

# Analysis of Differential Proteins in Two Wing-Type Females of *Sogatella furcifera* (Hemiptera: Delphacidae)

Zi-Qiang Liang,<sup>1</sup> Shao-Yun Song,<sup>1</sup> Shi-Ke Liang,<sup>1</sup> and Fang-Hai Wang<sup>1,2</sup>

<sup>1</sup>State Key Laboratory for Biocontrol and Institute of Entomology, Sun Yat-sen University, Guangzhou 510275, People's Republic of China (616661857@qq.com; songshaoyun66@163.com; 1014079026@qq.com; lsswf@mail.sysu.edu.cn) and

<sup>2</sup>Corresponding author, e-mail: lsswf@mail.sysu.edu.cn

Subject Editor: Qili Feng

Received 27 December 2015; Accepted 6 March 2016

## Abstract

*Sogatella furcifera* (Horvath) is an important rice pest with the wing dimorphism, including macropterous and brachypterous morphs. The protein expression profiles in two wing-type adults and two wing-type disc fifth-instar nymphs were analyzed using two-dimensional gel protein electrophoresis and mass spectrometry. In adults and fifth-instar nymphs, 127 and 162 protein spots were detected, respectively. Fifty-five differentially expressed protein spots were identified between the long-winged adults and the short-winged adults, and 62 differentially expressed protein spots were found between the long-winged disc fifth-instar nymphs and short-winged disc fifth-instar nymphs. In long-winged and short-winged adults, six and seven specific protein spots were identified, respectively, with five and seven protein spots having more than threefold increased level, respectively. In long-winged and short-winged disc morph nymphs, 8 and 12 specific protein spots were identified, respectively, with 11 and 17 spots containing more than threefold increased level, respectively. Among the 16 identified proteins, five proteins are associated with muscle function, suggesting that muscle is a main tissue where the genes were differentially expressed between the two wing types. In addition, the content of a peptidase with an insulinase domain was higher (by  $3.02 \pm 0.59$  fold) in the short-winged fifth-instar nymphs than in the long-winged fifth-instar nymphs, which suggests that this peptidase may be involved in wing differentiation by regulating insulin receptors. The results of this study provide some genetic clues for the wing differential development in *S. furcifera* and provide more references for future studies.

**Key words:** *Sogatella furcifera*, wing dimorphism, differential two-dimensional gel electrophoresis, mass spectrometry

*Sogatella furcifera* (Horvath) is one of the rice planthoppers and it sucks rice phloem sap as food. The pest causes poor plant growth and putrescence when it outbreaks. This rice planthopper has a wing dimorphism phenomenon. The macropterous morphs are good at migrating, while the brachypterous morphs have a strong ability to reproduce (Denno et al. 1989, Ayoade et al. 1999). Therefore, the ratio of the wing-type fluctuations has a significant impact on *S. furcifera* population and can help us predict the occurrence and outbreak of the pest.

Differences in the external environmental factors (light, nutritional condition, etc.) can affect *S. furcifera* wing development. Short exposure to light is conducive to the emergence of short-winged individuals, while long time light exposure increases the number of long-winged individuals (Liu et al. 2010). Malnutritional rice plants can directly induce the development of long-winged morphs (Saxena et al. 1981). In addition, in a high population density, the proportion of long-winged *S. furcifera* increases (Matsumura 1996). There are many reports on the wing-type development of *S. furcifera* at molecular level. For example, a wingless gene has

been found to manipulate the wing dimorphism (Yu et al. 2014). Differential DNA methylation is found between the two wing types *S. furcifera* (Zhou et al. 2013). Recently, the wing polyphenism of the rice planthopper is reported to be controlled by two insulin receptors (Xu et al. 2015), which is an excellent explanation for the wing dimorphism. However, fewer researches are carried out to investigate the mechanism of wing dimorphism at protein level and two-dimensional (2D) electrophoresis on *S. furcifera* wing type has not been reported so far.

2D electrophoresis can be used to compare differential protein expression profiles in individuals and cells under different physiological or pathological conditions. Differentially expressed proteins can be identified followed by functional and interaction analysis. 2D electrophoresis has been widely used in insect studies, including neurobiology, immunology, toxicology, etc. (Vierstraete et al. 2003, Baggerman et al. 2005, Shevchenko et al. 2005). For the study of insect wing, the differentiation of the *Drosophila melanogaster* wing disc is explored with this method (Alonso and Santaren 2005). We have compared protein expression profiles between the two

wing-type females of *S. furcifera* by SDS-PAGE electrophoresis and found three significantly differentially expressed protein bands. The protein expression profiles in the two wing types of *S. furcifera* are not the same as indicated by SDS-PAGE. However, SDS-PAGE has a much lower resolution of protein separation than 2D electrophoresis and it is difficult to separate a single protein for further study. In this study, 2D electrophoresis was performed to investigate the protein expression profiles associated with the wing dimorphism.

