Tissue-dependence and sensitivity of the systemic RNA interference response in the desert locust, *Schistocerca gregaria*

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ARTICLE INFO

Article history:

Received 29 June 2012

Received in revised form

10 September 2012

Accepted 13 September 2012

ABSTRACT

We report on a comprehensive study of the systemic RNAi-response in the desert locust, *Schistocerca gregaria*. Upon intra-abdominal injection of dsRNA for two housekeeping genes (*alpha-tubulin 1a* and *gapdh*) in the range of pg amounts of dsRNA per mg tissue, a potent reduction of their corresponding mRNA was obtained. Moreover, the observed transcript knockdown significantly increased in at least a 10 days period and eventually resulted in high mortality upon silencing of the *alpha-tubulin 1a* gene. A more moderate RNAi-response was however observed in the reproductive systems. Analysis of the tissue-dependent transcript level profile of several putative RNAi-genes indicated reduced levels of two genes, namely *sg-dicer-2* and *sg-argonaute-2*, in the reproductive systems. By silencing these components, we confirmed their importance in the RNAi-process and suggest that their expression levels are determinant for tissue-dependent differences in the potency of RNAi in the desert locust.

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1. Introduction

Several studies have shown that double stranded (ds)RNA can initiate the activation of an innate immune response, resulting in the sequence-specific inhibition of cellular gene expression at the post-transcriptional level (Hammond et al., 2001). This process, better known as RNA interference (RNAi), has become a widely used reverse genetic tool to knockdown and analyse the function of genes. Besides functioning as a research tool, RNAi may also contribute to novel strategies for selectively controlling agricultural pests, including a number of insect species (Mao et al., 2007; Baum et al., 2007; Whyard et al., 2009). In contrast to conventional insecticides, RNAi is a nucleic acid sequence dependent process. Consequently, RNAi is a highly species-specific technology, the use of which should result in fewer non-target effects and reduced harm to agricultural ecosystems. However, in order to induce an RNAi-response, dsRNA must enter the cell. Fortunately, some organisms are able to import extracellular dsRNA via dsRNA-specific transporters. One well-known uptake mechanism is transmembrane channel-mediated dsRNA-transport exemplified by the *Caenorhabditis elegans* SID-1 protein (Winston et al., 2002). A second uptake pathway was discovered in *Drosophila melanogaster* Schneider 2 (S2) cells, which employ receptor-mediated

endocytosis (Ulvila et al., 2006; Saleh et al., 2006). In addition, in several plants and nematodes, secondary dsRNA molecules are produced by RNA dependent RNA Polymerases (RdRP), whereby even with the presence of very low dsRNA amounts, a robust and persistent RNAi-response will be achieved (Sijen et al., 2001; Schiebel et al., 1998). However, until now no canonical RdRP orthologous sequences have been found in the genome of insects (Belles, 2010).

In many insect species, representing the orders of *Lepidoptera*, *Coleoptera*, *Diptera*, *Hemiptera*, *Hymenoptera*, *Dictyoptera*, *Isoptera* and *Orthoptera*, systemic RNAi-responses have been reported (Belles, 2010). However, the degree of sensitivity toward RNAi varies strongly among insect species (Miller et al., 2008; Belles, 2010; Terenius et al., 2011). The best-known example of a species with a reduced sensitivity towards systemic RNAi is the fruit fly *D. melanogaster* (Miller et al., 2008). In its larval stage, only the hemocytes seem to be capable of responding in a systemic manner. Furthermore, in many *Lepidoptera*, such as *Bombyx mori* and *Spodoptera littoralis*, most tissues also seem to be rather refractory to a systemic response (Terenius et al., 2011). On the other hand, recent reports suggest that some *Orthoptera*, *Dictyoptera* and *Coleoptera* respond very well to systemically applied dsRNA (Badisco et al., 2011b; Dong and Friedrich, 2005; Belles, 2010; Miller et al., 2008). Here, we report on the first detailed description of the systemic RNAi-response in a member of the *Orthoptera*, namely *Schistocerca gregaria*, a voracious pest insect and an important research organism. Although previous studies have proven the

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presence of a robust systemic RNAi-response in *S. gregaria* (Badisco et al., 2011b; Marchal et al., 2011a, b; Ott et al., 2012; Van Wielendaele et al., 2012; Marchal et al., 2012), its sensitivity, persistence and tissue dependency have not been investigated in detail so far. In this study, we demonstrate that injection of nano-mole amounts of dsRNA can silence up to 90% of the gene transcript level, with a knockdown effectiveness that increases over a ten-day period. Furthermore, a clearly reduced susceptibility towards RNAi was observed in the reproductive system, which may be caused by lower expression levels of components of the core RNAi-machinery.

