Research Article

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RNA interference of NADPH–cytochrome P450 reductase of the rice brown planthopper, *Nilaparvata lugens*, increases susceptibility to insecticides

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Abstract

BACKGROUND: NADPH–cytochrome P450 reductase (CPR) is essential for numerous biological reactions catalysed by microsomal cytochrome P450 monooxygenases (P450s). Knockdown of CPR in several insects leads to developmental defects and increased susceptibility to insecticides. However, information about the role of CPR in the brown planthopper, *Nilaparvata lugens*, is still unavailable.

RESULTS: A full-length cDNA encoding CPR was cloned from *N. lugens* (*NlCPR*). The deduced amino acid sequence showed marked features of classical CPRs, such as an N-terminus membrane anchor, conserved domains for flavin mononucleotide, flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide phosphate binding, as well as an FAD-binding motif and catalytic residues. Phylogenetic analysis revealed that NlCPR was located in a branch along with bed bug and pea aphid hemipteran insects. *NlCPR* mRNA was detectable in all tissues and developmental stages of *N. lugens*, as determined by real-time quantitative PCR. *NlCPR* transcripts were most abundant in the abdomen in adults, and in first-instar nymphs. Injection of *N. lugens* with double-strand RNA (dsRNA) against *NlCPR* significantly reduced the transcription level of the mRNA, and silencing of *NlCPR* resulted in increased susceptibility in *N. lugens* to beta-cypermethrin and imidacloprid.

CONCLUSION: The results provide first evidence that *NlCPR* contributes to the susceptibility to beta-cypermethrin and imidacloprid in *N. lugens*.

Supporting information may be found in the online version of this article.

Keywords: NADPH–cytochrome P450 reductase; *Nilaparvata lugens*; double-strand RNA; insecticide susceptibility; beta-cypermethrin; imidacloprid

1 INTRODUCTION

NADPH–cytochrome P450 reductase (CPR) (EC 1.6.2.4) is an essential enzyme in all kingdoms of life and involved in a variety of biological reactions catalysed by microsomal cytochrome P450 monooxygenases (P450s).[1,2] CPR contains an N-terminal transmembrane region (known as the membrane anchor), which contributes to localisation in the endoplasmic reticulum, and conserved domains for flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide phosphate (NADP) binding, which are vital for the supply of electrons derived from NADPH to its redox partner, various P450s.[3] Several CPRs have been identified and characterised from vertebrates,[3,4] insects,[5–8] plants[9–11] and fungi,[12,13] revealing an essential role for CPRs in P450-system-mediated detoxification of exogenous xenobiotics and regulation of endogenous substrates.

The insect P450/CPR complex has been intensively studied in relation to metabolic turnover of insecticides and host-plant allelochemicals. For example, several reports have shown that heterologous expression of P450s required the addition of CPR to reconstitute an effective system to metabolise insecticides and plant toxins, whereas lack of CPR resulted in no or low metabolic activity for these chemicals.[14,15] Moreover, evidence for the predominant role of CPRs in the biosynthesis of endogenous

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compounds, such as pheromone and cuticular hydrocarbon, have also been described in the pine bark beetle, *Ips pini*,[16] and in the fruit fly, *Drosophila melanogaster*.[17] In addition, the robust expression of CPR in the antennae of *D. melanogaster*[18] and the moths *Mamestra brassicae*[19] and *Spodoptera* littoralis,[20] along with P450s, indicated that the gene is potentially involved in odorant inactivation, thus protecting the olfactory neurons.

As involvement of CPR is required in a diverse array of reactions catalysed by P450s during metabolism *in vivo*, inhibition of CPR may effectively shut down all P450 activity. Henderson *et al.*[21] reported that hepatic P450 activity of the CPR-deleted mouse was completely inactivated. Recent studies have demonstrated that silencing CPR by double-strand RNA (dsRNA) injection in the mosquito *Anopheles gambiae*[22] and the bed bug *Cimex lectularius*[6] increases susceptibility to pyrethroid insecticides. In addition, oenocyte-directed RNAi of *CPR* of *D. melanogaster* results in a significant decrease in desiccation tolerance, as the biosynthesis of cuticular hydrocarbon is severely reduced.[17] These facts highlight the significance of CPRs in the P450/CPR-mediated biochemical process in insects, and it would be of great interest to develop synthetic inhibitors targeting this gene for use in insect pest management.

The brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), is a notorious rice phloem sap-sucker and plant virus vector in the East Asian countries, causing great economic losses to cultivated rice crops by direct feeding and by virus transmission through its long-distance migration.[23] In various geographical regions of China, the application of insecticides has been effective in the control of *N. lugens*. However, because of the indiscriminate usage of pesticides, some of the chemicals have become ineffective, even at relatively high doses.[24,25] Previous research suggested that P450 enzymes of *N. lugens* may contribute to the metabolic detoxification of several insecticides.[26,27] Therefore, it is important to understand whether CPR of *N. lugens* is involved in insecticide susceptibility.

RNA interference (RNAi) is a powerful tool for studying the function of a gene by suppressing its expression[28] and, potentially, for developing novel pest management strategies by the modification of plants.[29,30] RNAi has been broadly used to investigate the functions of essential genes in Insects.[31] RNAi has also been used in silencing vital genes of *N. lugens*. Wang *et al.*[32] reported that suppression of the chitin synthase 1 (*CHS1*) gene and its variant in *N. lugens* led to an inability to moult and increased mortality; Zhou *et al.*[33] showed that knockdown of two GST genes, *NlGSTe1* and *NlGSTm2*, significantly increased the sensitivity to chlorpyrifos.

In the present study, a full-length cDNA encoding CPR of *N. lugens* (*NlCPR*) was cloned, and the gene expression pattern in various tissues and at different developmental stages was analysed. The transcription of *NlCPR* mRNA was knocked down by RNAi, using dsRNA synthesised from the coding region of *NlCPR*. Thereafter, bioassays were carried out to compare the insecticidal susceptibility between gene knockdown and control *N. lugens*. The present study provides first direct evidence that suppression of *NlCPR* transcription contributes to insecticide susceptibility in *N. lugens*.

2 MATERIALS AND METHODS

2.1 Insects

The *N. lugens* used in this study was originated from a field population collected in Huajiachi Campus, Zhejiang University, Hangzhou, China. Insects were reared on rice variety Taichung Native 1 (TN1, susceptible to almost all herbivores of rice) seedlings in an insectary at 25 ± 1 °C and 80% relative humidity under a 16:8 h light:dark regime for at least 40 generations.

2.2 RNA extraction and cDNA synthesis

Total RNA was extracted from the third-instar nymphs of *N. lugens* using TRIzol reagent (Invitrogen, Carlsbad, CA) and treated with RNase-free DNase I (Takara, Dalian, China) to remove genomic DNA contaminants. The quality and concentration of RNA samples was determined using a Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE). A quantity of 1 μg of the total RNA was reverse transcribed to first-strand cDNA by using PrimeScript First-strand cDNA Synthesis kit (Takara, Dalian, China).

2.3 Cloning of *NlCPR*

Based on the transcriptome data of *N. lugens*,[34] a pair of gene-specific primers were designed to amplify the complete open reading frame (ORF) of *NlCPR* (supporting information Table S1). KOD FX DNA polymerase (Toyobo, Osaka, Japan) was used for amplification. The PCR product was gel purified, ligated into the vector pMD18-T (Takara, Dalian, China), transformed into *Escherichia coli* DH5α competent cells (Novagen, Darmstadt, Germany) and sequenced.

2.4 Bioinformatic analyses

The theoretical isoelectric point (pI) and molecular weight (MW) of the deduced NlCPR protein were calculated using ExPASy (www.expasy.org/tools/protparam.html). Sequence identification and searching for orthologues were performed using BLAST (blast.ncbi.nlm.nih.gov/Blast.cgi). Putative transmembrane domains and signal peptides were predicted respectively with TMHMM (www.cbs.dtu.dk/services/TMHMM) and signalP (www.cbs.dtu.dk/services/SignalP/). The binding domains and catalytic residues were predicted by Conserved Domain Search (www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml). Amino acid sequences of NlCPR and orthologues from other insect species were aligned using ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/), and a phylogenetic tree was constructed by the neighbour-joining method (1000 bootstrap replications) using MEGA 5.05 software.[35]