In rice planthopper, the nymph stage is a critical period of wing determination. Until the fifth-instar nymph, we can clearly observe the long-winged and short-winged disc differentiation under the stereoscope. Two wing morphs of female adults and two wing disc morphs of female fifth-instar nymphs were chosen as research objects, so that a more comprehensive analysis of wing development can be done.

## Materials and Methods

### Insects and Culture Conditions

Insects were collected from the rice field located in the South China Agricultural University, Guangzhou, China. The rearing condition was under a 16:8 (L:D)h photoperiodic regime at  $28 \pm 2^\circ\text{C}$ . The fifth-instar nymphs or adults were collected and cryopreserved. The two-wing phenotypes of female adults and the two-wing phenotypes disc of female fifth-instar nymphs, whose front wing disc extending to the fourth or second abdominal segment, were used as experimental samples.

### Sample Preparation and 2D PAGE

Protein samples were prepared from long-winged adults, short-winged adults, long-winged disc fifth-instar nymphs, and short-winged disc fifth-instar nymphs of female *S. furcifera* (equivalent of ~50 mg). Samples were ground to powder in liquid nitrogen and extracted with lysis buffer (8 M urea, 2 M thiourea, 4% [w/v] CHAPS, 65 mM DTT, and 0.5% [v/v] IPG buffer) for 3 h on ice (Wang et al. 2011). The protein concentration was estimated using Bradford protein assay. Typically ~500  $\mu\text{g}$  of each extract was supplemented to 250  $\mu\text{l}$  with rehydration buffer. Iso-electric focusing was performed using Ettan IPGphor IEF system and 13 cm linear Immobiline DryStrips pH 3–10 for a total of 38.8 kVh. IEF gel strips were placed onto the second dimension 10% SDS-PAGE gels and run until the dye reached the bottom of the gel. Each experiment was repeated at least three times.

### Image Acquisition and Spots Analysis

The gels were scanned with gel imager (GS-800, Bio-Rad). Three biological replicates of 2D gel had a correlation coefficient value above 0.65. For each protein sample, spots were detected and matched automatically and edited manually with PDQuest 2D gel analysis software (version 8.1.0, Bio-Rad). The detected spots presented in all the replicate gels to be accounted. Student's *t*-test was used to determine statistical significance ( $P < 0.05$ ).

### MALDI-TOF/TOF Analysis and Protein Identification

The selected protein spots were washed in  $\text{ddH}_2\text{O}$  and 50 mM  $\text{NH}_4\text{HCO}_3$  for 30 min. each. Both of them were digested overnight at  $37^\circ\text{C}$  with trypsin digesting (in-gel). In-gel digested peptides were analyzed with a MALDI MS/MS mass spectrometer, Autoflex-TOF/TOF (Bruker Daltonics, Bremen, Germany). The peak list was generated with the Flex Analysis (version 3.3, Bruker Daltonics). Peptide mass finger (PMF) printing and MS/MS ion search were

performed with the MASCOT software (version 2.2, Matrix Science). Mascot searches were conducted using from NCBI non-redundant database (released January 2012 or later). The settings chosen for identification were as follows: one missed cleavage site; carbamidomethyl as fixed modification of cysteine; variable modification of oxidation on methionine residue; MS tolerance of 100 ppm and MS/MS tolerance of 0.6 Da; enzyme used as trypsin, and a peptide charge setting as +1. A match protein with the best score was accepted as successful identification. Protein identification was considered to be significant with at least two of the following three criteria: PMF and MS/MS ion searches were statistically significant ( $P < 0.05$ ); sequence coverage  $\geq 13\%$ ; corresponding location of the protein spots.

