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Research paper

A small set of differentially expressed genes was associated with two color morphs in natural populations of the pea aphid *Acyrthosiphon pisum*

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ABSTRACT

Color polymorphism is an ecologically important trait, which is related to local adaptation and ecological speciation. The pea aphid *Acyrthosiphon pisum* shows color polymorphism: the red and green color morphs where differences in ecological adaptation have been observed. Here, we measured genome-wide gene expression profiles of two color morphs in natural populations of *A. pisum* to explore the genetic basis of differentiated ecological adaptation. The results showed that only 32 genes were significantly differentially expressed between the two morphs, of which 18 had functional annotations. Among them, 13 genes were up-regulated [e.g. genes encoding protoheme IX farnesyltransferase (LOC100570971), carotene dehydrogenase (*tor*) and V-type proton ATPase subunit B (LOC100169462)] and 5 genes were down-regulated in the red morph (e.g. genes encoding transcription factors and heat shock proteins). To assess the functional importance of these differentially expressed genes (DEGs), we selected three highly expressed DEGs (LOC100169462, LOC100570971 and *tor*) with functional annotations and analyzed their expression levels in the red morph under three low temperatures (1 °C, 4 °C, and 8 °C) for 24 h. These three DEGs showed an interesting expression response to the cold acclimating conditions which resulted in an obvious phenotypic change of the red individuals to be greenish variants. This study suggests a link between gene expressions and body color polymorphisms in the pea aphid and provides important clues for further studying molecular mechanisms of ecological adaptation in aphids.

1. Introduction

Body colors are ubiquitous in animals, even some appearing with color polymorphisms, which continue to be of interest to evolutionary biologists (McKinnon and Pierotti, 2010; Wellenreuther et al., 2014). Color polymorphism is an ecologically important trait, which is generally related to species recognition, sexual selection, mimicry, aposematism and crypsis (Ruxton et al., 2004; Wellenreuther et al., 2014; Keren-Rotem et al., 2016). As an important life strategy used by various species, color polymorphism is also widespread in insects (McKinnon and Pierotti, 2010; Tanaka et al., 2016; Yin et al., 2016). The pea aphid (PA), *Acyrthosiphon pisum* (Hemiptera: Aphididae), characterized by the complex life cycle that includes for example: two types of reproduction (sexual and asexual), polymorphism, and symbiotic relationship with bacteria, provides an ideal ecological model to understand the mechanism and process of local adaptation and speciation (Consortium, 2010; Moran and Jarvik, 2010; Tsuchida et al., 2010; Tsuchida, 2016). This aphid displays two body color morphs (red and green) in field populations and each color morph is stable within each parthenogenetic clone (Tsuchida et al., 2010; Valmalette et al., 2012).

Previous studies have indicated divergent adaptation between these two color morphs (Tsuchida et al., 2010): the red morph on green plants tends to be preyed by ladybird beetles (Losey et al., 1997), whereas the green morph is preferentially attacked by parasitoid wasps (Libbrecht et al., 2007). Under divergent selection pressures, a differential population density has been observed between the two morphs in the field.

Abbreviations: DEGs, differentially expressed genes; HSPs, heat shock proteins; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MRSA, methicillin resistant *Staphylococcus aureus*; nr, NCBI non-redundant protein sequences; PARF, the four biological replicates of the red color morphs of the pea aphid; PAGF, the four biological replicates of the green color morphs of the pea aphid; qRT-PCR, quantitative real-time PCR; *tor*, carotene dehydrogenase

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The red morph exhibits the higher density under some diverse conditions (such as low quality of host plant, crowded condition, and natural enemy attack) (Kugler and Ratcliffe, 1983; Braendle and Weisser, 2001; Ahsaei et al., 2013).

As an important insect pest on many crops and forages, the pea aphid not only causes serious alfalfa losses, but also is the vector of many plant viruses (He and Zhang, 2006; Emden and Harrington, 2007). In Northwest China, this aphid is one of the most important pests in alfalfa (*Medicago sativa*) fields (He and Zhang, 2006; Wu, 2011). Recently, the red morph shows a significant trend of population expansion from west to east of China and its population density has extensively increased over the green morph in alfalfa fields of the Western China (Wu, 2011).

Body coloration in aphids results from pigments in cuticle cells, internal organs, tissues, hemolymph, and bacteriocytes (Shamim et al., 2014; Tsuchida, 2016). The major pigments in aphids belong to three categories: melanins, aphins and carotenoids (Tsuchida, 2016). Among them, melanins are the most widespread pigment in nature and also affect sclerotization and melanization of aphids (True et al., 1999; Wittkopp et al., 2003; Y. Zhang et al., 2016). Aphins have been detected in all known aphid species (Brown, 1975), and they are a variety of polycyclic quinone pigments in aphid's hemolymph (Bowie et al., 1966). Aphins exhibit various colors, from light yellow through orange and red to deep blue-green (Tsuchida, 2016). The pea aphid exhibits the higher amounts of green pigments, presumed to be aphins, in the green form than in its red form (Tsuchida et al., 2010).

