 | SI.MKN.01301 ³ | fatty-acid amide hydrolase 2 (possible transcript1) | SiJWA12ADF_pcr_1 | 0.97 | 2.61 | 2.54 | 0.930 | < 0.001 | < 0.001 |
| | | fatty-acid amide hydrolase 2 (possible transcript2) | 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 |
| 20 | SI.MKN.00856 | long-chain-fatty-acid coa ligase | SiJWA07AAE_pcr_2 | 0.92 | 0.64 | 0.59 | 0.703 | < 0.001 | < 0.001 |
| | | | 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 |
| 21 | SI.MKN.02780 ³ | long-chain-fatty-acid ligase 3 (possible transcript1) | SiJWD07ACW_pcr_2 | 0.90 | 0.58 | 0.52 | 0.817 | 0.009 | 0.002 |
| | | | SiJWD11BAI_pcr_2 | 1.34 | 1.61 | 2.16 | 0.243 | 0.003 | < 0.001 |
| | | | SiJWH06ABK_pcr_2 | 1.37 | 1.91 | 2.61 | 0.257 | < 0.001 | < 0.001 |
| 22 | SI.MKN.01188 | cytochrome p450 | SiJWA10ABR_pcr_2 | 1.08 | 0.41 | 0.44 | 0.824 | < 0.001 | < 0.001 |
| | | | SiJWH06ABH_pcr_2 | 0.99 | 0.63 | 0.62 | 0.981 | < 0.001 | < 0.001 |
| 23 | SI.MKN.04072 | cytochrome p450 | SiJWC03BCF_pcr_1 | 0.99 | 1.30 | 1.29 | 0.970 | 0.006 | 0.008 |
| 24 | SI.MKN.04463 | cytochrome P450 | SiJWF12BCR_pcr_1 | 0.89 | 2.34 | 2.09 | 0.771 | < 0.001 | < 0.001 |
| 25 | SI.MKN.04572 | cytochrome P450 | SiJWB04BCA_pcr_1 | 1.01 | 0.44 | 0.44 | 0.982 | < 0.001 | < 0.001 |
| 26 | SI.MKN.00079 | cytochrome p450 4c1 | SiJWD04ABB_pcr_2 | 0.63 | 0.52 | 0.33 | 0.104 | < 0.001 | < 0.001 |
| | | | SiJWE01BEA_pcr_2 | 0.64 | 0.86 | 0.55 | 0.063 | 0.369 | < 0.001 |
| 27 | SI.MKN.00426 | cytochrome p450 4c1 | SiJWH09ACV_pcr_2 | 1.12 | 1.75 | 1.96 | 0.703 | < 0.001 | < 0.001 |
| 28 | SI.MKN.00428 | 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 |

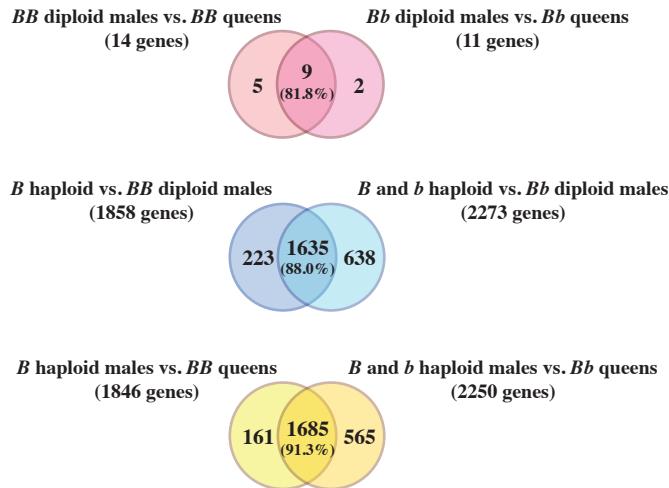
¹ Expression ratio for the first category relative to the second category, dM = diploid male, hM = haploid male, Qn = queen.² Genes that were both ploidy- and sex-specific.³ Possible alternatively spliced variants.