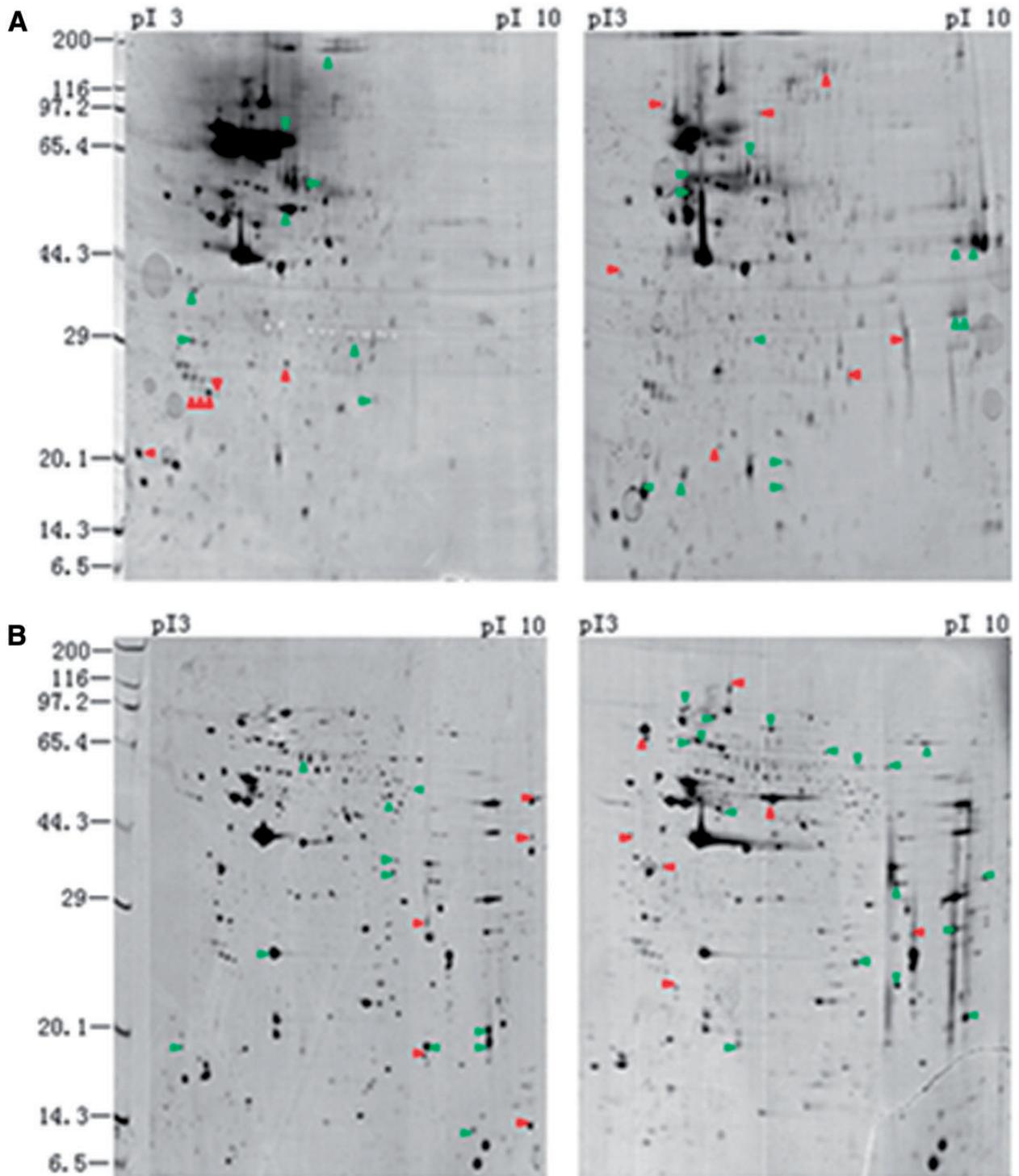
### Bioinformatics Analysis

GI numbers of differential identified proteins by 2D electrophoresis were uploaded to the uniprotKB database (www.uniprot.org) to obtain gene ontology (GO) annotation and functional enrichment analysis using DAVID v6.7 bioinformatics resources. The enrichment analysis was done using Fisher exact test (Zhou et al. 2005). Functional categories with  $P < 0.05$  were considered to be significant after multiple term testing by Bonferroni correction (Forstner et al. 2015). The STRING database was used to construct the protein-protein interactions (PPIs) networks of identified proteins.