2. Materials and methods

2.1. Rearing of *Schistocerca gregaria*

Gregarious *S. gregaria* were reared under crowded conditions with controlled temperature ($32 \pm 1^\circ\text{C}$), light (13 h photoperiod) and relative humidity (40–60%). They were fed daily with fresh cabbage and dried oat flakes. Directly after their final moult, locusts were synchronized to obtain adult animals of equal age.

2.2. Sequence information

Transcript sequence information of *S. gregaria* *glyceraldehyde phosphate dehydrogenase* (*gapdh*) (GenBank: HQ851387), *alpha-tubulin 1a* (GenBank: HQ851397), *dicer-2* (*dcr2*) (GenBank: JX516787), *argonaute-2* (*ago2*) (GenBank: JX516788), *enhanced RNA interference family member* (*eri1*) (GenBank: JX516790) was retrieved from the annotated *S. gregaria* EST-database (Badisco et al., 2011a). There was no sequence available for the *sg-sid1* transcript. However, based on the orthologous sequence found in *S. americana* by Dong and Friedrich (2005), a fragment of 435 bp was amplified (GenBank: JX516789). The DNA fragments were cloned into the pCR[®]4-TOPO[®] vector by means of the TOPO TA Cloning[®] Kit for Sequencing (Invitrogen). The sequences of the inserted DNA fragments were determined using the ABI PRISM BigDye Terminator Ready Reaction Cycle Sequencing Kit (Applied Biosystems). The obtained sequences were subsequently compared with BLAST to NCBI nucleotide databases of other insects and *in silico* translated into the corresponding amino acid sequences. The latter were then aligned against orthologous sequences of other insects (ClustalW), and the predicted protein domains were identified (NCBI, CD-search). In addition, since Dicer-1 (*Dcr1*) and Argonaute-1 (*Ago1*) are related to *Dcr2* and *Ago2* proteins, respectively, we have constructed an unrooted phylogenetic tree (ExpASy, ETH Zurich) to determine the correct identity of the sequences of *S. gregaria* (Supplementary data in Fig. S3).

2.3. Synthesis of dsRNA

Double stranded RNAs for *alpha-tubulin 1a* (545 bp), *gapdh* (447 bp), *gfp* (589 bp), *dcr2* (330 bp) and *ago2* (461 bp) were synthesised using the MEGAscript RNAi kit (Ambion). For production of *alpha-tubulin 1a*, *gapdh*, *dcr2* and *ago2* dsRNA, a DNA template flanked by two T7 promoter sequences was generated. A PCR reaction was performed using adult desert locust cDNA and gene specific primers containing a T7 promoter sequence at the 5' end. In the supplementary data (Fig. S1), the different PCR primers are displayed. REDTaq mix (Sigma–Aldrich co.) was used as a source of DNA Taq polymerase, dNTPs and PCR buffer. Next, the amplification products were analysed by 1% agarose gel electrophoresis and then visualised with UV-light. Finally, bands of the desired size were cut out and purified using GenElute[™] Gel extraction Kit (Sigma–Aldrich Co.). A TOPO 4.1 sequencing vector (Invitrogen) containing a *gfp* transcript sequence was used as template for *gfp* dsRNA

production. Since only one T7 promoter site is present in this vector, the fragment was cloned both in the sense and antisense direction. RNA was then synthesised by the T7 Enzyme Mix of the MEGAscript RNAi kit (Ambion). Both *gfp* RNA strands were first synthesised independently before being mixed to anneal, while transcripts made from a single template with opposing T7 promoters were hybridized during the transcription reaction. After the production of dsRNA, the remaining DNA and ssRNA was removed by nuclease treatment, and proteins and mono/oligonucleotides were removed by solid phase adsorption purification, according to the manufacturers' specifications (Ambion). The dsRNA-concentration was determined with a Nanodrop spectrophotometer (Thermo Fisher Scientific, Inc.), and the integrity of the dsRNA was assessed with gel electrophoresis using a 1% agarose gel. The dsRNA was stored at -20°C until further usage.

