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Proteomic identification of a potential sex biomarker for 2 fruit fly species at pupal stage

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Abstract

Fruit flies are one of the most destructive pests worldwide. The sterile insect technique (SIT) has been one of the most effective fruit fly control techniques. Genetic sexing strains have been developed to facilitate the separation of males and females based on color at the pupal stage for the oriental fruit fly, *Bactrocera dorsalis*, and the medfly, *Ceratitis capitata*, known as dorsalis translocation white pupae (DTWP) and temperature sensitive lethal mutation strain (TSL). In both cases, the females are white and the males are brown. By releasing only sterile males, the control efficiency was increased, and fruit damage due to sterile females reduced. There remains, however, a gap in our understanding of this phenomenon because there is no information on the physiological basis of the sex differences. Here, we report the outcome of a study designed to identify pupal proteins related to the sex differences using these two strains. We collected whole pupae of both species each day for first 10 days for protein analysis. Protein expression was analyzed by 2D electrophoresis, gel densitometry and mass spectrometry. The general odorant binding protein, OBP56d, was differentially expressed in white pupae versus brown pupae for both species. We also confirmed this result with phenotypical characteristics to prove these proteins were not derived from puparia. These proteins had molecular weights between 10-15 kDa and a pI of 6.73 for DTWP and 5.71 for TSL. We discuss the significance of this protein in relation to sex/color of pupal stage.

KEY WORDS dorsalis translocation white pupae (DTWP), temperature sensitive lethal mutation strain (TSL), oriental fruit fly, brown and white pupae, proteomics, biomarker

Introduction

The sterile insect technique (SIT) is an effective component of several insect pest control programs, including some deployed to manage tephritid fruit fly pests. Conventional SIT programs are based on mass rearing males and females, sterilizing, transporting, and releasing them into the wild. The sterile males compete for and mate with wild females, resulting in reduced fertility at the individual and population levels. These systems have been improved by increasing the field competitiveness and survival of released males. One approach is artificial selection for appropriate phenotypes. For example, McInnis et al. (2002) selected male medfly, *Ceratitis capitata*, for increased survivorship under field conditions. Development of male-only strains led to increased cost effectiveness in rearing and to reduced fruit damage caused by released sterile females.

Genetic sexing strains based on pupal coloration have been developed for the mediterranean fruit fly, *Ceratitis capitata* (Widemann) known as temperature sensitive lethal [TSL] (Robinson et al., 1999, Fisher 2000) and the oriental fruit fly, *Bactrocera dorsalis* (Hendel), known as dorsalis white pupal translocation [DWPT] (McCombs and Saul 1995). In each species, chromosomal translocations link a gene for normal brown pupae to males, and mutant white pupae to females. This translocation-based gender sorting system relies on automated photoelectric scanners to separate female-determined from male-determined pupae (McCombs and Saul 1995; McInnis et al., 2004). The TSL strain facilitates killing female-determined pupae, by heating to 34 °C for 12 h, thereby creating a male-only colony for release.

The sex-linked pupal coloration opens questions about the underlying mechanisms of the color differences. Some proteins are directly linked to insect color patterns. Insecticyanin, for example, is a blue biliprotein in hemolymph of the tobacco hornworm, *Manduca sexta* (Riley et

al., 1984). Two proteins, yellow and ebony, direct pigment patterns in fruit flies, *Drosophila melanogaster* (Wittkopp et al., 2002). Wild-type *D. melanogaster* have a stripe of dark pigment near the trailing edge of the anterior abdominal segments (A2-A6). The stripe is lost in *yellow* mutants and very much darker in *ebony* mutants. The stripe is completely missing in double *yellow; ebony* mutants. These two proteins also influence larval colors in silkworms, *Bombyx mori* (Futahashi et al., 2008). Pea aphids, *Acyrtosiphon pisum*, occur in red-green polymorphs. The carotenoid torulene is responsible for the red morph (Moran and Jarvik, 2010). More recently, a single gene, *cortex*, has been identified as regulator of wing pattern changes in many, possibly all, lepidopteran species (Nadeau et al., 2016). More to the point, insertion of a transposable element into *cortex* is responsible for the evolution of industrial melanisms in the peppered moth, *Biston betularia* (Van't Hof et al., 2016). *Cortex* is a member of the fizzy family of cell-cycle regulators and its cognate protein probably acts during early wing disc development. We infer that various proteins and their cognate genes are responsible for coloration and sex/color morphs in insects generally, which led us to pose the hypothesis that one or more proteins associated with sex/color formation is differentially expressed between male- and female-determined pupae. We tested our hypothesis by analyzing protein expression in male- and female-determined pupae. Here, we report on the outcomes of our analysis.