## Results

### Protein Modulation in Two Wing-Types Female Adults and Two Wing-Types Disc Female Fifth-Instar Nymphs

After the 2D electrophoresis, protein spots were visualized through Coomassie G-250 staining. The representative 2D protein profiles were shown in Fig. 1. Overall, 127 and 162 protein spots were detected in the profiles of female adults and fifth-instar nymphs using PDQuest 2D analysis software. The numbers of the protein spots that were detected in biological triplicates of 2D gels from long-winged adults, short-winged adults, long-winged disc fifth-instar nymphs, and short-winged disc fifth-instar nymphs were 120, 121, 155, and 157, respectively. The protein responses of long-winged adults and long-winged disc fifth-instar nymphs were compared with those of short-winged adults and short-winged disc fifth-instar nymphs. The numbers of significant differentially expressed protein spots ( $P < 0.05$ ) in adults and fifth-instar nymphs were 55 (22 [40%] in the long-winged adults and 33 [60%] in the short-winged adults) and 62 (25 [40.32%] in the long-winged disc fifth-instar nymphs and 37 [59.68%] in short-winged disc fifth-instar nymphs), respectively. Depending on the content of these spots, we divided them into three groups: those whose abundance increased by one- to twofold, those whose abundance increased by two- to threefold and those whose abundance increased by more than threefold (Fig. 2). Furthermore, the long-winged adults and long-winged disc fifth-instar nymphs had six and five specific protein spots, respectively, while the short-winged adults and short-winged disc fifth-instar nymphs both had seven specific protein spots. Twenty-five spots from specific proteins and 48 spots that showed more than threefold increase in abundance were cut from the gels for mass spectrometric analysis. Finally, 20 protein spots were identified (Table 1). The spots from long-winged adults or long-winged disc fifth-instar nymphs had a much higher success rate of identification than those short-winged adults or short-winged disc fifth-instar nymphs. Myosin light chain (MLC) and Myosin



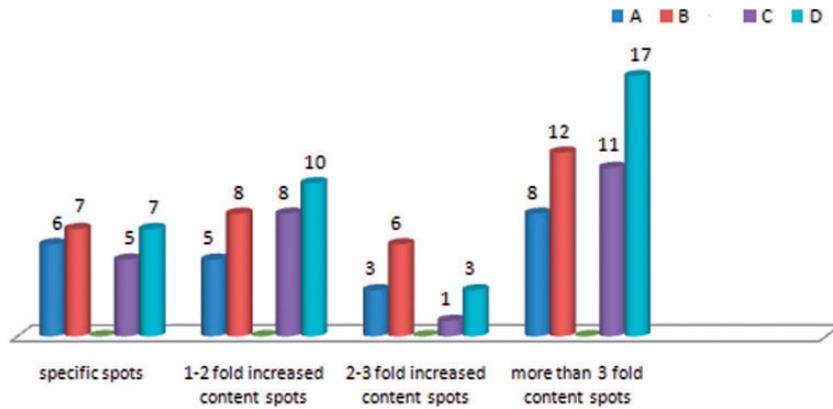
**Fig. 1.** Representative 2D profiles of female *S. furcifera*. (A) Long-winged adult (left) compared with short-winged adult. (B) Long-winged disc fifth-instar nymph (left) compared with short-winged disc fifth-instar nymph. Specific proteins are marked by red arrow. The proteins having more than triple content increasing are marked by green arrow. Molecular masses of marker proteins and pI are mentioned on left-hand side and at the top, respectively.

RLC2 (MRLC2) were both identified in three spots, respectively, and other proteins were identified in one spots.

#### Functional Annotation Clustering

Based on sequence similarities to *Drosophila* protein sequences, 16 identified proteins were used for GO analysis. These proteins were categorized into molecular functions (2 proteins), cellular

component (2 proteins), and biological processes (12 proteins), suggesting that proteins involved in biological processes had a great influence on wing dimorphism. Using the functional annotation clustering tool from DAVID v6.7 bioinformatics resources, the 16 identified proteins (five of them were not clustered) were divided into four clusters: muscle, phosphorylation, organelle lumen, and ion binding. The enrichment scores of four clusters were 3.27, 1.12,



**Fig. 2.** Content classification of significant ( $P < 0.05$ ) differentially expressed proteins. (A) Long-winged adult. (B) Short-winged adult. (C) Long-winged disc fifth-instar nymph. (D) Short-winged disc fifth-instar nymph.