2.5 Relative expression levels of *NlCPR*

Tissue- and development-related expression of *NlCPR* was examined by real-time quantitative PCR (qPCR). Primers for qPCR are listed in supporting information Table S1. Equal amounts of total RNA (1 μg) isolated from five tissues of *N. lugens* adults (heads, thoraces, abdomens, legs and wings) and nine different life stages (first- to fifth-instar nymphs, and the macropterous and brachypterous forms of both male and female adults) were reverse transcribed using ReverTra Ace qPCR RT kit (Toyobo, Osaka, Japan). The *α-tubulin* gene of *N. lugens* was used as the internal control gene to normalise the target gene expression and to correct for sample-to-sample variation. qPCR was performed in a 20 μL reaction volume that contained 10 ng of cDNA template, 10 μL of SYBR Green Real-time PCR Master Mix (Toyobo, Osaka, Japan) and 0.2 μM of each primer target for *NlCPR* or the *α-tubulin* gene of *N. lugens*. Reactions were performed in triplicate in a Bio-Rad CFX96 real-time system (Bio-Rad Laboratories, Hercules, CA) with the following parameters: 1 cycle at 95 °C for 2 min, 40 cycles of 95 °C for 10 s, and 60 °C for 20 s. Three independent biological repeats were done, and levels of *NlCPR* expression were calculated by the $2^{-\Delta\Delta C_t}$ method.[36]

2.6 Silencing of *NlCPR* by RNAi

NlCPR and the green fluorescent protein (*GFP*) gene were amplified by PCR using primers containing T7 RNA polymerase promoter (supporting information Table S1). Products were gel purified and used as templates to synthesise dsRNA, using MEGAscript T7 High Yield Transcription kit (Ambion, Austin, TX). dsRNAs were dissolved in ultrapure water, and the quality and concentration were determined by agarose gel electrophoresis and Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE). Approximately 100 ng of dsRNA (4 mg mL^{-1}) of either *NlCPR* or *GFP* was injected into each of the third-instar nymphs of *N. lugens*, following the protocol described by Wang *et al.*[32] After injection, nymphs were kept on fresh TN1 seedlings to recover for 48 h. Thirty insects were randomly collected, at 48 and 72 h after injection, to determine the reduction in *NlCPR* transcription levels using qPCR. The qPCR primers (supporting information Table S1) were from a separate region of *NlCPR* to those used for RNAi, to avoid hampering the qPCR reactions.

2.7 Bioassays after RNAi

Two chemicals, beta-cypermethrin and imidacloprid, were purchased from Xinnong Chemical Co., Ltd (Taizhou, Zhejiang, China) and were all ≥95% pure. Chemicals were diluted in acetone to produce a stock solution from which serial decimal dilutions were made. By using microtopical application, a sublethal dose[33] of each insecticide was applied on each insect from either an *NlCPR* dsRNA-injected or a *GFP* dsRNA-injected group 48 h after injection. A group of non-injected (naive) insects were also tested. The mortality was assessed 24 h after insecticide exposure. Four independent bioassays were performed, and in each bioassay at least 90 insects were tested.

2.8 Data analyses

Data analyses were carried out using Data Processing System (DPS) software v.9.5.[37] For qPCR results, Student's *t*-test (two-tailed paired *t*-test) and one-way analysis of variance (ANOVA) with the least significant difference (LSD) test were applied, respectively, for comparing the difference between two samples and the differences among multisamples. Mortality data were arcsine square root transformed and subjected to ANOVA and the LSD test. The level of significance was set at $P < 0.05$.

3 RESULTS AND DISCUSSION

3.1 Cloning and sequence analysis of *NlCPR*

In a previous investigation, a transcriptome dataset for *N. lugens* was constructed in the authors' laboratory using a mixture of whole bodies at all developmental stages.[34] From the transcriptome data, it was possible to identify and clone the cDNA containing complete ORF of *NlCPR* in the present study. The 2070 bp cDNA was deposited into GenBank (accession number KF591574). The authors also searched other transcriptome and expressed sequence tag (EST) datasets of *N. lugens*,[38,39] as well as the newly finished genome dataset of *N. lugens* (Zhang *et al.*, *N. lugens* Genome Project, Zhejiang University, China, unpublished); the results indicated the presence of only one *CPR* gene in the *N. lugens* genome. Also, it was found that *N. lugens* has only one isoform of *NlCPR*. However, the fruit fly, *D. melanogaster*, expresses multiple transcript isoforms of CPR. In addition, some vascular plants, such as hybrid poplar (*Populus trichocarpa*),[9] cotton (*Gossypium hirsutum*)[10] and rice (*Oryza sativa*),[11] each contain two or more *CPR* paralogues, and these enzymes were classified into distinct groups according to their amino acid sequences and expression profiles. The presence of a single form of CPR in *N. lugens* may reflect the unique function of the enzyme involved in the interaction with its redox partner, various P450s.