Materials and Methods

Insects sample collection

Newly laid eggs (< 6 h) of the oriental fruit fly, *B. dorsalis* (DTWP) were provided by the DTWP colony rearing group in the Tropical Crop and Commodity Protection Research Unit (USDA Agricultural Research Service, Hilo, Hawaii). Hatched larvae were reared on a liquid

diet supplemented with WGO (0.66%, v:v) *ad lib* (Chang and Vargas, 2007). Upon pupation, pupae were collected at the same time every day for the first 10 days of the pupal stage and sorted by color. Colored pupae of medfly TSL strain were provided by California Department of Food and Agriculture, Waimanalo, Hawaii. Brown and white pupae (400 each day) were quick-frozen in liquid nitrogen and stored at -80°C for protein analysis. Protein expression was analyzed by 2D electrophoresis, gel densitometry and mass spectrometry. Identified proteins were confirmed with qPCR as well. We also confirmed our finding with phenotypical characteristics to exclude the hypothesis that these proteins may be derived from color of puparia. All protocols were described below:

Sample preparation

Pupae (0.2 g whole pupae/ml buffer, approximately 20-30 pupae) were homogenized 3x in 1 ml 10mM Tris-HCl (pH 7.0) containing protease inhibitors (final dilution =1:100; Sigma, St. Louis, #P8340 for Mammalian Cell and Tissue Extracts) using a Fast Prep-24 instrument (MP Biomedicals, Solon, OH). Homogenates were centrifuged twice at 15,294 x g for 15 min at 4°C. The resulting infranatants (middle clear layer, just below the supernatant) were transferred to new vials on ice for immediate use. Three independent biological replicates were processed for each day.

2D-electrophoresis

Electrophoresis and mass spectrometric protocols followed published procedures (Stanley et al., 2008; Chang et al., 2012). Five µl of 2D gel protein standards (Bio-Rad, Hercules, CA, #161-0320) were added to each sample tube. Protein concentration (5 µg/µl) was determined using the Pierce Micro BCA Protein Assay Kit, using BSA as a quantitative standard (Rockford,

IL). A Protean IEF cell system (Bio-Rad, Hercules, CA) was used to perform IEF using the standard protocol and a pre-set linear volt ramp program (8,000 V and 50 μ A/strip max, 35,000 vH).

For the second dimension, the IPG strips were equilibrated (15 min in buffer: 6 M urea, 2% SDS, 20% glycerol, 130 mM DTT, 0.375 M Tris-HCl, pH 8.7 [Buffer I] followed by 6 M urea, 2% SDS, 20% glycerol, 135 mM iodoacetamide, 0.375 M Tris-HCl, pH 8.7 [Buffer II]). Prior to running, molecular weight standards (10 μ l/lane, Bio-Rad #161-0363) were applied to each gel (precast gels, 8-16% Tris-HCl, Bio-Rad #345-0105), and proteins were separated on SDS-PAGE using the Criterion Cell system (Bio-Rad, Hercules, CA, #165-6001). Gels were stained with Coomassie Blue G-250 (BioSafe Stain, Bio-Rad) and analyzed using Delta 2D software (Decodon GmbH, Greifswald, Germany). Protein spots with densities significantly different between treatments (Students' t-test, $p < 0.05$) were removed from the gels using a 1.5 mm spot picker (The Gel Company, San Francisco, CA) and stored at -80°C . One gel was run for each independent biological replicate and three independent biological replicates were performed.

MS/MS analysis

Mass spectrometric protocols followed published procedures (Stanley et al., 2008; Chang et al., 2012). Selected protein spots were prepared for mass spectrometry by transferring them to clean tubes, digesting with trypsin, extracting peptides from the gels, and then lyophilizing and reconstituting the peptides with water for MS/MS analysis as described (Stanley et al., 2008). A portion of each protein was mixed with alpha-cyano-4-hydroxycinnamic acid matrix and applied to the MALDI target for analysis. The resulting sequence data, combined with observed MW and pI values, were used to establish protein identities. MS/MS ion searches were performed for each

spot using the Mascot search engine v. 2.4 (www.matrixscience.com) on an in-house server against a custom *B. dorsalis* protein database derived from mRNA sequence and the NCBI Inr Metazoa protein database. E-values and frequency of matches to a specific protein were the primary criteria for these determinations. All reported matches in the species specific *B. dorsalis* database had higher significance levels compared to the Metazoa database.

