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Horizontally transferred fungal carotenoid genes in the two-spotted spider mite *Tetranychus urticae*

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Carotenoids are organic pigments commonly synthesized by plants, algae and some microorganisms. Through absorption of light energy, carotenoids facilitate photosynthesis and provide protection against photo-oxidation. While it was presumed that all carotenoids in animals were sequestered from their diets, aphids were recently shown to harbour genomic copies of functional carotenoid biosynthesis genes that were acquired via horizontal gene transfer from fungi. Our search of available animal transcripts revealed the presence of two related genes in the two-spotted spider mite *Tetranychus urticae*. Phylogenetic analyses suggest that the *T. urticae* genes were transferred from fungi into the spider mite genome, probably in a similar manner as recently suggested for aphids. The genes are expressed in both green and red morphs, with red morphs exhibiting higher levels of gene expression. Additionally, there appear to be changes in the expression of these genes during diapause. As carotenoids are associated with diapause induction in these animals, our results add to recent findings highlighting the importance of eukaryotic horizontal gene transfer in the ecology and evolution of higher animals.

Keywords: *Acyrthosiphon pisum*; carotene; lateral gene transfer; mites; pigments

1. INTRODUCTION

Plants and algae, as well as several bacteria and fungi, can synthesize carotenoids. These pigments serve important functions, including absorption of light energy transferred to chlorophyll and protection from photo damage by reactive oxygen species [1]. In many animals, these pigments underlie important ecological processes, including vertebrate night vision and crustacean, insect and bird coloration [2]. Until recently, it was assumed that animals were unable to produce their own carotenoids and that instead they sequestered carotenoids from their diet. Recently, however, Moran & Jarvik [3] showed that pea aphids (*Acyrthosiphon pisum*) are capable of synthesizing carotenoids. Carotenoid synthesis in aphids is feasible owing to horizontal transfer of biosynthesis genes from fungi into the insect genome. Both pea aphids and green peach aphids (*Myzus persicae*) harbour horizontally transferred genes encoding carotenoid desaturases and carotenoid cyclase–carotenoid synthase. These pigments underlie pea aphids' red and green body colour polymorphism, which affects interactions with aphid natural enemies, including beetles and wasps [3,4].

The two-spotted spider mite *Tetranychus urticae* (Acari, Tetranychidae) is a widespread agricultural pest that infests over 120 plant families [5]. Spider mites harbour a variety of carotenoids, some of which underlie the characteristic bright red colour of many mite species [6,7]. In *T. urticae*, there are green and red colour morphs as well as colour polymorphism within morphs; these differences are developmentally regulated [7]. Green morphs, for example, are typically faintly yellowish, with two dark spots on the abdomen, but diapausing females are more orange. This pigment change is associated with a more than twofold increase in ketocarotenoid content [7], and β-carotene and its derivative vitamin A are thought to function as the photoreceptor pigments for the photoperiodic induction of diapause [8].

Given the importance of carotenoids in the ecology of many animals, we explored available sequence data to determine if carotenoid synthesis genes similar to those in aphids were present in other animals. We provide evidence that the spider mite *T. urticae* harbours at least one carotenoid desaturase and one carotenoid cyclase–carotenoid synthase gene of fungal origin. Both genes are more highly expressed in red than in green spider mites, and exhibit changes in expression during diapause. This second case of acquisition of functional fungal carotenoid biosynthetic machinery by animals highlights the ecological importance of horizontal gene transfer in animals.