NlCPR encoded a deduced peptide of 689 amino acid residues, with predicted MW and pI of 77.87 kDa and 5.37 respectively. An amino acid homology analysis (Blastp) showed that the protein shared a high identity with the other heteropteran *C. lectularius* (73%), and an identity of at least 65% with respective orthologues from other insect species (supporting information Table S2). No signal peptide was predicted at the N-terminus of NlCPR, while a hydrophobic transmembrane region consisting of 22 amino acids was identified (Fig. 1A), indicating that this region may function as a membrane anchor involved in location on the endoplasmic reticulum membrane. The anchor region is essential for normal interaction between CPR and P450s. Without the anchor, the remaining soluble domain is capable of transferring electrons to cytochrome *c* and other electron acceptors, but is incapable of passing electrons to P450s.[3,13,14]

NlCPR contained several marked structural features of classical CPRs, including the FMN-, FAD- and NADP-binding domains (Fig. 1A). The FMN domain is located near the N-terminus of the protein, connected to the FAD domain via a flexible loop, and the NADP-binding domain is present at the C-terminal end (Fig. 1A). Multiple alignment showed that the amino acids constituting the FMN-, FAD- and NADP-binding pocket in the NlCPR tertiary structure are conserved among CPRs of insects, rats and humans (Fig. 1B). The interface of the FMN domain of NlCPR comprises two FMN-binding sites, which is structurally similar to yeast (*Saccharomyces cerevisiae*) CPR and interacts with the site of redox partner binding of its physiological substrate P450s (Fig. 1A).[40] The movement of the loop region linking the FMN and FAD domains facilitates conformational changes in the position of the FMN domain to interact with various P450s.[3,41] Consensus binding sites of the FAD-binding motif, composed of three conserved amino acid residues (Arginine 453, Tyrosine 455 and Serine 456), is ubiquitous in the FAD-binding domain.[42] Four catalytic residues (Serine 456, Cysteine 641, Aspartic 686 and Tryptophan 688) constitute the active site conserved between NlCPR and rat CPR, which was demonstrated to be essential in the hydride transfer reaction.[43,44] As a well-known electron supplier, NlCPR is presumed to transfer a hydride ion derived from NADPH to FAD, then to FMN and finally to a wide variety of P450s.[45] These domains contribute to electron passing and are involved in several metabolic pathways such as sterol biosynthesis and xenobiotic metabolism.[1,2]

3.2 Phylogenetic relationship of insect CPRs

To date, more than 25 CPRs, including ten reported and 15 predicted from genomic sequences, are known from insects. In order to elucidate evolutionary relationships among insect CPRs, the authors constructed a neighbour-joining phylogenetic tree of NlCPR along with its annotated orthologue proteins from hemipteran, lepidopteran, dipteran, coleopteran and hymenopteran insects (Fig. 2). The result shows that, although identity of these proteins is quite high (supporting information Table S2), they were well segregated from each other. CPRs from the same insect order were clustered together within a branch with strong bootstrap support, and the branch pattern was largely in accordance with the accepted phylogeny of the insect species. In this tree, NlCPR fell into a branch with CPRs of other hemipterans, the bed bug *C. lectularius* and the pea aphid *Acyrthosiphon*