**Table 1.** Identified proteins from female adults and fifth-instar nymphs of *S. furcifera*

Identified protein	Expression level (fold change with SE values)	Function	<i>t</i>	df	<i>P</i>	Categories <sup>a</sup>
<b>Adult</b>						
Myosin RLC2 (MRLC2) #	$\infty^b$	Somatic muscle development				B
	$3.67 \pm 0.58^b$		4.64	2	0.044	
	$4.10 \pm 0.27^b$		5.82	2	0.028	
Myosin light chain (MLC) #	$\infty^b$	Muscle myosin complex				C
	$3.44 \pm 0.26^b$		9.31	2	0.011	
	$3.39 \pm 0.32^b$		7.47	2	0.017	
ATP synthase delta chain (ATP5DC)	$3.81 \pm 0.53^b$	ATP synthesis coupled proton transport	5.31	2	0.034	B
Ferritin subunit (Fer5)	$3.34 \pm 0.17^b$	Cellular iron ion homeostasis	13.86	2	0.005	B
14-3-3 Protein zeta isoform X1 (14-3-3zeta)	$3.91 \pm 0.67^b$	Activation of tryptophan 5-monooxygenase activity	4.35	2	0.049	B
Glutathione Transferase (GluT)	$3.12 \pm 0.47^c$	Glutathione metabolic process	4.57	2	0.045	B
Tropomyosin 2 (Tm2)	$3.27 \pm 0.12^c$	Muscle thin filament tropomyosin	18.25	2	0.030	C
<b>Fifth-instar nymph</b>						
Flightin (Fln)	$\infty^b$	Flight				B
Pupal cuticle protein C1 (PCPC1)	$3.32 \pm 0.51^b$	Structural constituent of chitin-based cuticle	4.56	2	0.046	M
Elongation factor 1-alpha (EF1A)	$4.01 \pm 0.21^b$	Translation	14.10	2	0.005	B
Carboxylesterase (Car)	$3.89 \pm 0.66^b$	Carboxylic ester hydrolase activity	4.41	2	0.048	M
Electron transfer flavoprotein subunit alpha (ETFSA)	$3.56 \pm 0.58^b$	Ectodermal digestive tract morphogenesis	4.37	2	0.049	B
Transketolase (Tra)	$3.35 \pm 0.29^b$	Cytoplasmic microtubule organization	0.14	2	0.015	B
Imaginal disc growth factor (IDGF)	$4.11 \pm 0.42^b$	Carbohydrate metabolic processes	7.36	2	0.018	B
Actin-depolymerizing factor 1 (ADF1)	$4.51 \pm 0.42^c$	Actin filament depolymerization	8.38	2	0.014	B
Peptidase from Family M16 (Pep)	$3.02 \pm 0.59^c$	Peptidase	4.30	2	0.049	B

#, the identified proteins are present in more than one spot;  $\infty$ , specific proteins.

<sup>a</sup>Categories: molecular functions (M); biological processes (B); and cellular component (C).

<sup>b</sup>A higher content of protein in long-winged adults or long-winged disc fifth-instar nymphs.

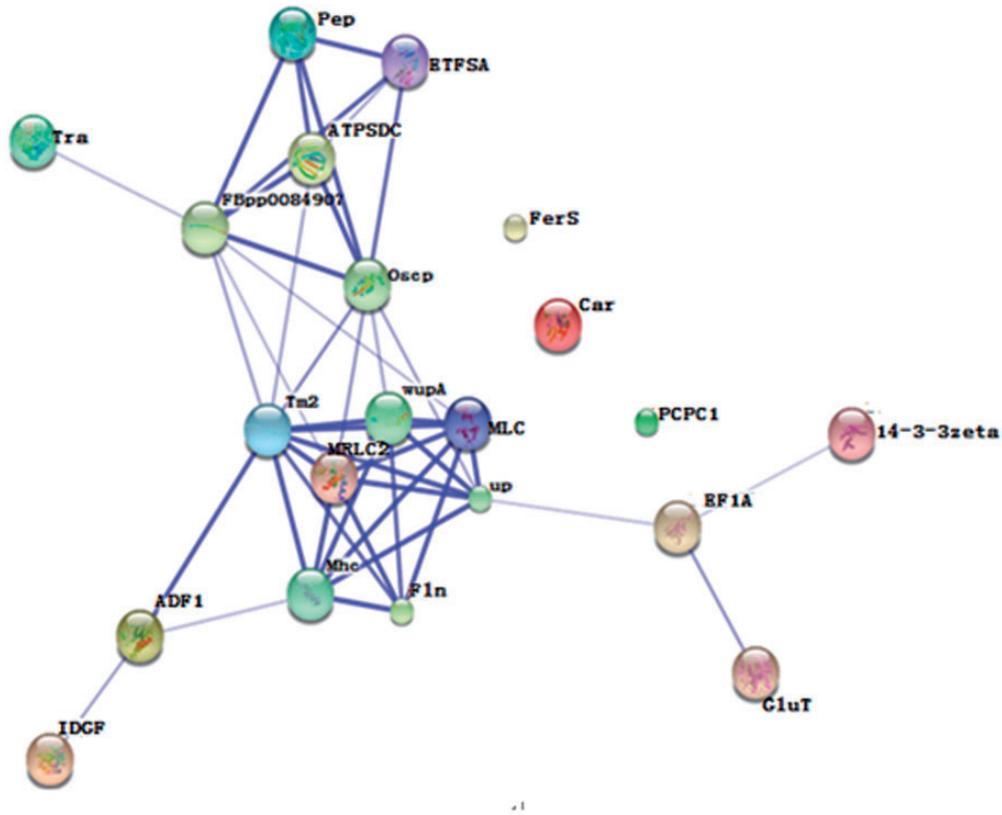
<sup>c</sup>A higher content of protein in short-winged adults or short-winged disc fifth-instar nymphs.