Table S8. (Continued)

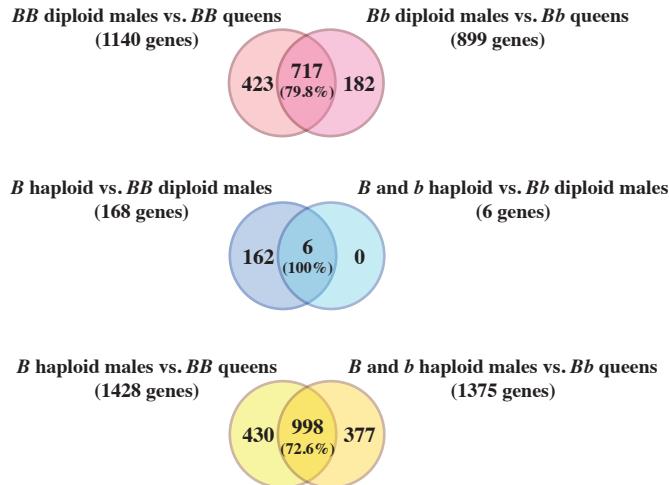
| No. | gene ID | description | spot ID | expression ratio ¹ | | | p-value | | |
|-----|--------------|---|------------------|-------------------------------|-------------|-------------|--------------|--------------|--------------|
| | | | | dM/Qn | hM/dM | hM/Qn | Qn-dM | dM-hM | hM-Qn |
| 29 | SI.MKN.02054 | cytochrome p450 4c1 | SiJWE03ABR_pcr_2 | 0.78 | 0.63 | 0.49 | 0.407 | 0.011 | < 0.001 |
| | | | SiJWH11ACV_pcr_2 | 0.86 | 0.56 | 0.48 | 0.672 | 0.002 | < 0.001 |
| 30 | SI.MKN.04318 | cytochrome p450 4c1 | SiJWB02BBZ_pcr_1 | 0.84 | 0.59 | 0.49 | 0.546 | 0.002 | < 0.001 |
| 31 | SI.MKN.04334 | cytochrome p450 4c1 | SiJWH08BCX_pcr_1 | 1.00 | 0.69 | 0.69 | 0.996 | 0.007 | 0.008 |
| 32 | SI.MKN.05047 | cytochrome P450 4C1 | SiJWD12BBE_pcr_1 | 0.96 | 1.47 | 1.41 | 0.896 | 0.002 | 0.005 |
| 33 | SI.MKN.02795 | cytochrome p450 4g15 | SiJWE06ABI_pcr_2 | 1.08 | 5.61 | 6.05 | 0.730 | < 0.001 | < 0.001 |
| | | | SiJWE09ACN_pcr_2 | 0.89 | 3.49 | 3.09 | 0.694 | < 0.001 | < 0.001 |
| | | | SiJWF04BAD_pcr_1 | 0.90 | 4.33 | 3.88 | 0.759 | < 0.001 | < 0.001 |
| | | | SiJWB05ADH_pcr_1 | 1.10 | 0.68 | 0.75 | 0.668 | 0.001 | 0.013 |
| 34 | SI.MKN.00599 | cytochrome p450 6k1-like | SiJWA10AAK_pcr_2 | 1.18 | 1.58 | 1.86 | 0.536 | 0.002 | < 0.001 |
| 35 | SI.MKN.04264 | cytochrome p450 6k1-like | SiJWB02AAG_pcr_2 | 0.93 | 2.04 | 1.90 | 0.851 | < 0.001 | < 0.001 |
| 36 | SI.MKN.00844 | cytochrome p450 6a1; 6a2-like | SiJWA01CAH_pcr_1 | 0.93 | 3.61 | 3.37 | 0.744 | < 0.001 | < 0.001 |
| | | | SiJWE05ADV_pcr_1 | 1.65 | 0.49 | 0.82 | 0.004 | < 0.001 | 0.097 |
| 37 | SI.MKN.01627 | probable cytochrome p450 6a13 | SiJWA11BCJ_pcr_1 | 0.96 | 1.65 | 1.59 | 0.936 | 0.003 | 0.006 |
| 38 | SI.MKN.04965 | cytochrome P450 9e2electron carrier activity | SiJWB01AAC_pcr_1 | 0.81 | 0.45 | 0.36 | 0.500 | < 0.001 | < 0.001 |
| | | | SiJWB01AAC_pcr_2 | 0.81 | 0.54 | 0.44 | 0.430 | < 0.001 | < 0.001 |