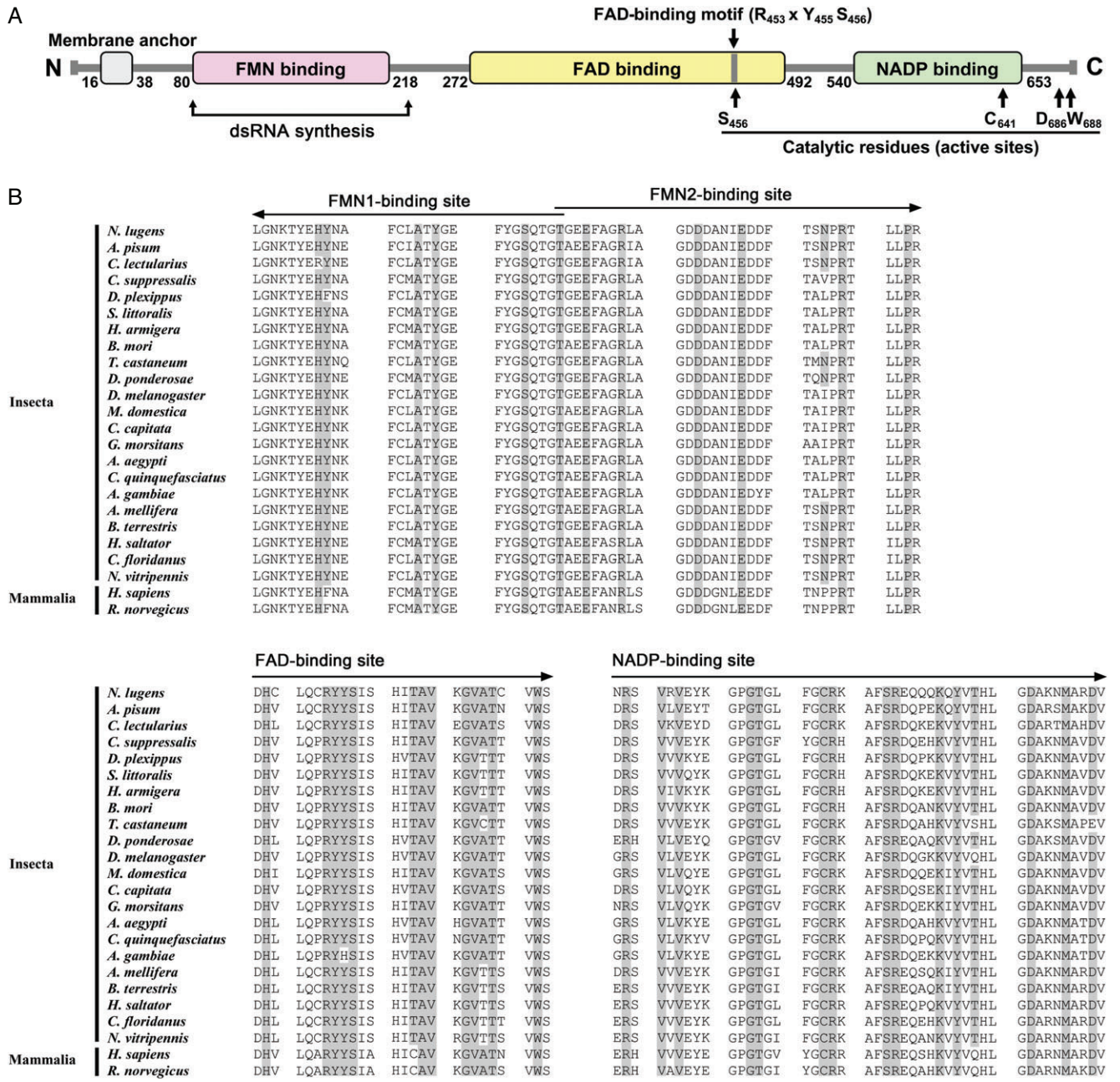

Figure 1. Sequence analysis of NlCPR. (A) Schematic drawing of NlCPR. Membrane anchor, conserved FMN-, FAD- and NADP-binding domains, FAD-binding motif (Arg_{453} × $Tyr_{455}Ser_{456}$) and catalytic residues (Ser_{456}, Cys_{641}, Asp_{686} and Trp_{688}) are shown; the region used for dsRNA synthesis is also indicated. (B) Alignment of FMN-, FAD- and NADP-binding sites in insect and mammal CPRs. The arrows show the direction from the N-terminus to the C-terminus. Residues constituting the binding site in each domain are highlighted in grey. All CPR amino acid sequences were extracted from GenBank (the accession numbers are listed in supporting information Table S2).

pisum. It is worth mentioning, however, that CPRs of *N. lugens* and *C. lectularius* are in one subbranch, while *A. pisum* CPR is in the neighbouring subbranch, indicating the evolutionary relationship within these enzymes.

3.3 Spatial and temporal expression profiles of *NlCPR*

Insect P450s catalyse a diverse array of metabolic reactions during the insect life cycle. Catalytic activities of P450s require involvement of their redox partner, CPR. Therefore, tissue- and development-related expression of the *CPR* gene is a reflection of P450 activity. To study the expression pattern of *NlCPR* in different tissues of brown planthopper, total RNA from heads (with antennae), thoraces, abdomens, legs and wings of *N. lugens* adults was extracted and used as template for qPCR. It was found that *NlCPR* transcripts can be detected in all tissues tested (Fig. 3A). The highest *NlCPR* level was found in the abdomen, significantly higher than in other tissues ($P < 0.05$). *NlCPR* transcripts were also abundant in the thorax, but relatively scarce in the wing and leg (Fig. 3A).