2. MATERIAL AND METHODS

Carotenoid biosynthesis genes in *T. urticae* were detected (tBlastx) using aphid carotenoid desaturase (XM_001950729.1, XM_001946654.1 and XM_001943190.1) and carotenoid cyclase–carotenoid synthase (XM_001943135.1, XM_001950833.1 and XM_001950752.1) transcripts to screen mRNA sequences available at NCBI (www.ncbi.nlm.nih.gov, May 2010). We combined overlapping cDNA sequences of the *T. urticae* carotenoid desaturase gene and the carotenoid cyclase–carotenoid synthase gene into contigs (see the electronic supplementary materials for detailed methods and accession information). We used the deduced protein sequences, along with homologous sequences of other organisms (electronic supplementary material, table S2), aligned with blosum62 (http://mafft.cbrc.jp/alignment/server/index.html), to construct both Bayesian and maximum-likelihood phylogenies. To verify the presence of these genes within the spider mite genome, we assembled paired sequence reads downloaded from NCBI's sequence read archive (SRA; project ID 32849).

We conducted expression studies of both genes across several life stages. The two-spotted spider mite has two predominant colour morphs, red and green; the red morph is sometimes referred to as a separate species, *T. cinnabarinus*, though molecular phylogenetic analyses suggest that this species delineation is inaccurate [9,10]. Individual leaves from *Rosa* and *Musa* spp. were collected from plants harbouring spider mites in Atlanta, GA, USA. Spider mite species identification was verified through sequencing portions of cytochrome oxidase I and of the internal transcribed spacer region of nuclear ribosomal DNA (see the electronic supplementary material for details). After rearing mites for several generations under controlled laboratory conditions (28°C, 12 h photoperiod) on *Vicia faba*, we collected samples of red and green non-diapausing mites (three red and four green samples, 100 pooled individuals each). To induce diapause, we reared 20 adult egg-laying females on *V. faba* at 28°C (12 h photoperiod) for 3 days and then changed

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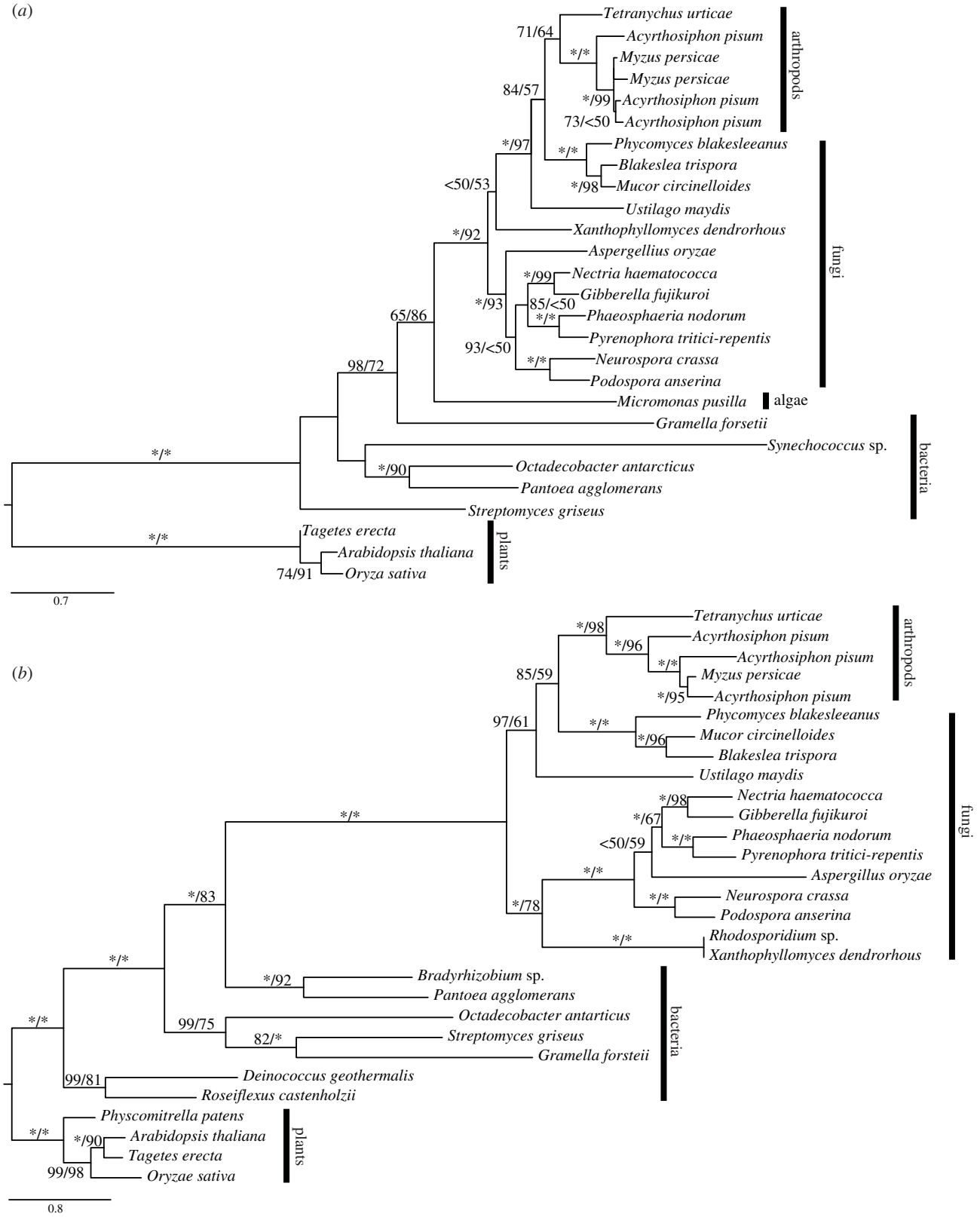