| 39 | SI.MKN.03889 | nadph-cytochrome p450 reductase | SiJWC01ACM_pcr_2 | 1.01 | 0.54 | 0.54 | 0.982 | < 0.001 | < 0.001 |
| | | | SiJWE03ABW_pcr_2 | 1.00 | 0.65 | 0.65 | 0.995 | < 0.001 | < 0.001 |
| | | | SiJWG12CAX_pcr_1 | 1.04 | 0.60 | 0.63 | 0.847 | < 0.001 | < 0.001 |
| | | | SiJWH02BAB_pcr_1 | 0.91 | 0.56 | 0.51 | 0.532 | < 0.001 | < 0.001 |
| | | | SiJWH10CAX_pcr_1 | 1.04 | 0.51 | 0.53 | 0.859 | < 0.001 | < 0.001 |
| 40 | SI.MKN.04671 | odorant binding protein SiOBP1 | SiJWA03ABL_pcr_2 | 0.99 | 0.73 | 0.73 | 0.988 | 0.005 | 0.004 |
| 41 | SI.MKN.90805 | odorant binding protein (OBP2 in Wang et al, 2008) | SiJWA08ABR_pcr_2 | 0.93 | 0.41 | 0.38 | 0.858 | < 0.001 | < 0.001 |
| | | | SiJWD04CAE_pcr_1 | 0.98 | 0.47 | 0.46 | 0.952 | < 0.001 | < 0.001 |
| | | | SiJWD11BBJ_pcr_1 | 1.08 | 0.38 | 0.41 | 0.883 | < 0.001 | < 0.001 |
| | | | SiJWF11BCW_pcr_1 | 0.92 | 0.44 | 0.40 | 0.699 | < 0.001 | < 0.001 |
| | | | SiJWA03ACS_pcr_1 | 1.01 | 0.69 | 0.70 | 0.965 | < 0.001 | < 0.001 |
| 42 | SI.MKN.00700 | odorant binding protein SiOBP13 | SiJWD07ABA_pcr_2 | 1.18 | 0.47 | 0.55 | 0.364 | < 0.001 | < 0.001 |
| 43 | SI.MKN.05034 | odorant binding protein SiOBP10 | SiJWF11BCE_pcr_1 | 0.92 | 0.58 | 0.53 | 0.778 | < 0.001 | < 0.001 |
| 44 | SI.MKN.04549 | chemosensory protein | SiJWA03BCG_pcr_1 | 0.94 | 0.61 | 0.58 | 0.888 | 0.007 | 0.003 |
| 45 | SI.MKN.04697 | chemosensory protein SiCSP2 | SiJWD02BAY_pcr_1 | 1.02 | 1.42 | 1.45 | 0.952 | 0.005 | 0.003 |
| 46 | SI.MKN.00127 | sol s 2 allergen | SiJWF02ABD_pcr_2 | 1.01 | 3.06 | 3.09 | 0.976 | < 0.001 | < 0.001 |
| 47 | SI.MKN.00134 | protein g12; contains allergen domain | SiJWH04BCY_pcr_1 | 0.95 | 0.31 | 0.29 | 0.936 | < 0.001 | < 0.001 |
| 48 | SI.MKN.04507 | obstructor C1 (peritrophin), chitin binding protein | SiJWH07ABA_pcr_2 | 1.07 | 2.72 | 2.90 | 0.836 | < 0.001 | < 0.001 |
| 49 | SI.MKN.04650 | chemosensory protein SiCSP6 | | | | | | | |