0.43, and 0.35, respectively. In addition, peptidase from Family M16 (Pep) was gathered in three clusters: phosphorylation, organelle lumen, and ion binding. MLC and MRLC2 were clustered into muscle and organelle lumen. Importantly, the contents of MLC and MRLC2 in the long-winged adults were three times higher than that in the short-winged adults. Myosin's main function is in muscle contraction and is mainly divided into two categories, muscle myosin and nonmuscle myosin. Flying movement requires the support of

formidable muscle tissue (Huxley and Niedergerke 1954). Thus, the different content of the two myosin light chains may come from muscle myosin.

### Network Analysis

First, 16 identified proteins were uploaded to String database (<http://string-db.org/>). A functional interaction network of the 21 proteins (five were predicted functional partners), with 42 PPIs based on

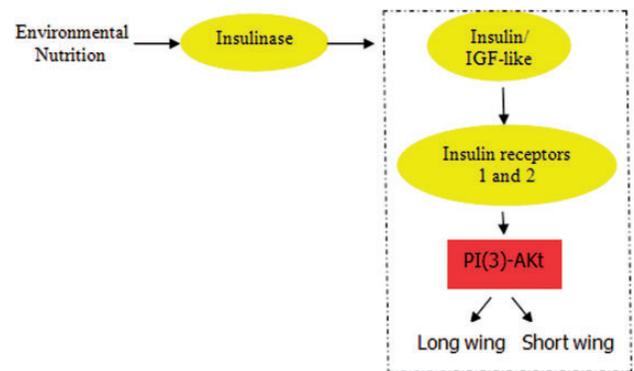


**Fig. 3.** PPIs network of identified and predicted proteins. Stronger associations are represented by thicker lines. Five predicted partners: ATP synthase gamma subunit (FBpp0084907), ATP synthase OSCP (Oscp), Upheld (up), troponin (wupA), and myosin heavy chain (Mhc).

*Drosophila* database, was constructed (Fig. 3). The network had two strong connection parts; one part contained eight proteins associated with muscle and another part contained five proteins involving in ATP synthase. The two parts linked together through ATP synthase gamma subunit and ATP synthase OSCP. Myosin movement required a lot of energy from ATP hydroxylation, which could explain the higher content of ATPSDC, OSCP, and MLC in long-winged adults.

**Discussion**

This study analyzed *S. furcifera* differential proteins in two wing morphs of female adult and two wing disc morphs of female fifth-instar nymph, using 2D electrophoresis for the first time. In general, the number of protein spots detected in adults was less than that in fifth-instar nymphs, and the contents of significant differentially proteins were generally higher in short-winged adults or short-winged disc fifth-instar nymphs compared with long-winged adults or long-winged disc fifth-instar nymphs. One of the reasons for this may be the storage proteins (SPs), which are usually higher in fifth-instar nymphs than in adults. In the last instar nymph, the concentration of SPs is at its peak, after which SPs are consumed during development and reproduction (Levenbook et al. 1985). Thus, more protein spots could be detected in fifth-instar nymphs. The SP Calliphorin was injected into the body of *Calliphora vicina* with a radioactive marker and degradation product (phenylalanine) was detected to be involved in the formation of the flight myosin (Levenbook and Bauer 1984). The decrease of significant differential



**Fig. 4.** A possible regulating mechanism of wing dimorphism in *S. furcifera*. IIS pathway is inside the dotted box. Regulatory proteins or factors are marked by yellow oval. Signaling cascades are marked by red rectangle.

protein content in long-winged adults or long-winged disc fifth-instar nymphs seems to derive from flight myosin.

The insect wing dimorphism is represented not only by the wing shape, but also by the muscle function (Braendle et al. 2006). In the long-winged and short-winged *Nilaparvata lugens*, some differential expressed genes were found to be associated with muscle formation, for example flightin, troponin C4, titin, and myosin heavy chain (Xue et al. 2010). In the 2D profiles of two wing-types adults and two wing-types disc fifth-instar nymphs of female *S. furcifera*, we found several differential proteins associated with muscle function, including Fln, MRLC2, MMLC, actin-depolymerizing factor 1