Figure 1. Maximum-likelihood protein phylogenies of carotenoid biosynthesis enzymes. (*a*) Carotenoid desaturases and (*b*) fused carotenoid cyclase–carotenoid synthases. Branches with support values greater than 50 are labelled with Bayesian posterior probabilities followed by maximum-likelihood bootstrap support values (asterisks denote a value of 100). Scale bars represent replacements per site.

the temperature to 18°C with a 10 h photoperiod. We collected pooled samples of 100 red and green spider mites at 10, 15 and 28 days into diapause (two samples per time point per colour morph; electronic supplementary material, figure S2).

Expression studies used the ΔΔCT method on an Applied Biosystems Step One Plus. For relative quantification analysis, three separate reactions per sample (three technical replicates) were run for each primer pair and the comparative threshold cycle (Ct) was averaged. The ΔCt values for both genes of interest were generated by subtracting the average Ct for the endogenous control gene (ef1α) from the average Ct for each target gene. We then standardized each ΔCt value by subtracting the ΔCt for one green morph

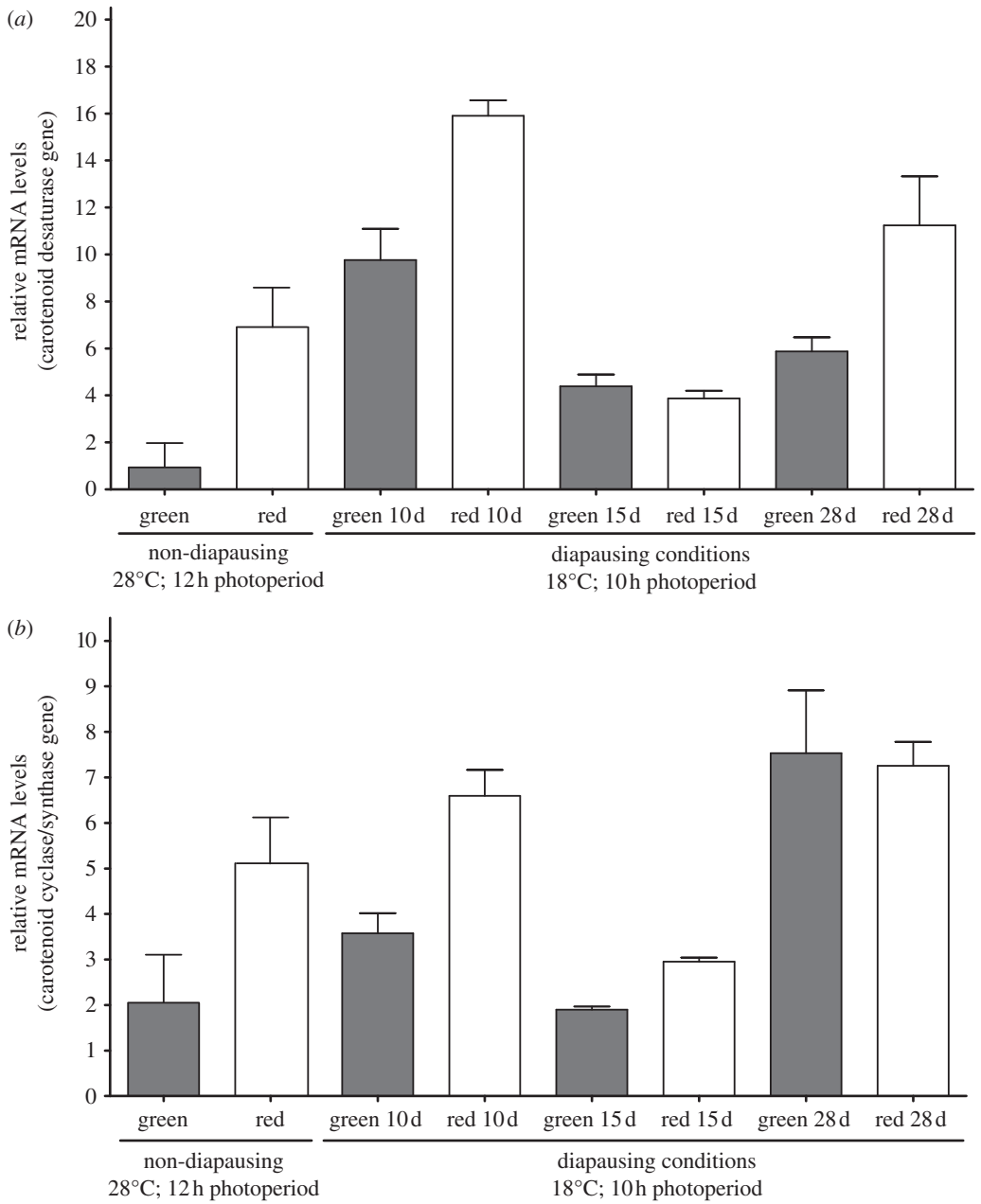

Figure 2. Expression of carotenoid biosynthesis genes across morphs and life stages in *T. urticae*. Relative quantification of (*a*) carotenoid desaturase and (*b*) carotenoid cyclase–carotenoid synthase. Grey bars represent the mean expression of two to four samples of 100 green spider mites each (±s.e.m.; three technical replicates per sample); white bars represent red spider mites.

non-diapausing sample, yielding the ΔΔCt value. Presented relative expression values ($2^{-\Delta\Delta Ct}$) are averaged across biological replicates. We used a nested ANOVA (sample type nested within spider mite colour) to analyse differences between ΔCt values of all red and green samples, and also used a *t*-test to analyse differences between green and red non-diapausing samples only.

3. RESULTS

We identified multiple *T. urticae* mRNA sequences encoding fungal-like carotenoid cyclase–carotenoid synthase and carotenoid desaturase. These mRNAs assembled into two gene contigs, one for each enzyme. Both genes are located on a single 11 637 bp scaffold (mean coverage = $6.9x \pm 3.4$, electronic supplementary material, figure S1) of the spider mite genome that includes an insect-like amino acid transporter gene, verifying that the scaffold is of arthropod origin and is not a contaminant.