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.

Pupae



1 day-old adults



11 day-old adults

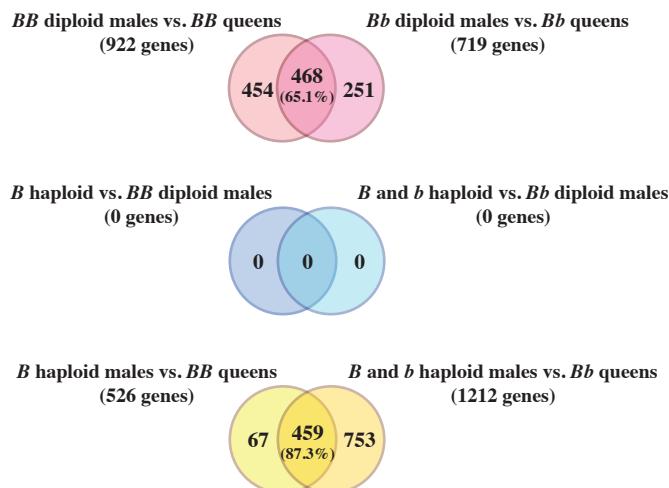


Figure S1. Venn diagram showing the numbers of genes differentially expressed between *S. invicta* individuals of different categories with different *Gp-9* genotypes. Because haploid males can have either the *B* or *b* genotype, we compared both types of males to *Bb* diploid individuals.