The authors also examined *NlCPR* expression levels in *N. lugens* across nine different life stages, including first- to fifth-instar nymphs and macropterous adult males and females, as well as brachypterous adult males and females (Fig. 3B). The results

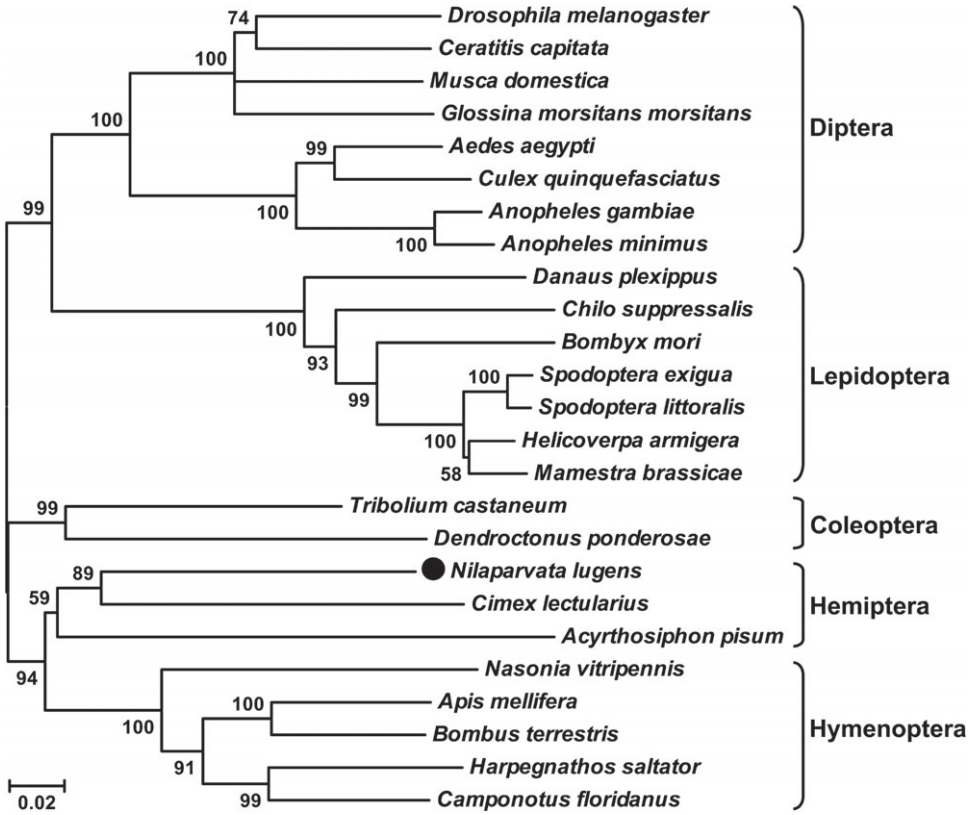

Figure 2. Phylogenic analysis of insect CPRs. The neighbour-joining tree was constructed by MEGA 5.05,[35] and testing of phylogeny was done by the bootstrap method with 1000 replications. Bootstrap values of >50% are shown on each node. *N. lugens* CPR is indicated by a solid circle. GenBank accession numbers of the sequences used are listed in supporting information Table S2.

clearly showed that *NlCPR* transcription levels fluctuated through different developmental stages of the insect species. The highest expression of *NlCPR* was found in the first-instar nymphs; the mRNA transcription then decreased from second- to fourth-instar nymphs, but the differences were not significant ($P > 0.05$). Thereafter, the transcription level increased slightly at the fifth-instar nymph stage (Fig. 3B). In the adult stage, as a whole, *NlCPR* levels were significantly higher in brachypterous adults than in macropterous adults ($P < 0.05$). Low *NlCPR* levels were detected in macropterous adult males and females (Fig. 3B). The wide distribution of *NlCPR* in a variety of tissues and across different developmental stages of *N. lugens* probably indicates its involvement in various demands of different P450s for driving biosynthesis and metabolic processes, and it improves the adaptability of insects to different ecological niches.

3.4 Silencing of *NlCPR* by RNAi

Previously, Liu *et al.*[46] reported that microinjection with a high dsRNA concentration (250 ng) into *N. lugens* induced high RNAi efficiency of several genes, but caused high mortality of Insects. A very low dose (0.1 ng), however, could result in a lethal phenotype.[32] Based on these studies, a volume of 100 ng of *NlCPR*- or *GFP*-dsRNA was chosen for injection. qPCR data showed that the *NlCPR* mRNA levels decreased dramatically at 48 h after injection of *NlCPR* dsRNA compared with the *GFP* dsRNA-injected insects, and the effect was still strongly evident at 72 h (Fig. 4). Injection of *GFP*-dsRNA did not affect *NlCPR* expression (Fig. 4). Therefore, injection of 100 ng of dsRNA was effective in silencing the transcription of *NlCPR*.