2.4. Injection and tissue collection of adult locusts

S. gregaria Ringer solution (1 L: 8.766 g NaCl; 0.188 g CaCl_2 ; 0.746 g KCl; 0.407 g MgCl_2 ; 0.336 g NaHCO_3 ; 30.807 g sucrose; 1.892 g trehalose; pH 7.2) was used to dilute the dsRNA to the desired concentration. Locusts were then each injected with 10 μl dsRNA-solution. The locust tissues of interest were micro-dissected in *S. gregaria* Ringer solution under a binocular microscope and immediately transferred to liquid nitrogen to prevent RNA degradation. All samples were stored at -80°C until further processing.

2.5. RNA extraction and cDNA synthesis

The Lipid tissue extraction kit (Qiagen) was utilized to extract RNA from larger tissues, such as gut, gonadal, muscle and fat tissue, as well as all pooled samples, while the RNAaqueous[®]-micro kit (Ambion) was employed to extract RNA from the relatively smaller brain and malpighian tubules. This was followed by an additional DNase treatment to remove genomic DNA contamination. Quality and concentration of the extracted RNA were assessed using a Nanodrop spectrophotometer. Next, equal amounts of RNA were used as template to produce cDNA. The cDNA synthesis was performed using the Superscript III reverse transcriptase kit (Invitrogen), random hexamer primers (Invitrogen) and dNTPs (Roche diagnostics GmbH) as described in the corresponding protocol. Finally, the cDNA-solution was 10-times diluted with MilliQ water. A calibrator cDNA sample was also made based on a pooled tissue sample containing all investigated tissues.

2.6. Quantitative real time-PCR

Primer express software (Applied Biosystems) was used to design quantitative real time (RT-)PCR primers. The corresponding primer sequences are displayed as supplementary data in Fig. S2. The primers were validated with a standard curve based on a serial dilution of cDNA to determine the primer annealing efficiency, and a dissociation protocol was performed to detect the presence of primer dimers and production of a single PCR product. For all transcripts, only a single melting peak was found. In addition, the qRT-PCR products were run on a 1% agarose gel containing Gel-Red[™] (Biotium) that resulted in a single band. Each reaction was performed in duplicate and contained 10 μl SYBR green solution (Invitrogen), 0.75 μl of 10 μM forward primer (Sigma–Aldrich), 0.75 μl of 10 μM reverse primer (Sigma–Aldrich), 3.5 μl milliQ water and 5 μl cDNA. Seven household genes were chosen, based on the study of Van Hiel and co-workers (Van Hiel et al., 2009). The geNorm program (Pattyn et al., 2003) was used to determine the most stable reference genes in the designed experiments. The four genes that were most stably expressed were, respectively, *ubiquitin*

conjugating enzyme 10 (*ubi*), elongation factor 1a (*ef1a*), *gapdh* and *alpha-tubulin 1a*. In order to correct for sample-to-sample variations, the relative expression levels were normalized against the two most stably expressed reference genes, *ubi* and *ef1a*. The data were further normalized against a calibrator cDNA sample to account for variations in the PCR-efficiency in different PCR runs. In every experiment, no-template and no-RT controls were included to check for possible contaminations. The PCR reaction was performed and analysed in a 96 well plate and by the StepOne System (ABI Prism, Applied Biosystems). Since the efficiency of the different primers was the same, the relative transcript quantity was calculated according to the delta–delta Ct method.

2.7. Statistical analysis

All data were analysed by non-parametric statistics in GraphPad prism 5 (GraphPad). In addition, the change of transcript knock-down efficiency over time (Section 3.2) was assessed by linear regression analysis.