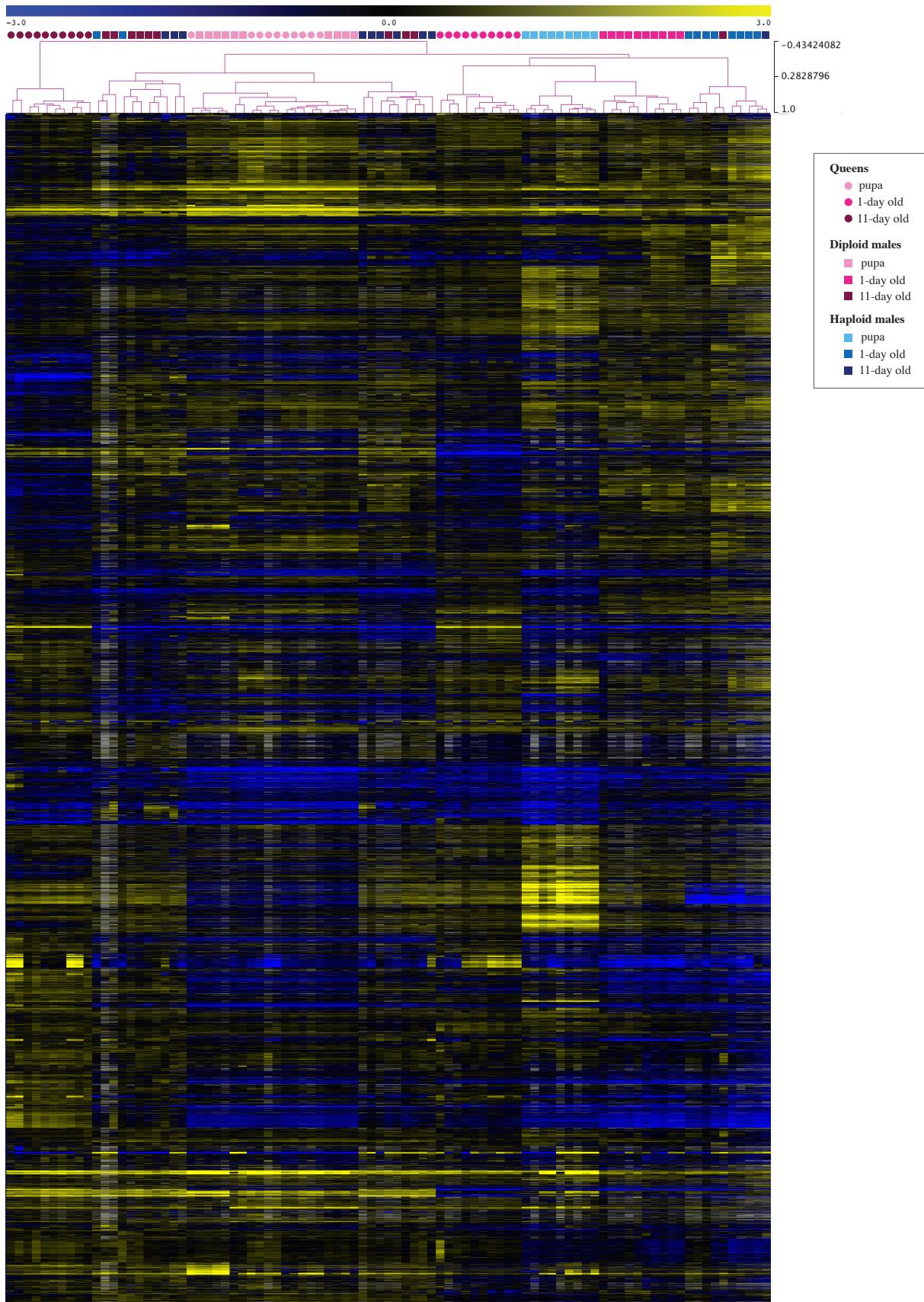


Figure S2 Hierarchical clustering of gene expression profiles of individual queens, diploid males and haploid males at three developmental timepoints in *S. invicta*. Colors in the heat map represent relative levels of expression: blue=highly expressed, yellow=lowly expressed, gray = NA (data not available). Numbers for heat map are \log_2 transformed relative expression levels. Scale bar on the right of the cluster represents an index of similarity between samples.