3.5 Knockdown of *NlCPR* and susceptibility to insecticides

Knockdown of *NlCPR* in the nymphs of *N. lugens* significantly increased their susceptibility to beta-cypermethrin and imidacloprid compared with control insects (Fig. 5). Among the *NlCPR*-knockdown insects, 59.5% died after beta-cypermethrin exposure, significantly higher than in the control groups ($P < 0.05$), of which only 26.2% of the *GFP* dsRNA-injected and 19.6% of the naive insects died at this time point (Fig. 5A). Similarly, the sensitivity of *NlCPR* dsRNA-injected nymphs to imidacloprid was enhanced, and the differences among *NlCPR* dsRNA-injected, *GFP* dsRNA-injected and naive individuals were significant ($P < 0.05$), with a mortality of 52.2, 20.6 and 11.9% respectively (Fig. 5B).

In insects, overexpression of P450 genes is often associated with enhanced detoxification of insecticides or plant secondary metabolites,[47] and *in vivo* inactivation of P450 or its redox partner, CPR, by application of inhibitors or knockdown of target genes by RNAi results in increased susceptibility to synthetic and natural compounds. In the mosquito *A. gambiae*, injection of dsRNA into general populations significantly reduced CPR content in the abdomen and led to increased susceptibility to permethrin.[22] In *CPR*-silenced *C. lectularius* also, significantly enhanced susceptibility to deltamethrin was observed.[6] The present results showed *NlCPR* contributing in a similar manner to susceptibility not only to beta-cypermethrin but also to imidacloprid (Fig. 5). This is to the authors' knowledge the first *in vivo* evidence that knockdown of *CPR* in *N. lugens* increases the susceptibility of the two insecticides. The phenomenon could be caused by several possible mechanisms. Firstly, the P450/CPR complex could be a major factor involved in the detoxification of the two insecticides.

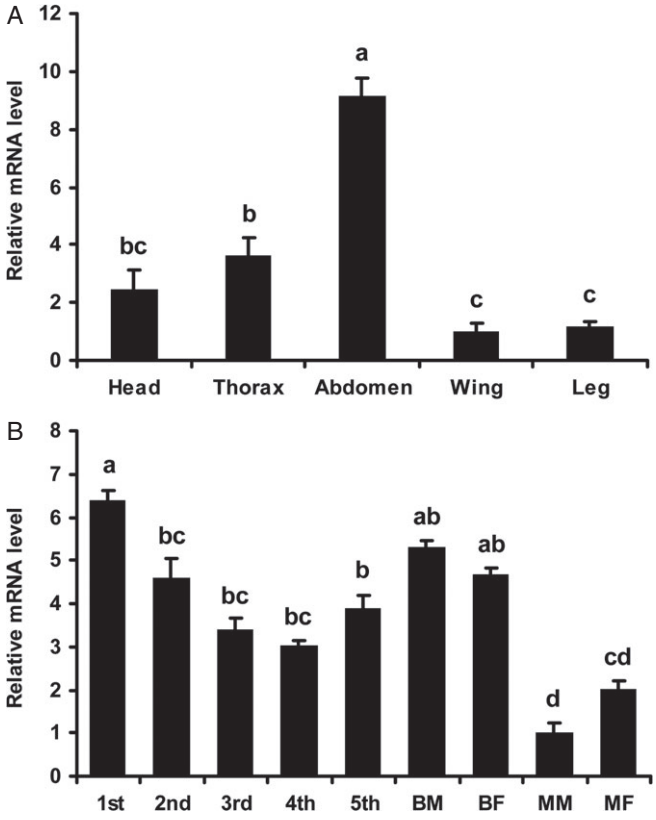

Figure 3. Relative expression levels of *NlCPR* in various tissues (A) and at different developmental stages (B) of *N. lugens*, as determined by real-time quantitative PCR (qPCR). 'Head' refers to the head with antennae; 1st to 5th, first- to fifth-instar nymphs; BM, brachypterous adult male; BF, brachypterous adult female; MM, macropterous adult male; MF, macropterous adult female. The levels of *NlCPR* expression in various tissues and at different developmental stages were normalised relative to the level in the wing and macropterous adult male (MM) respectively. Data are presented as mean ± SE; different letters denote a significant difference among different samples ($P < 0.05$, one-way ANOVA with LSD test).