3. Results

3.1. Tissue-dependence of the systemic RNAi-response

Five μg of *sg-gapdh* dsRNA was injected into the haemocoel of four-day old adult locusts. Three days later, the tissue-dependent knockdown was determined by comparing the relative *gapdh* transcript level between the test and control group, i.e. locusts injected with *gfp* dsRNA. The data (Fig. 1) indicate that a very robust knockdown was achieved for most tissues investigated, with the exception of the male and female reproductive systems. While a significant knockdown was still observed in the reproductive system, it was tempered in comparison to the other tissues investigated.

3.2. dsRNA-dose and time dependence of the RNAi-response

The dsRNA-dose dependence was studied by intra-abdominal injection using various amounts of *gapdh* dsRNA. After three days, the knockdown for two distinct tissues was examined: the female reproductive system, which is known to be more resistant towards RNAi, and the brain, which is one of the more responsive tissues (Section 3.1). The data demonstrate that the RNAi-response was extremely sensitive (Fig. 2a). Injection of only 1 ng of *gapdh* dsRNA

Fig. 1. Tissue-dependence of systemic RNAi, three days after injection of 5 μg of dsRNA. Seven different tissues were examined: Fb (fatbody), Mu (muscles), Mg (midgut), Br (brain), Mt (malpighian tubules), Frs (Female reproductive system) and Mrs (Male reproductive system). Significantly higher transcript levels were observed in the Mrs and Frs. The knockdown is represented as the mean percentage of the relative *gapdh* transcript levels in the test group in comparison to the mean relative transcript levels in the control groups that were injected with *gfp* dsRNA (mean $\pm$ SEM, $n \geq 5$, **: $p < 0.01$).

Fig. 2. RNAi-response three days after injection of different amounts of dsRNA for (a) *gapdh* and (b) *alpha-tubulin 1a* in the Female reproductive system (Frs) and brain. The knockdown is displayed as the mean percentage of the transcript levels present in the test groups in comparison to the mean relative transcript levels in the control groups that were injected with *gfp* dsRNA (mean $\pm$ SEM, $n \geq 4$).

was sufficient to induce a significant knockdown. Moreover, the maximum concentration dependent silencing was observed upon administration of as little as 30 ng dsRNA, an amount that corresponds to an average of 15 pg per mg tissue. Remarkably, despite the reduced knockdown robustness in the female reproductive system, both tissues displayed a similar dsRNA-dose-response relationship. To ascertain that these observations were not specific for *gapdh* gene silencing, a similar experiment was conducted using *alpha-tubulin 1a* dsRNA. Silencing of *alpha-tubulin 1a* resulted in a similar dose-dependent response (Fig. 2b), suggesting that the reported sensitivity is not specific to the *gapdh* gene or dsRNA sequence.

Similarly, by measuring the knockdown after three different durations; 1-day, 3-days and 10-days post injection (p.i.), the time-dependency of the RNAi-response was assessed. Five μg *gapdh* dsRNA was applied to saturate the RNAi-machinery, and four different tissues were examined, brain and midgut representing the more responsive tissues and male and female reproductive systems as less robust responders (Section 3.1). Our data show that the silencing response increased significantly with time, for all four tissues (Fig. 3a). A severe knockdown was seen 1-day p.i. in the brain and midgut, while this was minimal at this time point in the reproductive tissues. Similar time dependence was observed for *alpha-tubulin 1a* (Fig. 3b). However, in contrast to silencing the *gapdh* gene, injection of *alpha-tubulin 1a* dsRNA resulted in locust mortality, with the bulk of the locust population being dead 14-days p.i.. Since initial lethality occurred 7-days after injection of the dsRNA, the latest assessed time point for *alpha-tubulin 1a* was 6-days, instead of 10-days p.i..

The transcript levels of *alpha-tubulin 1a* and *gapdh* in midgut, brain, female reproductive system and male reproductive system

Fig. 3. Time-dependence of the RNAi-response after the injection of 5 µg dsRNA for (a) *gapdh* and (b) *α-tubulin 1a* transcript sequence in the Female reproductive system (Frs), male reproductive system (Mrs), brain and midgut. The knockdown efficacy is displayed as the mean percentage of the transcript levels present in the test groups in comparison to the mean relative transcript levels in the control groups that were injected with *gfp* dsRNA, i.e. at each time point (mean ± SEM, $n \geq 6$). The *gapdh* transcript levels decreases in all four tissues in course of time (Frs: $p < 0.0001$, Mrs: $p < 0.0001$, midgut: $p < 0.005$ and brain: $p < 0.0005$). For *α-tubulin 1a* the transcript amount decreases in the Frs ($p < 0.001$), the Mrs ($p < 0.005$) and the brain ($p < 0.005$).

can be found as supplementary data in Fig. S4. This demonstrates that the observed differences in RNAi-potency are not due to significantly higher expression levels of these genes.