(ADF1), and Tropomyosin 2. The interaction of flightin with myosin has a pivotal role in flight muscle activation (Vigoreaux 2001, Ayer and Vigoreaux 2003), long-winged adults have flight capacity, and short-winged adults do not; thus, there are lower levels of flightin, MRLC2, and MLC in short-winged adults than in long-winged adults. Tm can inhibit actin by activating the  $Mg^{2+}$ -ATPase activity of myosin (Liu and Bretscher 1989); therefore, Tropomyosin 2 should have a higher expression in short-winged adults than in long-winged adults with flight capacity. Moreover, the expression of imaginal disc growth factor (IDGF) in long-winged disc fifth-instar nymphs was  $4.11 \pm 0.42$  times higher than in short-winged disc fifth-instar nymphs. IDGF can affect proliferation of wing disc cell and increase its movement ability (Kawamura et al. 1999). At the same time, ADF1 have a  $4.51 \pm 0.42$  fold increasing in short-winged disc fifth-instar nymphs. ADF can depolymerize microfilaments. We speculate that both IDGF and ADF1 might be involved in the wing dimorphism. In a future investigation, the detailed muscle composition of the two wing morphs of *S. furcifera* should be determined, which would help us to understand the long distance flying mechanism of the insects and muscle differences of insects with wing dimorphism.

Population density and nutritional quality of host plants are generally considered to be related to wing dimorphism (Kisimoto 1956, Saxena et al. 1981, Iwanaga et al. 1985, Syobu et al. 1986). The insulin/insulin-like growth factor signaling pathway (IIS pathway) is one of the nutritional pathways thought to be involved in wing dimorphism. Recently, the wing type of rice planthopper was confirmed to be regulated by IIS pathway, which has two insulin receptors to control wing dimorphism (Xu et al. 2015). We have found that Pep (Family M16), containing an insulinase domain, has a  $3.02 \pm 0.59$  fold increasing protein content in short-winged disc fifth-instar nymphs. We hypothesized that the concentration of insulinase in *S. furcifera* nymphs increases substantially and remains at a high level under an adequate nutrition. This insulinase affects the degradation of insulin or insulin/insulin-like growth factor, which regulates the IIS pathway and PI (3) K-Akt signaling cascade to control wing dimorphism, leading to the appearance of short-winged adults. A regulating mechanism of wing polyphenism is shown in Fig. 4. In future studies, we will clone insulinase gene and analyze its exact role in wing dimorphism.

Due to the lack of *S. furcifera* proteome and genome databases, although the successful rate of protein identification is only 21.92% we still identified some proteins that are related with the wing dimorphism. These proteins provide a reference for understanding the mechanism of wing dimorphism in planthopper. The genome (Xue et al. 2014) and transcriptome (Xue et al. 2010) of *N. lugens* have been published. These achievements can accelerate the establishment of various databases of rice planthopper and helpful to protein research in *S. furcifera*.

## Acknowledgments

This research was supported by the National Natural Science Foundation of China (31171844) and Natural Science Foundation of Guangdong Province (2015A030313114).