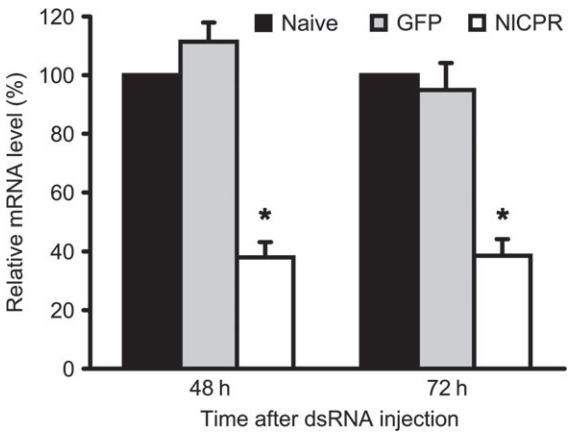

Figure 4. *NlCPR* mRNA levels of *N. lugens* after dsRNA injection. GFP, nymphs injected with dsRNA of green fluorescent protein (*GFP*); NlCPR, nymphs injected with dsRNA of NlCPR; naive, non-injected nymphs. The levels of *NlCPR* transcription in *NlCPR* dsRNA-injected and *GFP* dsRNA-injected insects were normalised relative to the naive individuals. Data were presented as mean ± SE; * denotes a significant difference in *NlCPR* expression levels between naive and injected insects ($P < 0.05$, two-tailed paired *t*-test).

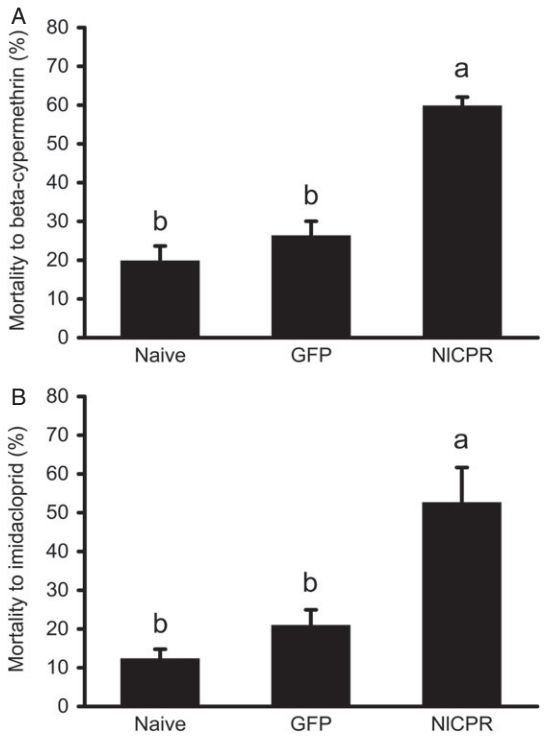

Figure 5. Mortality of *NlCPR*-silenced *N. lugens* nymphs to beta-cypermethrin (A) and imidacloprid (B). Naive, non-injected nymphs; GFP, nymphs injected with dsRNA of *GFP* gene; NlCPR, nymphs injected with dsRNA of *NlCPR*. Data were presented as mean ± SE. Different letters denote a significant difference ($P < 0.05$, one-way ANOVA with LSD test).

Secondly, as CPR is heavily involved in hydrocarbon production in insects,[17] silencing of CPR in *N. lugens* may enhance the permeability of the defective cuticle, thus causing increased penetration of insecticide molecules. Thirdly, apart from P450s, CPR also donates electrons to other oxygenases such as haem oxygenase and cytochrome b_5.[48,49] Therefore, NlCPR is likely to participate in reactions involving haem oxygenase and/or cytochrome *b5*, indirectly influencing the sensitivity to insecticides. On the other hand, in the present research, only the silencing of the *CPR* gene in a general population of *N. lugens* was investigated, and not of an insecticide-resistant population. Further RNAi studies on the insecticide-resistant strains of *N. lugens* are urgently needed to elucidate the particular role of NlCPR in insecticide resistance.

In conclusion, the present study provides preliminary information on the sequence, phylogenicity and expression pattern of *NlCPR* in *N. lugens*, and, more importantly, on knockdown of *NlCPR* in *N. lugens* increasing the susceptibility to beta-cypermethrin and imidacloprid. Further studies are needed to investigate the specific function of NlCPR in P450-mediated detoxification pathways and other physiological processes in *N. lugens*, especially in insecticide-resistant populations of the insect species.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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