3.3. The reproductive system differentially expresses *ago2* and *dcr2* transcript levels

In order to investigate the underlying causality of the reduced RNAi-susceptibility in the reproductive system, we assessed the tissue-dependent transcript profile of several putative components of the RNAi-process. The systemic RNAi-process can be subdivided in two steps: first, the transport and spreading of the dsRNA into the tissues, and secondly, the cell-autonomous RNAi-response exerted by the core RNAi-machinery. We tested if different tissues show differences in expression levels for genes involved in these processes. As previously mentioned, SID-1 is a putative dsRNA-transporter (Winston et al., 2002). Furthermore, two genes well known to play a crucial role in the core RNAi-machinery of *D. melanogaster* and *Tribolium castaneum* are *dcr2* and *ago2* (Tomoyasu et al., 2008; Tomari and Zamore, 2005). As pointed out by several reports (Kennedy et al., 2004), dsRNA-degrading enzymes, such as ERI-1, may also negatively regulate the silencing process. Therefore, we measured the tissue-distribution of *dcr2*, *ago2*, *sid1* and *eri1* transcript levels in four-day old *S. gregaria*. Our results (Fig. 4) show that the transcript levels of *dcr2* were relatively lower in female and higher in male reproductive organs, while significantly lower *ago2* expression levels were observed in the male reproductive system. On the other hand, the transcript levels of *sid1* and *eri1* remained indistinguishable from the different tissues investigated.

Fig. 4. Tissue-dependent transcript level profile of several putative RNAi-genes. The relative transcript levels of *dcr2* are higher in the male reproductive system (Mrs) and reduced in the female reproductive system (Fr), while *ago2* transcript levels are reduced in the Mrs. Each bar represents the mean of three independent pools of adult males (40, 10 and 10 animals/pool) and three independent pools of adult females (40, 10 and 10 animals/pool). For the male and female reproductive systems only males or females were used, respectively (mean $\pm$ SEM, **** p < 0.0005).

3.4. The ovaries are the less responsive part of the female reproductive system

The female reproductive system was further subdivided into ovaries and oviducts. Measuring the systemic RNAi-susceptibility in these tissues revealed a very strong knockdown in the oviducts, while a significantly lower knockdown was obtained in the ovaries (Fig. 5a). Next, we tested if the reduced *dcr2* expression level in the female reproductive system is also limited to the ovaries. The expression levels of *dcr2* and *ago2* were measured, and as displayed

Fig. 5. Reduced RNAi-susceptibility and lower transcript levels of *dcr2* in the ovaries in comparison to the oviducts. (a) Knockdown of *gapdh* in oviducts and ovaries (mean $\pm$ SEM, $n \geq 6$, $^{**}p < 0.01$). (b) Relative transcript levels of *dcr2* and *ago2* in the oviducts and ovaries (mean $\pm$ SEM, $n \geq 6$, $^{*}p < 0.05$).

in Fig. 5b the ovaries contain relatively less *dcr2* transcript levels, while no significant differences could be reported for *ago2*.

3.5. Lower *dcr2* or *ago2* transcript levels result in reduced RNAi-susceptibility

In order to investigate whether the expression levels of either *dcr2* or *ago2* can influence the RNAi-susceptibility, we silenced these components by injecting 100 ng of gene specific dsRNA. As a control, 100 ng of *gfp* dsRNA was injected. Six days later, a second injection was performed with 100 ng of *alpha-tubulin 1a* dsRNA. To be able to determine the knockdown effectiveness, a second control group was taken into account that was treated with a double *gfp* dsRNA injection. The data in Fig. 6a show a successful knockdown of *ago2* and *dcr2* in midgut tissue and, as demonstrated in Fig. 6b, this resulted in a reduced knockdown of the *alpha-tubulin 1a* gene

expression. It is, however, possible that silencing *dcr2* or *ago2* directly influences the *alpha-tubulin 1a* gene expression level, rather than the RNAi-susceptibility. Therefore, the *alpha-tubulin 1a* expression level was also determined 6 days after injection of *dcr2* or *ago2* dsRNA, without injection of *alpha-tubulin 1a* dsRNA. As demonstrated in Fig. 6c, no differential *alpha-tubulin 1a* transcript levels were reported.