Phylogenetic analyses revealed that *T. urticae* carotenoid synthesis genes cluster with those in the aphid genome, which are similar to carotenoid genes within fungi (figure 1). Of note, *T. urticae* has a fused carotenoid cyclase–carotenoid synthase gene, which is characteristic of fungi but not of plants or bacteria. Both the carotenoid cyclase–carotenoid synthase and carotenoid desaturase genes were expressed in all samples tested (figure 2), and across both non-diapausing and diapausing samples, red morph samples had higher expression than green morph samples for both genes (nested ANOVA: desat $F_{1,19} = 10.2$, $p = 0.02$; cyc/syn $F_{1,19} = 11.1$, $p = 0.01$). Specifically, relative to non-diapausing green morph samples, non-diapausing red morph samples exhibited a significant threefold increase in expression for carotenoid cyclase-carotenoid and a significant sixfold increase in expression for carotenoid desaturase (figure 2; *t*-test:

cyc/syn d.f. = 5, $p = 0.03$; desat d.f. = 5, $p = 0.01$). Interestingly, both red and green morphs samples displayed increased expression of both genes at day 10 of diapause, followed by a drop in expression at day 15, subsequent to a final rise in expression (figure 2).

4. DISCUSSION

We found evidence for transfer of two functional carotenoid biosynthesis genes (one carotenoid cyclase–carotenoid synthase and one carotenoid desaturase) into the genome of the two-spotted spider mite, *T. urticae*. While transfer of these genes could have occurred from fungi into a single arthropod ancestor of both spider mites and aphids, this scenario would require subsequent loss of these genes in countless extant arthropod taxa. Alternatively, horizontal transfer could have occurred directly between aphids and spider mites, or through some microbial intermediary. Such a sequential transfer could involve organisms like *Wolbachia* bacteria, which are known to infect both animal groups [11,12] and which are involved in diverse horizontal gene transfer events in arthropods [13,14]. It is perhaps more likely that these arthropods acquired genes from fungal symbionts, either beneficial or pathogenic, independently; both aphids and spider mite populations are frequently infected with fungal pathogens [15,16]. Interestingly, it has been suggested that the phoretic and parasitic feeding behaviours of many mite species, including spider mites, coupled with their frequent association with viruses, make them ideal horizontal gene transfer vectors, and may also result in the incorporation of mobile genes into their own genomes [17].

Carotenoids underlie important biological processes in arthropods. They underlie colour differences between and within species, may serve a protective role against oxidative stress and ultra-violet damage [1,18], and influence interactions with predators [4]. *Tetranychus urticae* harbours many carotenoids (e.g. α-carotene, β-carotene, diverse keto-carotenoids). The relative abundance of the predominant pigments is similar in red and green non-diapausing *T. urticae* but differs substantially between non-diapausing and diapausing individuals [6,7]. These differences in pigment may be influenced by increased expression of carotenoid synthesis genes during diapause, as is suggested here for both red and green morphs at some stages. As the pigment composition of mites is complex, these genes may also influence the composition of the less predominant pigments that underlie differences between red and green morphs. Ultimately, a complex set of multiple carotenoid biosynthetic genes, coupled with sequestration of environmental carotenoids, may dictate colour variation throughout the life cycle and between morphs. Complete annotation of the *T. urticae* genome, currently underway (http://www.jgi.doe.gov/sequencing/why/50028.html), combined with comprehensive expression and proteomic analyses in diverse morphs, will provide important additional insights into the ecological and developmental importance of carotenoid biosynthesis machinery in spider mites.

In conclusion, our present findings coupled with recent studies [3] demonstrate that carotenoid biosynthesis genes have been transferred multiple times into the genomes of higher animals. Given the ubiquity of carotenoids in arthropods, and the protective benefits provided by carotenoids, it is likely that these genes are present in the genomes of other arthropods as well. The knowledge that even complex biosynthetic pathways can be gained by horizontal gene transfer changes our understanding of evolutionary processes underlying animal adaptation.

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