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A rapid method to monitor parasitoid infestations in *Drosophila suzukii* populations

Eine Methode zum schnellen Nachweis von Parasitoiden in *Drosophila suzukii* Populationen

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Abstract

Parasitoid wasps are efficient natural enemies of other insects. They are in focus as an important biological pest management element to control invasive pest species. While spreading into new areas these invasive pests are often followed by their natural parasitoids from their home regions. The monitoring of native and adventive parasitoids, and their success in controlling the pest populations in the field, is cumbersome and relies mainly on hatching experiments. Here, we present a rapid method to detect parasitoid infestations in the invasive agricultural pest *Drosophila suzukii*. We specifically amplified a hymenopteran 28S rRNA sequence from DNA or RNA extracted from fly pupae. This allowed the determination of parasitoid infestations at the species level.

Keywords

***Drosophila suzukii*, parasitoids, Spotted wing Drosophila, monitoring**

Zusammenfassung

Parasitoide Wespen tragen zur Kontrolle von Insektenpopulationen bei und sind daher ein wichtiger Baustein zur biologischen Bekämpfung invasiver Schädlinge. Während der Ausbreitung von invasiven Schädlingen in neue Gebiete folgen ihnen häufig Parasitoide aus ihren Heimatregionen nach. Die Überwachung der einheimischen und der adventiven Parasitoidpopulationen und die Auswertung ihres Erfolgs bei der Reduzierung von Schädlingspopulationen im Freiland ist aufwändig, und beruht meist auf langwierigen Parasitierungs- und Schlupfversuchen. Wir haben eine schnelle und kostengünstige Methode zum Nachweis von Parasitoiden bei der invasiven Kirschessigfliege, *Drosophila suzukii*, entwickelt. Sie basiert auf dem Amplifizieren der Hymenoptera-spezifischen 28S rRNA Sequenz von DNA oder RNA aus Fliegenpuppen, welche den Nachweis von Parasitoiden bis auf Artniveau ermöglicht.

Stichwörter

***Drosophila suzukii*, Kirschessigfliege, Parasitoide, Monitoring**

Introduction

Drosophila suzukii (Matsumura, 1931), the spotted wing Drosophila (SWD)(Diptera: Drosophilidae), is an invasive pest responsible for substantial agricultural revenue losses (Bolda et al., 2009). Originating from Asia, SWD invaded North America and Europe between 2008 and 2011 (Asplen et al., 2015). In contrast to other fruit-feeding drosophilids that lay their eggs in rotten or overripe fruit, SWD has a specialized enlarged, serrated and hardened ovipositor, which allows the female to oviposit into intact, ripe fruits (Walsh et al., 2011). Population reduction and seasonal control is difficult due to its rapid development with five to eight generations per season in Germany, depending on yearly climatic conditions (Vogt, 2020; Weber & Kockerols, 2016). All life stages are present simultaneously on host plants and oviposition occurs in undamaged fruits just before or at harvest time. Thus, the use of insecticides is limited by potentially harmful residues on the treated fruits which can interfere with the scheduled harvest e.g. due to required pre-harvest waiting periods. This conflict raises the demand for alternative control measures (Eben et al., 2020; Schetelig et al., 2018).

Parasitoid wasps are potential candidates for biological control of SWD as part of integrated pest management methods. Especially two species native to Europe, *Pachycrepoideus vindemiae* (Rondani, 1875) (Hymenoptera: Pteromalidae) and *Trichopria drosophilae* (Perkins, 1910) (Hymenoptera: Diapriidae), have shown promising results infesting SWD in laboratory and field experiments (Englert & Herz, 2016; Esteban-Santiago et al., 2021; Gonzalez-Cabrera et al., 2019; Häussling et

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al., 2021, 2022; Mazzetto et al., 2016; Rossi Stacconi et al., 2018; Wang et al., 2016). Additionally, two foreign species, *Leptopilina japonica* and *Ganaspis brasiliensis (*Hymenoptera: Figitidae), that contribute to control *D. suzukii* populations in their native regions, seem to be following SWD's invasive paths and have for example been observed in Canada, Italy and Germany (Abram et al., 2022; Martin et al., 2023; Nair & Peterson, 2023; Puppato et al., 2020).