4. Discussion

In this paper, we report on a detailed examination of the systemic RNAi-response in the desert locust, *S. gregaria*. Our results demonstrate that, in contrast to the relatively high resistance against systemic RNAi in many dipteran and lepidopteran species (Terenius et al., 2011; Miller et al., 2008; Belles, 2010), application of as little as 15 pg of dsRNA per mg tissue is sufficient to almost completely silence specific gene expression in adult desert locusts (Fig. 2). This finding is in accordance with the recently published highly sensitive RNAi-response in the migratory locust, *Locusta migratoria* (Luo et al., 2012). In addition, we show that the reduction in transcript levels persists for a long time, with an increase in knockdown efficacy during (at least) a ten-day period (Fig. 3), thereby rendering the desert locust an extremely RNAi-sensitive and -persistent insect species. Targeting the *alpha-tubulin 1a* mRNA gene product resulted in mortality in the locusts' population (Section 3.2), confirming knockdown at the protein level and suggesting that RNAi may contribute to promising strategies for selectively controlling locust pests. Its highly effective systemic RNAi-response, in combination with the available *S. gregaria* EST-sequence information (Badisco et al., 2011a), makes the desert locust an attractive organism for 'loss of function' analyses.

On the other hand, a more moderate RNAi-response is observed in the male and female reproductive systems (Fig. 1). This effect is most pronounced shortly after injection of dsRNA, but eventually also a clear knockdown effect will be reached in these organs (Fig. 3). By investigating the tissue-dependent transcript profile of several putative RNAi-genes, we assessed the possible influence of (a) the core RNAi-machinery, (b) dsRNA-transport from the hemolymph into the tissue, and finally, (c) dsRNA-degradation in the tissues. This analysis revealed reduced *dcr2* and *ago2* transcript levels in the female and male reproductive systems, respectively (Fig. 4). Since their function in locusts was still unclear, we silenced *dcr2* and *ago2* and confirmed their role in the RNAi-process in *S. gregaria* (Fig. 6). This is in agreement with the role of these genes in *D. melanogaster* and *T. castaneum* (Tomoyasu et al., 2008; Lee et al., 2004; Czech et al., 2009), and is the first report on their function in a member of the Orthoptera. These components clearly play a crucial role in the core RNAi-pathway of *D. melanogaster* (Kim et al., 2006). However, silencing *dcr2* or *ago2* in the desert locust did not completely abolish the RNAi-response (Fig. 6). This should not necessarily imply that their involvement in RNAi is less important in *S. gregaria*. First, RNAi only generates a silencing effect at the post-transcriptional level. Second, previous studies have demonstrated that the protein turnover rate can be a determinate factor for the potency of the knockdown at the (functional) protein level (Ott et al., 2012). A slow protein turnover rate may thus account for a rather moderate functional effect of the RNAi-response.

Although both male and female reproductive systems display less potent knockdown effects, their reported transcript profiles for the RNAi-genes *ago2* and *dcr2* are dissimilar. Whereas *dcr2* transcript levels are lower in the female reproductive system, the male reproductive system contains less *ago2* and more *dcr2* transcripts (Fig. 4). This finding illustrates the complexity of RNAi and indicates that the process may be regulated differently in different tissues.