Carotenoid pigments are responsible for many of red, orange and yellow colors, and are synthesized nearly exclusively by plants, bacteria and fungi, whereas animals acquire carotenoids almost entirely through their diets (Walsh et al., 2012; Toews et al., 2017). Strikingly, the pea aphid possesses several carotenoid related genes (such as carotene dehydrogenase: *tor*) and can endogenously synthesize carotenoids, like in a manner similar to the two-spotted spider mite *Tetranychus urticae* (Moran and Jarvik, 2010; Bryon et al., 2017). Carotenoid levels were shown to vary among the different color forms in the pea aphid (Tsuchida et al., 2010; Tsuchida, 2016). For example, the green clones contain mostly α-carotene, β-carotene and γ-carotene, whereas red forms comprise of torulene and dehydro-γ, Ψ-carotene (Moran and Jarvik, 2010).

In addition to genetic factors, body coloration of the pea aphid can be influenced by various biotic and abiotic environmental factors. Some environmental factors could lead to the shift between the two color morphs, such as ambient temperatures, light conditions and infections with symbiotic bacteria (Abdi, 2007; Valmalette et al., 2012; Tsuchida, 2016). A hypothesis of an epigenetic regulation in color polymorphisms (such as gene expression and DNA methylation) has been strongly suggested by several previous studies (Tsuchida et al., 2010; Valmalette et al., 2012; Tsuchida, 2016). However, the identification of more genes involved in pigment metabolism and measuring their expressions are very challenging (Lópezmaury et al., 2008; Pardodiaz et al., 2015; Byers et al., 2016; Ishikawa et al., 2017; Kim et al., 2017; Mack and Nachman, 2017).

The release of the genome sequence of the pea aphid provides a good opportunity to explore gene expression differences between the red and green morphs (Consortium, 2010). In the present study, we measured the genome-wide expression profiles of the red and green morphs of the pea aphid, collected from the same plant in an alfalfa field. Our results showed that 32 genes were significantly differently expressed between the two morphs, suggesting that they may contribute to divergent colors and susceptibility to the adversity of the aphids.

Valmalette et al. (2012) found that red individuals of the pea aphid can switch to be greenish variants under a sustained low temperature (8 °C). However, its singular pigmentation faded away when it was placed back in an optimal temperature condition (Valmalette et al., 2012). Therefore, we have assessed the importance of these significantly differentially expressed genes (DEGs) by measuring the expression changes of three highly up-regulated genes (LOC100169462, LOC100570971 and *tor*) in the red morph which was exposed to three low temperatures (1 °C, 4 °C, and 8 °C). Our results highlighted the importance of expression changes of these three DEGs in response to cold conditions. This study provides foundational information to further study on the genetic basis and molecular mechanism of body color polymorphism in the pea aphid.

2. Materials and methods

2.1. Sample collection

Adult pea aphids were sampled in the field from the same plant of alfalfa (*M. sativa*) in Yuzhong County (104°9′N, 35°56′E, 1720 m above sea level), Lanzhou City, China, in June 2016. Samples of red (PAR) and green (PAG) morphs with a visually similar body size were collected from the same plant to assume they belong to a clone of the pea aphid. All samples were immediately frozen in liquid nitrogen and transferred to −80 °C until RNA extraction. Four biological replicates (PARF: the four biological replicates of the red morph of the pea aphid and PAGF: the four biological replicates of the green morph of the pea aphid) were used for each color morph, with approximately 20 individuals for each replicate.

2.2. RNA sequencing

Total RNA of all samples was extracted using the Trizol Reagent (Ambion, USA) according to the manufacturer's instructions. The RNA concentration was measured with a Nanodrop 1000 spectrophotometer (Thermo Scientific, USA), and the RNA integrity was assessed using an RNA Nano 6000 Assay Kit with an Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA). Library construction and RNA sequencing on BGISEQ-500 were conducted at Beijing Genomics Institute (BGI). Genomic DNA was removed with two digestions using Amplification grade DNAse I (Invitrogen, USA). The RNA was sheared and reverse transcribed using random primers to obtain cDNA, which was used for library construction. The library quality was determined by using Bioanalyzer 2100 (Agilent Technologies, CA, USA). Then, the library was used for sequencing with the sequencing platform BGISEQ-500 (BGI, Shenzhen, China).

All the generated raw sequencing reads were filtered, by removing reads with adaptors, reads with more than 10% of unknown bases, and low quality reads. Clean reads were then obtained and stored as FASTQ format. HISAT (Kim et al., 2015) was used to map clean reads to the pea aphid genome (AphidBase Official Gene Set v2, http://www.aphidbase.com/). Gene expression levels were quantified by RSEM (Li and Dewey, 2011). The NOISeq method (Tarazona et al., 2011) was used to detect DEGs between the red and green morphs. Genes were considered as significantly differentially expressed if its fold change (M) ≥ 1 and diverge probability (P) ≥ 0.8. Functions of protein-coding genes were assigned according to the best match derived from alignments to proteins in nr (NCBI non-redundant protein sequences) database. Gene Ontology (GO) annotation was performed for all identified DEGs and the WEGO software (Ye et al., 2006) was used to conduct the GO functional classification. GO terms with a P value (≤ 0.05) corrected by Bonferroni (Abdi, 2007) were defined as significantly enriched GO terms in DEGs. Pathway enrichment analysis of DEGs was performed based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa et al., 2008).

2.3. Quantitative real-time PCR validation

To validate the reliability of the RNA-Seq data, qRT-PCR was performed on 18 DEGs with nr annotation, as previously described (Zhang et al., 2017). The total RNA (1 μg) extracted for RNA sequencing was