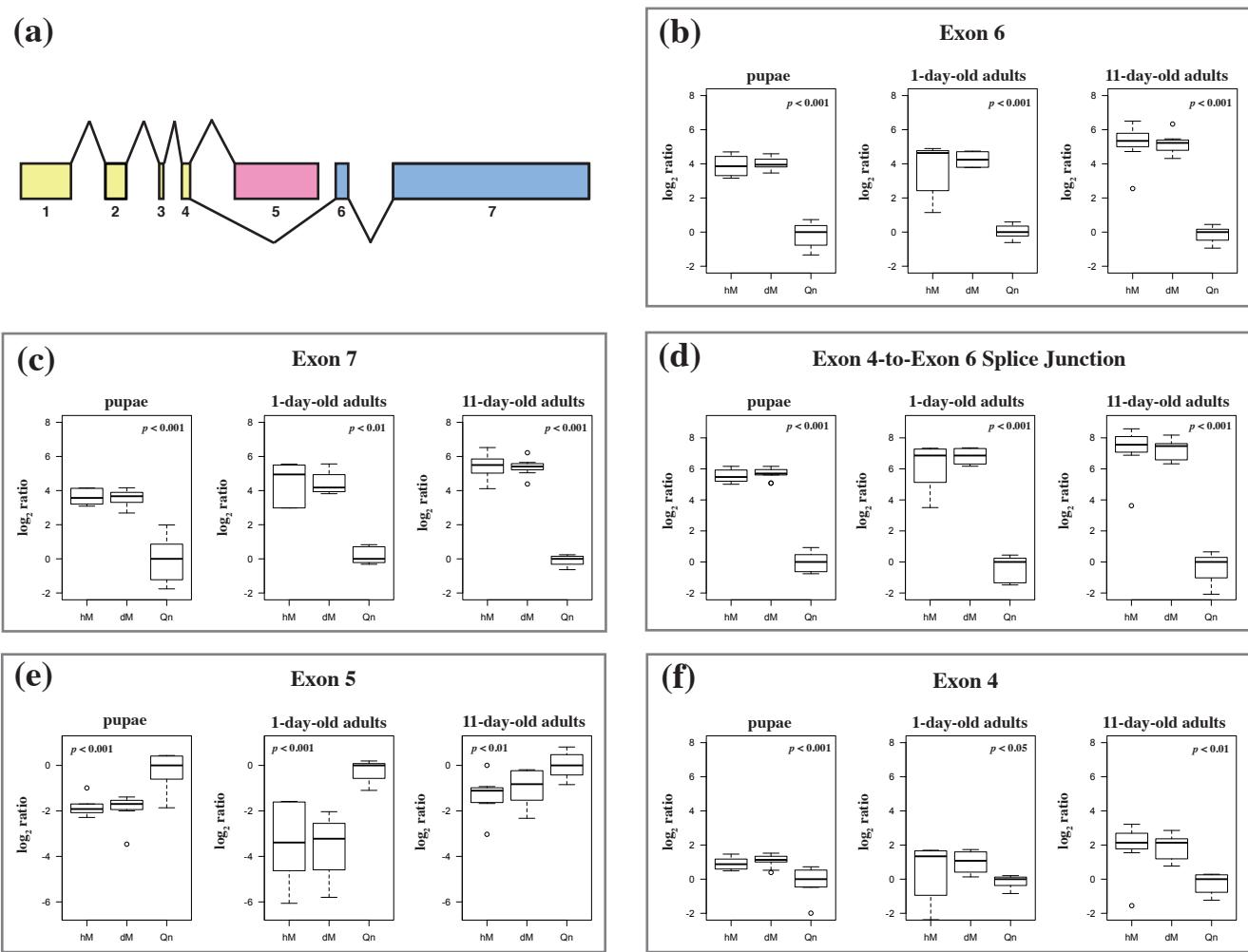


Figure S3. Alternative splicing of the *S. invicta* *dsx* gene in pupae. (a) Diagram depicting alternative splicing pattern of *dsx* (black, pattern for both sexes; gray, queen pattern; vertical stripe, male pattern). (b-f) Box-and-whisker plots of \log_2 transformed relative expression levels based 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.

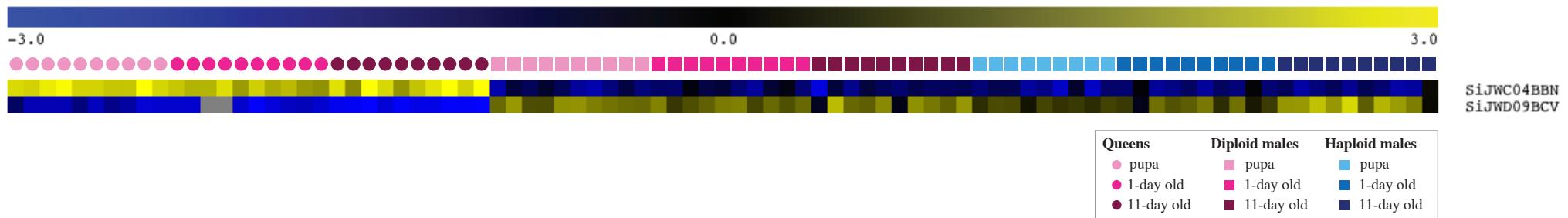


Figure S4. Hierarchical clustering of the two *dsx* microarray spots at three developmental timepoints in *S. invicta*. Colors in the heat maps represent relative levels of expression: blue=highly expressed, yellow=lowly expressed, gray = NA (data not available). Numbers are \log_2 transformed relative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