Quantitative PCR (qPCR)

Ten OBPs primers from *Drosophila* genes used in Zheng et al. (2013) for *B. dorsalis* genes along with chitin binding proteins and chitin synthases listed in Table 1 were used in this study for qPCR analysis to confirm our finding.

The qPCR protocol followed published procedures (Chang et al., 2012). Total RNA was isolated from separated brown and white DTWP pupae using a Macherey-Nagel NucleoSpin RNA II Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany). Four pupae of each color per day were homogenized in 600 µl of RA1 and 6 µl of β-mercaptoethanol using a RNase-free pestle and 1.5 ml microcentrifuge tube, following the manufacturer's protocol. Total elution volume was 60 µl. Total RNA concentrations were measured using a Qubit Fluorometer using Qubit RNA BR reagents (Invitrogen, Carlsbad, CA). The first-strand cDNA was synthesized using Moloney Murine Leukemia Virus Reverse transcriptase (M-MLVRT; Promega, Madison, WI) and Oligo (dT) primers. RNA was denatured and oligo (dT) primers were annealed by incubation at 70°C for 5 min with 2 µg of total RNA and 0.5 µg of Oligo dT primers. The components of the reaction were 5 µl of M-MLV 5x reaction buffer, 1.25 µl of dNTPs (10 µM each), 1 µl of RNasin (Qiagen, Valencia, CA, 4 Units/µl), 1 µl of M-MLV RT enzyme (20 Units/µl). Reverse transcription then took place at 37°C for 1 hour to synthesize 1st strand cDNA. Prior to use in qPCR, cDNA was diluted to 1:4000 with nuclease-free water (Qiagen

sciences, MA). Gene-specific primers (Table 1) were designed for a chitin binding protein, chitin synthase-2, BD1 chitinase, bangles and beads, and 10 odorant binding proteins (Zheng et al. 2013) from full-length gene sequencing from the draft assembly of the *B. dorsalis* genome (www.bactrobase.org). The housekeeping gene, actin was used as the reference gene for relative quantitation using the $2^{-\Delta\Delta C(T)}$ protocol (Livak and Schmittgen, 2001). Correct target amplification was verified by sequencing the reaction products from a subset of the qPCR reactions.

qPCR amplification and analysis were performed on a StepOne Plus Instrument and analyzed with StepOne Software v2.2 (Applied Biosystems, Life Technologies Corporation, Carlsbad, CA). qPCR was carried out using KAPA SYBR Fast Master Mix for ABI Prism (KABA Biosystems, Boston, MA). Components of the reaction mixture (total 20 μ l) for the actin reference gene were 10 μ l of KAPA SYBR Fast Master Mix (2x) ABI Prism, 0.4 μ l of forward primer (10 μ M), 0.4 μ l of reverse primer (10 μ M), 5.2 μ l of nuclease-free water, and 4 μ l of diluted (1:500) cDNA. Components for the target gene qPCR reactions were 10 μ l of KAPA SYBR fast master Mix (2x) ABI Prism, 0.1 μ l of forward primer (10 μ M), 0.1 μ l of reverse primer (10 μ M), 1.8 μ l of nuclease-free water, 8 μ l of diluted cDNA. The qPCR program used was: 95°C for 1 min initial denaturation followed by 40 cycles at 95°C for 5 s and 60°C for 30 s. The specificity of the gene specific primers was further confirmed by melting curve analysis. Gene expression was quantified as just described. The relative quantitation (RQ, $2^{-\Delta\Delta C_t}$) term was calculated using the same reference sample (Male, day 1, batch A) for all plates to allow for comparison between plates.

Pupal dissection

Dissection of 1 to 10-d-old brown and white pupae from their puparia was performed under a microscope (Leica) to demonstrate the phenotypic difference between brown and white pupae. Four and 8-d-old brown and white pupae without puparia were also processed for protein analysis to confirm the origin of these proteins.

