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DEVELOPMENTAL EXPRESSION OF A TRANSFERRIN IN THE RED IMPORTED FIRE ANT, *SOLENOPSIS INVICTA*

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Transferrin is an iron-binding protein considered to be a component of the innate insect immune response (Nichol et al. 2002). Although direct causal evidence is lacking, transferrin is thought to sequester the essential nutrient, iron, from invading pathogens (Yoshiga et al. 1997). Interestingly, a number of insect transferrins have been reported to be up-regulated in response to microbial challenge (Yoshiga et al. 1997, 1999; Yun et al. 1999; Kucharski & Maleszka 2003; Thompson et al. 2003; Ampasala et al. 2004; Valles & Pereira 2005). We recently reported the cDNA sequence, gene architecture, and induction of a transferrin from the fire ant, *Solenopsis invicta* Buren. Because transferrin has been shown to be up-regulated in response to pathogen attack, we were interested to know if the expression of *S. invicta* transferrin (SiTf) was developmentally regulated. In addition, we evaluated the effects of the insect hormones, juvenile hormone and 20-hydroxyecdysone, on SiTrf expression.

Expression of the SiTrf gene was quantified with real-time quantitative PCR (Q-PCR) by the comparative C_T method (Livak & Schmittgen 2001) using the 18S ribosomal RNA gene (Valles & Pereira 2003, GenBank accession number: AY334566) as our endogenous reference as described and validated previously (Valles & Pereira 2005).

The relative quantity of the SiTrf transcript (GenBank accession number AY940116) was determined in 5 fire ant developmental stages, including queen, early larvae (first and second instars), late larvae (third and fourth instars), early pupae (cuticle not darkened), and workers. Larval stages were identified as described by Petralia & Vinson (1979). Total RNA was extracted with Trizol reagent (Invitrogen) from 10 to 20 mg of ants or brood, or a single queen. The RNA was quantified spectrophotometrically and diluted to a concentration of 10 ng/µl for analysis.

Northern analysis was conducted with the different developmental stages (excluding the queen) following the general procedure of Sambrook & Russell (2001). Membranes were blotted with 3 µg of total RNA from early instars, late instars, white pupae, and workers from a monogyne colony. The 744-nucleotide probe was synthesized from oligonucleotide primers p295 (5'CCTCTGATTGATGGCAGGATCGGGTGAC) and p296 (5'CATGGACTGAAGGAGTAGAAAGGACAGTCTCGACA), ^{32}P-radiolabeled dCTP, and a clone from the 5' end of the SiTrf gene as template.

To determine the influence of insect hormones on SiTrf gene expression, as shown previously (Nascimento et al. 2004), worker ants were exposed to the insect hormones, juvenile hormone III (JH) and 20-hydroxyecdysone (20HE). Glass Mason jars (118 ml) were coated with 200µg of JH or 20HE. JH and 20HE were dissolved in 1 ml of acetone or ethanol, respectively, and subsequently applied to the jars. The jars were rolled continuously until the solvent evaporated. The inner, upper lips of the jars were coated with Fluon to prevent the ants from escaping. Forty large worker ants from a monogyne colony were placed in the jar with a small amount of water-soaked cotton. The ants were held in the treated jars for 6 d at 25°C without food. Q-PCR analysis was conducted as described (Valles & Pereira 2005). Control jars were treated with acetone or ethanol only. Three replications of each treatment were conducted with ants from 3 different colonies.

Among the developmental stages examined, early pupae exhibited the highest expression level for SiTrf (Fig. 1). Relative SiTrf expression was 180-fold higher in pupae compared with the calibrator, early larvae (relative expression level of 1). No significant differences were observed in expression level between late larvae (24-fold), queens (25-fold), and workers (39-fold). Results from Northern analysis of the SiTrf transcript corroborated the comparative C_T developmental data (Fig. 1 inset). A single band (2.58 kb) was observed in the autoradiogram that was most prevalent in pupae.

Developmental expression of transferrins has been reported in 3 insect species, *Drosophila melanogaster* (Yoshiga et al. 1999), *Choristoneura fumiferana* (Ampasala et al. 2004), and *Apis mellifera* (Kucharski & Maleszka 2003; Nascimento et al. 2004). Although there were differences in insect stage/age sampled from each study, a general trend in expression was apparent; transferrin transcript levels tended to increase through larval development, reaching a maximum in either the last instar or early pupal stage. SiTrf transcript level was highest in the early (white) pupal stage (180-fold over early larvae). Similar results were reported for *D. melanogaster*, *A. mellifera* (whole body and brain), and *C. fumiferana*. Nascimento et al. (2004) hypothesized that *A. mellifera* transferrin expression was negatively regulated by ecdysone. Indeed, they were able to experimentally repress transferrin expression by application (or artificial *in vivo* production) of 20HE.