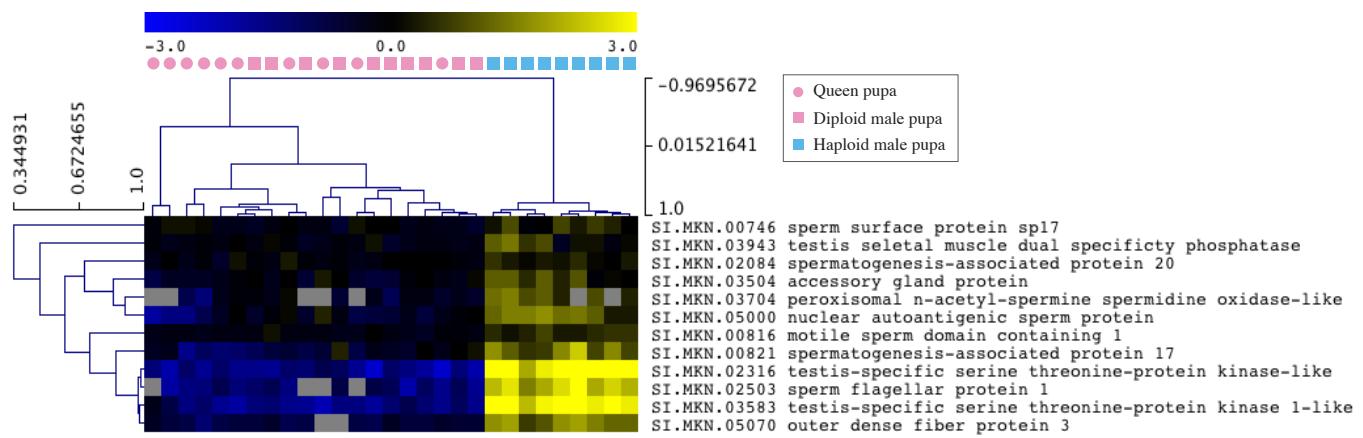


Figure S5. Hierarchical clustering of genes implicated in sperm production in *S. invicta* pupae. Each row in the heat map represents a gene and each column represents an individual sample. Colors in the heat maps represent relative levels of expression: blue = highly expressed, yellow = lowly expressed,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