Statistical analysis

All data presented here were repeated 3 times from separate batches of insects. Data are presented as mean $\pm$ standard errors (S.E.), and subjected to analysis of variance. Means separations for significant factors were done using a protected least significant difference test (SAS, 2008). For each protein, expression ratios above 1.50 or below 0.5 were considered significantly different.

Results

Differentially expressed proteins between brown and white *B. dorsalis* pupae

Gel densitometry revealed two proteins were differentially expressed between brown and white pupae from 1-7 d, but not days 8-10, and were identified as general odorant binding protein 56d (GOBP 56d) and pupal cuticle protein (Edg-84A) (Fig. 1). For each protein, expression ratios above 1.50 or below 0.5 and at $p > 0.05$ were considered significantly different.

Differentially expressed proteins between brown and white *C. capitata* pupae

One protein was differentially expressed between white and brown pupae at ages 1-8 days, identified as OBP56d-like. Expression ratios above 1.5 or below 0.5 and at $p > 0.05$ were considered significantly different (Table 2, Fig. 2).

Since OBP56d was expressed on white pupae of both *B. dorsalis* and *C. capitata* pupae, not brown pupae, we are going to focus on discussing OBP56d in this study only. As for the second protein of Edg-84A, pupal cuticle protein, that was also differentially expressed in the oriental fruit fly only. *Edg-84A* encodes a pupal cuticle protein and is expressed during mid- to late prepupal period in *Drosophila melanogaster* (Apple and Fristrom, 1991). While Edg-84A is a structural protein, it is expressed in a space-specific manner in the anterior epidermis, under regulation by at least six regulatory elements, four positive and two negative. This finding prompted Kayashima et al. (2005) to wonder why the expression is so complex. They suggested that a combination of elements may be required to achieve the very high expression recorded in their analysis. Adding to the complexity of the gene regulation, here we report that Edg-84A is differentially expressed during days 1 - 6 in white, but not brown pupae and expressed in similar levels in brown and white pupae during days 7 – 10 (Fig. 4). Due to the unknown and complexity nature of the interaction between the cuticle protein and chitin, we expect to run more thorough research in the near future (Hamodrakas et al. 2002). Therefore, we are focusing on OBP56d in this study only.

Quantitative PCR (qPCR) confirmation of Odorant Binding Protein (OBP)

OBP56d was differentially expressed between white and brown pupae and this difference was confirmed by qPCR analysis using the 10 primers listed in Table 1. The genes encoding *BdorOBP1* (*OBP8a*) and *BdorOBP2* (*OBP56d*) were differentially expressed between white and brown pupae in 8-d and 2-d pupae respectively (Fig. 3). *BdorOBP3* (*OBP56d*) was differentially expressed in 1 and 3-d pupae. *BdorOBP4* (*OBP56d*), *BdorOBP8* (*OBP83ef*), and *BdorOBP10* (*OBP99c*) were expressed differentially between day 2 white and brown pupae. *BdorOBP2* (*OBP56d*) was down-regulated and *BdorOBP4* (*OBP56d*), *BdorOBP8* (*OBP83ef*) were up-

regulated in white pupae. *BdorOBP6* (*OBP56h*) and *BdorOBP10* (*OBP99c*) were differentially up-regulated at 4, 5 d-old in favor of brown pupae and 2, 4-d-old in favor of white pupae. There were no differences in expression of *BdorOBP5* (*OBP56g*) and *BdorOBP7* (*OBP83g*). In conclusion, expression level of OBP56d mRNA was significantly differentially expressed at 2 - d-old. This finding confirmed with our results from proteome analysis. The protein expression level from OBP56d was shown the highest in both species at 2 d-old: 4.20 for *B. dorsalis* and 2.46 for *C. capitata* (Fig. 1 and Fig. 2).

Puparium experiment

We compared the differences of puparia proteins between white and brown pupae and pupae with and without puparium. Puparia were collected after adult emergence naturally. Pupae without puparia from both 4d and 8d old brown and white pupae were dissected out and protein analysis was performed. The protein images were compared between pupae with and without puparia on 4 and 8-d-old for both *C. capitata* and *B. dorsalis*. The spot of OBP56d were significantly expressed in both 4 and 8-d-old white pupae but not in brown pupae without puparia (Fig. 4 and Fig. 5).

To ensure OBP56d protein/biomarker was not the expression from “white color” puparium, a comparison of pupal proteins from puparia between white and brown was performed. OBP56d protein was not expressed on both puparia of both species. Therefore, this result further confirmed that these proteins were expressed in pupae without puparia.