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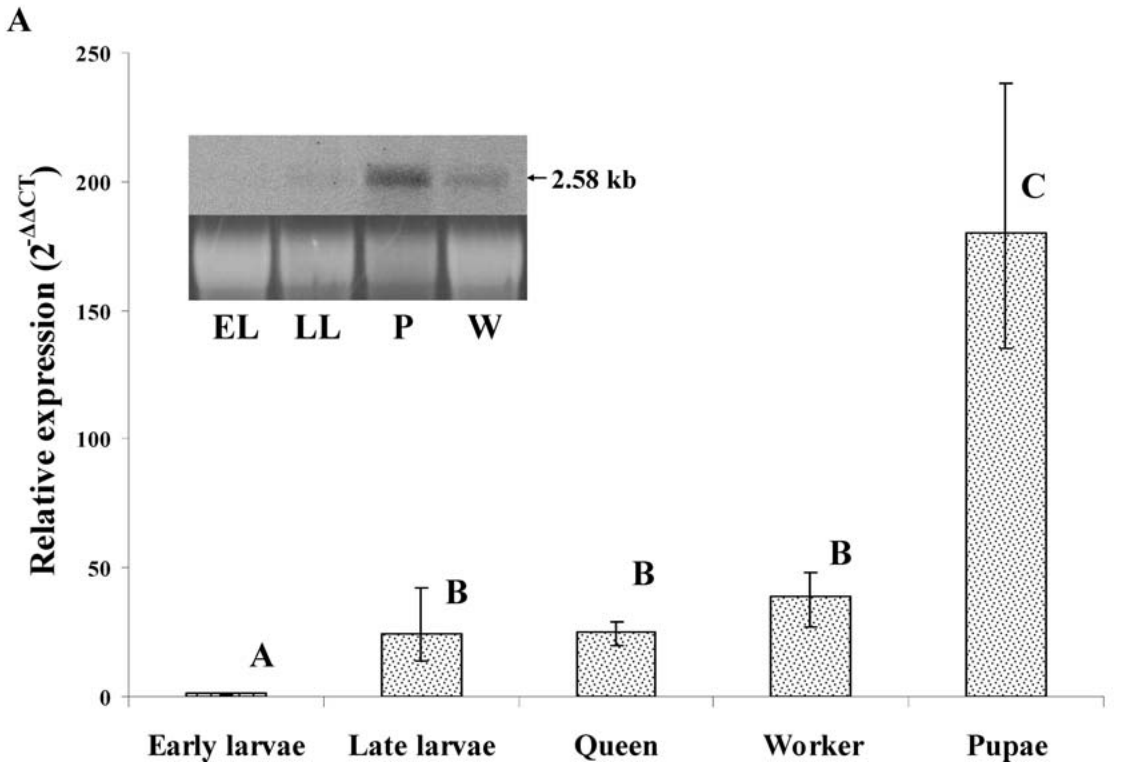

Fig. 1. Relative expression ($2^{-\triangle\triangle CT}$) of SiTrf in different developmental stages of *S. invicta* as determined by quantitative PCR. Inset is a corresponding Northern analysis. Top gel represents *S. invicta* mRNA (3 µg) separated on a 1.6% agarose gel and hybridized with a cloned SiTrf cDNA fragment. The bottom gel represents the 18S-rDNA subunit for normalization. Queens were excluded from the Northern analysis because of unavailability. Bars with different letters are significantly different by non-overlapping confidence limits (Livak & Schmittgen 2001).

Their hypothesis appears to be further corroborated by the intrastadial transferrin expression observed in *C. fumiferana*, in which the expression levels decreased prior to ecdysis in 4th-6th instars and pupae. In our case, despite constant exposure to 20HE for 6 days, the SiTrf expression level remained unchanged in worker ants when compared to a control group (Fig. 2). However, because of problems with high ant mortality by topical treatment methods, we were forced to use residual exposure to deliver 20HE. Thus, either SiTrf is not regulated by 20HE as in *A. mellifera*, or our residual exposure method did not deliver a sufficiently high dose of 20HE to repress SiTrf expression.

Although 20HE did not appear to alter SiTrf expression, JH did. As reported for *B. discoidalis* (Jamroz et al. 1993), *Riptortus clavatus* (Hirai et al. 2000), and *A. mellifera* (Kucharski & Maleszka 2003), JH repressed the expression of transferrin in *S. invicta* workers by greater than 50% (Fig. 2). Nascimento et al. (2004) suggest that most of the JH modulatory effect (in *A. mellifera*) on transferrin expression is attributable to an interendocrine effect on the hemolymph ecdysteroid titer. Additional studies are necessary to elucidate hormonal effects (and possible regulation) of this gene in *S. invicta* as well as other insects.

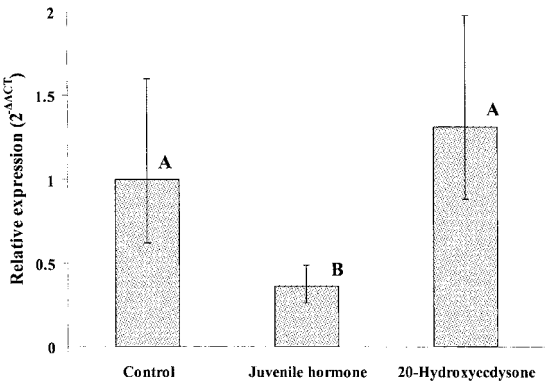

Fig. 2. Relative expression (2-△△CT) of SiTrf in worker ants exposed to juvenile hormone or 20-hydroxyecdysone for 6 d as determined by quantitative PCR. Bars with different letters are significantly different by non-overlapping confidence limits (Livak & Schmittgen 2001).

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SUMMARY

The developmental expression of a transferring gene (SiTrf) from the red imported fire ant, *Solenopsis invicta* was determined by quantitative PCR. Among the stages examined, early pupae exhibited the highest expression level (180-fold) followed by workers (39-fold), queens (25-fold), and late larvae (24-fold); early larvae exhibited the lowest level of expression.

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