## References Cited

- Alonso, J., and J. F. Santaren. 2005. Proteomic analysis of the wing imaginal discs of *Drosophila melanogaster*. *Proteomics* 5: 474–489.
- Ayer, G., and J. O. Vigoreaux. 2003. Flightin is a myosin rod binding protein. *Cell Biochem. Biophys.* 38: 41–54.
- Ayoade, O., D. Morooka, and S. Tojo. 1999. Enhancement of short wing formation and ovarian growth in the genetically defined macropterous strain of the brown planthopper, *Nilaparvata lugens*. *J. Insect Physiol.* 45: 93–100.
- Baggerman, G., K. Boonen, P. Verleyen, A. De Loof, and L. Schoofs. 2005. Peptidomic analysis of the larval *Drosophila melanogaster* central nervous system by two-dimensional capillary liquid chromatography quadrupole time-of-flight mass spectrometry. *J. Mass Spectrom.* 40: 250–260.
- Denno, R. F., K. L. Olmstead, and E. S. McCloud. 1989. Reproductive cost of flight capability—a comparison of life-history traits in wing dimorphic planthoppers. *Ecol. Entomol.* 14: 31–44.
- Forstner, A. J., A. Hofmann, A. Maaser, S. Sumer, S. Khudayberdiev, T. W. Mühleisen, M. Leber, T. G. Schulze, J. Strohmaier, F. Degenhardt, et al. 2015. Genome-wide analysis implicates microRNAs and their target genes in the development of bipolar disorder. *Transl. Psychiatry.* 5: e678.
- Huxley, A. F., and R. Niedergerke. 1954. Structural changes in muscle during contraction: interference microscopy of living muscle fibres. *Nature* 173: 971–973.
- Iwanaga, K., S. Tojo, and T. Nagata. 1985. Immigration of the brown planthopper, *Nilaparvata lugens*, exhibiting various responses to density in relation to wing morphism. *Entomol. Exp. Appl.* 38: 101–108.
- Kawamura, K., T. Shibata, O. Saget, D. Peel, and P. J. Bryant. 1999. A new family of growth factors produced by the fat body and active on *Drosophila* imaginal disc cells. *Development* 126: 211–219.
- Kisimoto, R. 1956. Effect of crowding during the larval period on the determination of the wing-form of an adult planthopper. *Nature* 178: 641–642.
- Levenbook, L., and A. C. Bauer. 1984. Fate of the larval storage protein calliphorin during adult development of *Calliphora vicina*. *Insect Biochem.* 14: 77–86.
- Levenbook, L., G. A. Kerkut, and L. I. Gilbert. 1985. Comprehensive insect physiology. In G. A. Kerkut and L. I. Gilbert (eds.), *Biochemistry and pharmacology*. Pergamon Press, UK.
- Liu, H., and A. Bretscher. 1989. Disruption of the single tropomyosin gene in yeast results in the disappearance of actin cables from the cytoskeleton. *Cell* 57: 233–242.
- Liu, J. N., F. T. Gui, and Z. Y. Li. 2010. Factors of influencing the development of wing dimorphism in the rice white-backed planthopper, *Sogatella furcifera* Horvath. *J. Plant Protect.* 37: 511–516.
- Matsumura, M. 1996. Genetic analysis of a threshold trait: Density-dependent wing dimorphism in *Sogatella furcifera* (Horvath) (Hemiptera: Delphacidae), the white-backed planthopper. *Heredity* 76: 229–237.
- Saxena, R. C., S. H. Okech, and N. J. Liquido. 1981. Wing morphism in the brown planthopper, *Nilaparvata lugens*. *Int. J. Trop. Insect Sci.* 1: 343–348.
- Shevchenko, A., M. M. de Sousa, and P. Waridel. 2005. Sequence similarity-based proteomics in insects: characterization of the larvae venom of the Brazilian moth *Cerodirphia speciosa*. *J. Proteome Res.* 4: 862–869.
- Syobu, S., H. Mikuriya, J. Yamaguchi, M. Matsuzaki, and M. Matsumura. 1986. Fluctuations dimorphism and oocyte development in the brown planthopper, *Nilaparvata lugens*. *Insect Physiol.* 32: 585–590.
- Vigoreaux, J. O. 2001. Genetics of the *Drosophila* flighty muscle myofibril: a window into the biology of complex systems. *Bioessays* 23: 1047–1063.
- Vierstraete, E., A. Cerstiaens, G. Baggerman, G. Van den Bergh, A. De Loof, and L. Schoofs. 2003. Proteomics in *Drosophila melanogaster*: first 2D database of larval hemolymph proteins. *Biochem. Biophys. Res. Commun.* 304: 831–838.
- Wang, G. B., Y.-H. Chen, J.-M. Wang, Z.-G. Wei, Y.-X. Xu, B. Li, and W.-D. Shen. 2011. Analysis of proteins in the antennae of *Bombyx mori* moths by two-dimensional electrophoresis. *Acta Entomol. Sin.* 54: 589–595.
- Xu, H. J., J. Xue, B. Lu, X. C. Zhang, J. C. Zhuo, S. F. He, X. F. Ma, Y. Q. Jiang, H. W. Fan, J. Y. Xu, et al. 2015. Two insulin receptors determine alternative wing morphs in planthoppers. *Nature* 519: 464–467.
- Xue, J., Y. Y. Bao, B. L. Li, Y.-B. Cheng, Z.-Y. Peng, H. Liu, H.-J. Xu, Z.-R. Zhu, Y.-G. Lou, J.-A. Cheng, et al. 2010. Transcriptome analysis of the brown planthopper *Nilaparvata lugens*. *PLoS One* 5: 1–11.
- Xue, J., X. Zhou, C. X. Zhang, L. L. Yu, H. W. Fan, Z. Wang, H. J. Xu, Y. Xi, Z. R. Zhu, W. W. Zhou, et al. 2014. Genomes of the rice-pest brown

- planthopper and its endosymbionts reveal complex complementary contributions for host adaptation. *Genome Biol.* 15: 521.
- Yu, J. L., Z. F. An, and X. D. Liu. 2014. Wingless gene cloning and its role in manipulating the wing dimorphism in the white-backed planthopper, *Sogatella furcifera*. *BMC Mol. Biol.* 15: 20.
- Zhou, F., Y. Xue, H. Lu, G. Chen, and X. Yao. 2005. A genome-wide analysis of sumoylation-related biological processes and functions in human nucleus. *FEBS Lett.* 579: 3369–3375.
- Zhou, X., J. Chen, M. S. Zhang, and F. Wang. 2013. Differential DNA methylation between two wing phenotypes adults of *Sogatella furcifera*. *Genesis* 51: 819–826.