Phenotypic differences between brown and white pupae of B. dorsalis

To further confirm our finding, the major phenotypic difference between brown and white pupae is the ovipositor located in the abdomen of white pupae. This ovipositor can be an indicator of sex between these two color pupae (Fig. 6). The ovipositor could be linked to the

cuticle protein at age 1 to 10-d-old of white pupae from fruit flies. Further investigation would be needed.

Discussion

The data presented in this paper supported our hypothesis that one or more proteins is differentially expressed between male- and female-determined pupae. Here we report on the odorant binding protein, OBP 56d, that were differentially expressed between brown, male-determined pupae and white, female-determined pupae on both species.

Insect odorant binding proteins (OBPs) are low-molecular-weight water-soluble proteins (15 kDa) that often consist of 120–150 amino acids present in the chemosensory organs of insects. OBPs are present in high concentration in olfactory and gustatory sensilla (Pelosi 1994). The olfactory signal transduction cascade in insects is facilitated by three main groups of molecules: odorant-binding proteins (OBPs), odorant receptors (ORs), and odorant-degrading enzymes (ODEs) (Justice et al. 2003). They solubilize hydrophobic odorant molecules and transport them through the hydrophilic hemolymph environment to the odorant receptors at the olfactory sensory neurons within antennae, which launches the olfactory signal transduction system. OBPs generally act in odorant transport, although they may also serve in other roles, because they are expressed in abdomens, legs and in seminal fluids (Zheng et al. 2013).

Our results on the expression of an OBP in fruit fly pupae may expand the biological significance of this protein. Although we cannot yet suggest a physiological role for these proteins in pupae, the broad idea that OBPs operate in additional areas of insect biology, specifically in pupae, is consistent with recent reports indicating OBPs are expressed in several tissues. Xu et al. (2003) reported that the mosquito, *Anopheles gambiae* expresses 57 genes encoding OBPs, including some atypical OBPs that are expressed in chemosensory organs of

adult and juvenile stages. Similarly, honeybees, *Apis mellifera*, have 21 genes encoding OBPs, the smallest set of known OBP-encoding genes. Nine OBPs are specific to antennae and the other 12 are expressed in other tissues and life stages (Forêt and Maleszka, 2006). He et al. (2011) found OBP1 was expressed in all life stages of the brown planthopper, *Nilaparvata lugens*. OBP2 was expressed mostly in adults and OBP3 was expressed almost exclusively in the nymphal stages. Within adults, OBP1 was expressed in male and female antennae and in the wings of both genders and OBP3 was expressed in antennae, abdomens and wings of both genders. This protein apparently acts in non-sensory functions because the dsRNA treatments led to increased nymphal mortality. These and other reports indicate OBPs are not limited to antennal expression and their biological actions are not limited to chemosensory physiology (Wang et al, 2001; Kim et al., 1998; Xu et al, 2005; Plettner et al., 2000; Pelletier et al., 2010; Biessmann et al., 2010; Zhong et al, 2012). Our data on an OBP in *B. dorsalis* pupae are consistent with the idea that these proteins act in roles outside of sensory physiology.

This is not the first report of OBPs in insect pupae or in multiple tissues within a single individual. In their work on OBPs in honeybees, Forêt and Maleszka (2006), *obp14* and *obp15* are expressed in larvae and expression disappears after pupation. *Obp 10* is expressed in pupae and its highest expression appears in newly emerged bees. *Obp13* is highly expressed throughout the pupal stages. It follows that several OBPs are expressed in honey bee pupae and are likely expressed in pupae of many, if not all, insect species.

Conclusion

This finding will not replace the color system as a tool for sexing fruit fly at pupal stage. However, the differential expression of OBPs in the pupae of genetic sexing strains of *B.*

dorsalis and *C. capitata* draws attention to the roles of these proteins may play more than what we have known, although it remains unclear whether they directly act in pupal sex morphs. However, we have proved that OBP56d was not involved with color morphs. Further investigation on whether these proteins are involved with sex determination is ongoing. Upon confirmation, we may be able to develop a novel rearing technology by manipulating this identified biomarkers/proteins at the molecular level to rear two sexes of flies.