Fig. 6. Both *ago2* and *dcr2* are involved in the RNAi-pathway. (a) An effective knockdown of *ago2* and *dcr2* transcript levels was obtained (mean $\pm$ SEM, $n \geq 6$; ***; $p < 0.001$). (b) The locusts were injected with either 100 ng of *dsgfp*, *dsago2* or *dsdcr2* and six days later followed by a second dsRNA injection of 100 ng of *alpha-tubulin 1a* (*dstubu*), indicated as respectively *dsgfp-dstubu*, *dsago2-dstubu* and *dsdcr2-dstubu*. In addition, a second control group that was twice injected with *dsgfp*, indicated as *dsgfp-dsgfp*, was also assessed. Fourteen hours after the second injection midgut tissue was microdissected and the relative transcript levels were then measured with real-time PCR. Statistical analysis was performed between the *dsago2-dstubu* or *dsdcr2-dstubu* group and the group *dsgfp-dstubu* (mean $\pm$ SEM, $n \geq 8$, ***; $p < 0.001$ and *; $p < 0.05$). (c) Silencing of *ago2* or *dcr2* transcript levels had no direct effect on the *alpha-tubulin 1a* transcript level in the midgut. The expression level was determined 6 days after injection of *dsgfp*, *dsago2* or *dsdcr2* (mean $\pm$ SEM, $n \geq 5$).

The reasons for this are currently not clear. One possible explanation could reside in the fact that, as reported in *D. melanogaster*, some cross-talk and overlap may exist between the short interference (si)RNA- and micro (mi)RNA-pathways (Zhou et al., 2008; Tomari et al., 2007). Whereas the siRNA-pathway is primarily an anti-viral immune response that recognizes dsRNA-molecules, the miRNA-pathway regulates the expression of endogenously produced mRNA-targets (Carthew and Sontheimer, 2009). This could mean that regulation of the expression level of components of the (si)RNAi-pathway might also affect the miRNA-pathway, or vice versa, and that compensatory control mechanisms might be required. An analysis of the components of the miRNA-pathway would therefore be of interest to verify this hypothesis. Unfortunately, sequence information of these components was either not available in the *S. gregaria* EST-database, or the fragments were too short to design suitable qRT-PCR primers. Nevertheless, it remains remarkable that specifically in the reproductive system a more moderate RNAi-potency is observed and that this coincides with differential transcript levels of *dcr2* or *ago2*. Since silencing either *dcr2* or *ago2* is sufficient to reduce the knockdown efficiency (Fig. 6), reduced transcript levels of one of these components may account for a lower RNAi-potency. It is therefore probable that each of these enzymes represents a rate-limiting step for the RNAi-process. However, it remains unclear to what extent an increase of *dcr2* transcripts (as observed in the male reproductive system) could (partially) undo the effects caused by lower *ago2* transcript levels.

Interestingly, low transcript levels of the core RNAi-components, *Bm-R2D2*, *Ms-dcr2* and *Ms-ago2*, have previously been considered as an explanation for the apparent inefficacy of RNAi in *B. mori* and *Manduca sexta*, respectively (Garbutt and Reynolds, 2012; Swevers et al., 2011). In *C. elegans*, the relative inefficiency of neuronal RNAi appears mainly due to the low expression levels of the *ce-sid1* dsRNA-transporter and high expression levels of the *ce-eri1* dsRNase (Calixto et al., 2010; Kennedy et al., 2004). In the present study, we have assessed the transcript levels of *sid1* and *eri1* in various tissues of the desert locust, but no tissue-dependent differences were observed (Fig. 4).

This article describes for the first time in detail the systemic RNAi-response in the desert locust. From this study, we can conclude that the desert locust possesses one of the most sensitive and persistent systemic RNAi-responses reported in an insect, and demonstrate for the first time tissue-dependency of RNAi in a member of the *Orthoptera*. Moreover, two main components of the RNAi-process are identified and a correlation between their transcript levels and the tissue-dependent RNAi-potency in the desert locust is described.

Acknowledgements

The authors gratefully thank J. Van Duppen, B. Deweer and F. Van Lommel for technical support; E. Marchal, L. Badisco and G. Perée for help with the dissections; R. Jonckers for taking care of the locust cultures and all lab members who took care of the locust cultures on holidays and in the weekends. In addition, the authors gratefully acknowledge the K.U. Leuven Research Foundation (GOA/11/02), the Research Foundation of Flanders (FWO-Flanders) and the Interuniversity Attraction Poles programme (Belgian Science Policy Grant P6/14) for financial support.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ibmb.2012.09.004>.

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