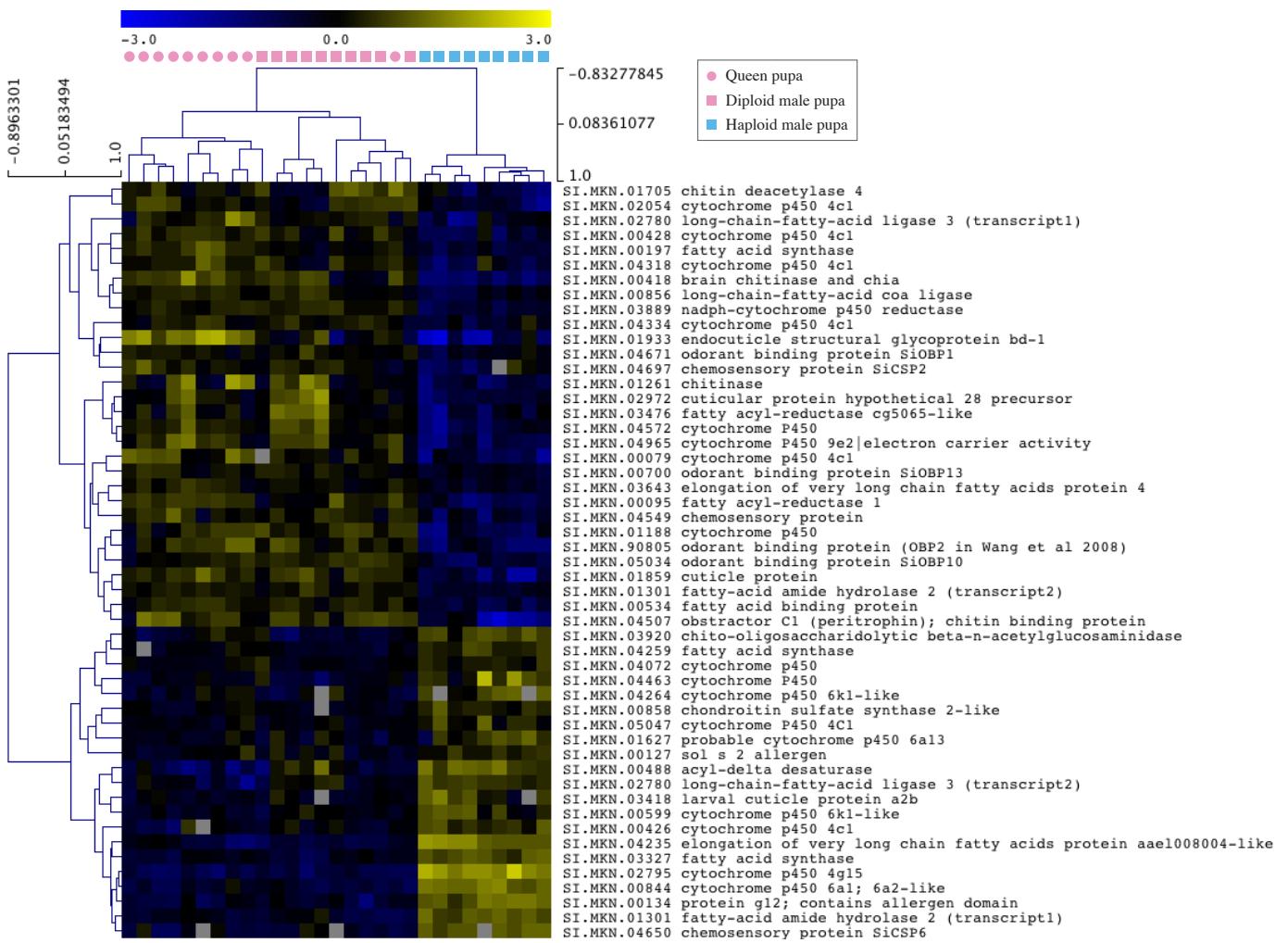


Figure S6. Hierarchical clustering of genes implicated in pheromone production/perception in *S. invicta* pupae.
 Each row in the heat map represents a gene and each column represents an individual sample. Colors in the heat maps represent relative levels of expression: blue = highly expressed, yellow = lowly expressed, gray = NA. Numbers are log₂ transformed relative expression levels. Scale bars of the dendrograms represent indices of similarity between samples and between genes.

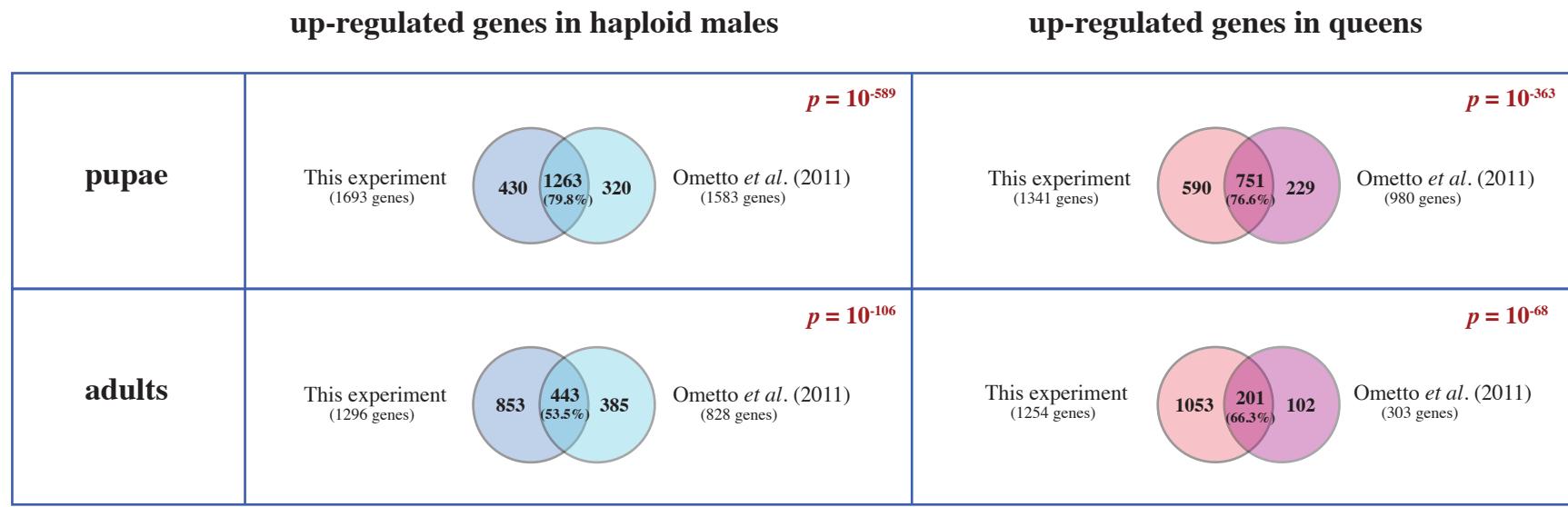


Figure S7. Venn diagram showing number of up-regulated genes in *S. invicta* haploid males and queens at three developmental time-points in this study and in Ometto *et al.* (2011). Percentages in the intersection areas are relative to Ometto *et al.* (2011). Hypergeometric tests (p -values given in 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.