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Figure legends

Figure 1. Two differentially expressed proteins with aging between white and brown pupae of *Bactrocera dorsalis*. W: white pupae; B: brown pupae; *: 95% significantly different in OBP56d; **: 95% significantly different in Edg84A.

Figure 2. One differentially expressed proteins with aging between white and brown pupae of *Ceratitis capitata*. W: white pupae; B: brown pupae; * 95% significantly different in OBP56d.

Figure 3. Relative 10 OBP mRNA expression levels in 1 to 10-day-old brown and white pupae of *Bactrocera dorsalis*. Histogram bars annotated with the same letters are not significantly different.

Figure 4. Representative 2-D images showing significantly differentially expressed proteins OBP56d (as indicated with arrows) from 4 (4B and 4W) and 8 d old (8B and 8W) brown (B) and white (W) pupae of *Ceratitis capitata* (TSL) without puparium.

Figure 5. Representative 2-D gel images showing significantly differentially expressed proteins OBP56d (as indicated with arrows) from 4 (4B and 4W) and 8 d old (8B and 8W) brown (B) and white (W) pupae of *Bactrocera dorsalis* (DTWP) without puparium.

Figure 6. Alternative way to differentiate male and female at pupal stage. This photo shows the ovipositor at the end of abdomen of 8 d old white (right) and brown (left) pupae. A lateral view of female abdomen is demonstrated in photo A.

Table 1. Primers used for gene expression analysis in pupae at the indicated ages (d) and colors. Abbreviations: B: Brown; W: White;

Primer names	Forward Nucleotide sequence (5’->3’)	Reverse Nucleotide sequence (5’->3’)	Significance	
			Age	Pupae
<u>Odorant binding proteins (<i>Drosophila</i> gene)</u>				
<i>BdorOBP1 (OBP8a)</i>	GGCGAGCGTTGTTTCCAG	ATCGGCACCAGCACTTCC	8	B
<i>BdorOBP2 (OBP56d)</i>	TTCTTCGCTGTTGCTGTTTTGC	AGAAGCATTTGACTTTGCCATC	2	B
<i>BdorOBP3 (OBP56d)</i>	TTGACCGAGGAGCAGAAAC	TTGACTTTGCCATCGACTT	1,3	B,B
<i>BdorOBP4 (OBP56d)</i>	GAACTTCTTCGCTGTTGCTG	CTTGACTGTCATCGCCTTTG	2	W
<i>BdorOBP5 (OBP56g)</i>	CCACGACTTCATTGAGGGTA	CCACAGCAACTAATTTATC	-	-
<i>BdorOBP6 (OBP56h)</i>	ACGAAGCCAAAGTCACGG	GCATCAAAGACGCCATCC	4,5	B,B
<i>BdorOBP7 (OBP83g)</i>	GTTGCAGGCAAATTCCAGAT	AGAGTCCCATTGCTCCACA	-	-
<i>BdorOBP8 (OBP83ef)</i>	CGAAGTCGGCAGCAAAGA	TCGTCCGCATCGAACCAG	2	W
<i>BdorOBP10 (OBP99c)</i>	GTGTCTTCTGCGAACATGAGG	CACTTGTGACCACGATAGGC	2,4,5	W,W,B
Actin (reference gene)	TCCATCATGAAGTGCGACGT	AGAAGCACTTGCGGTGGACGA	-	-

Table 2. Identification of OBP56d expression between white (W) and brown (B) *Ceratitis capitata*,

pupae. This protein was significantly expressed 1.5-fold or higher in white pupae on days 1-8 d old.

White pupal age	Protein score	Ion score	Protein Coverage	Expect Value	Peptide counts	Highest PI for Peptide sequence	W/B ratio	p value
1	407	412	83	2.80E-36	19	167	2.08	0.98311
2	418	425	83	2.20E-37	17	163	2.46	0.99967
3	434	440	66	5.60E-39	17	160	2.12	0.99998
4	372	377	66	8.90E-33	16	152	1.62	0.96434
5	457	464	68	2.80E-41	16	159	2.07	0.99759
6	423	431	81	7.10E-38	18	155	1.54	0.94570
7	439	444	77	1.80E-39	19	170	1.77	0.98539
8	401	406	72	1.10E-35	21	162	1.45	0.99207

Figure 1.

Figure 2.

Figure 3.

Fig. 4.

Fig. 5

Fig. 6

Genetic Sexing Strain

Proteome analysis

Bactrocera dorsalis

Ceratitis capitata