Currently, the detection of these parasitoid wasps relies mainly on collecting host larvae and pupae, followed by documentation of the hatching of flies or parasitoids. This experimental procedure can be lengthy, especially when parasitism occurs at the end of the fruiting season in late autumn, when parasitoids and their hosts can overwinter as pupae. It also requires broad taxonomic expertise to determine parasitoid wasps at the species level via morphological identification only. Here, we present an easy, rapid, and low-cost way to detect parasitoids in SWD pupae via PCR and Sanger sequencing. We utilized primers generated by Pook and colleagues (2017) which specifically amplify parts of the hymenopteran 28S rRNA, but do not produce an amplicon from their dipteran host *D. suzukii*. Subsequent commercial sequencing of the 28S rRNA fragment allows the detection of SWD parasitoids down to the species level within a day or two.

Methods

D. suzukii *and parasitoid wasp culture and sampling*

P. vindemiae and *T. drosophilae* were reared under laboratory conditions on *D. suzukii* flies, kept as laboratory colonies at the Julius Kühn-Institut, Institute for Biological Control, Dossenheim, Germany. Pupae were kept in a mass-rearing cage of each respective parasitoid and were collected between 24 and 48 hours after initial wasp exposure.

DNA & RNA extraction

Genomic DNA of *D. suzukii* pupae was extracted by homogenizing a single pupa in lysis buffer (100mM Tris-HCL, 100mM NaCl, 50mM Na2EDTA and 1%SDS) and 0.5 mg/mL Proteinase K (Qiagen), incubated at 37 °CC for 20 minutes, followed by deactivation of proteinase K at 95 °C for 5 minutes. A 1/10 volume of 3.3 M sodium acetate was added and samples were incubated at 65 °C for one hour. DNA was then precipitated using the phenol-chloroform method. We added the equal volume (lysis buffer) phenol and 1/5th volume chloroform, mixed rigorously and centrifuged at 13.000 RPM for 2 minutes to separate the phases. The aqueous phase containing the DNA was transferred to a new tube and mixed with 2.5 volumes of absolute EtOH and 1 µl of glycogen (Thermo Scientific). DNA was precipitated at -20 °C for 2 hours and pelleted by centrifugation for 20 minutes at 13.000 RPM and 4 °C. The pellet was subsequently washed twice with 70% ethanol and finally eluted in 15 µl of H_2O. RNA of pupae was extracted with the TRIzol/chloroform method. One pupa was homogenized with a bead beater by adding ~5-10 1 mm zicronia/silica beads (BioSpec) in tubes with 500 ul TRIzol and 200 µl chloroform. Samples were incubated at room temperature for five minutes and phases were separated by centrifugation at 13.000 RPM for 15 minutes at 4 °C. The clear phase containing the RNA was transferred to a new reaction tube and mixed with 1 volume of isopropanol and 1 µl of glycogen (ThermoFisher). An RNA pellet was obtained through centrifugation at 13.000 RPM for 10 minutes at 4 °C and then washed with 70% ethanol twice. RNA was eluted in 15 µl H_2O.

cDNA synthesis, PCR and sequence analysis

cDNA was synthetized by using 0,5-1 µg of total RNA with FastGene Scriptase II (NIPPON Genetics) according to specifications. Polymerase chain reaction (PCR) was then performed by using cDNA (1:5 dilution) or genomic DNA using Taq DNA Polymerase with Standard Taq Buffer (NEB) to amplify hymenopteran specific 28S rRNA (F: GTAAACCTGAGAAACCCAAAAGAT, R: CCTGAAAGTACCCAAAGCAGTAG (Pook et al., 2017)) for parasitoid barcoding and mitochondrial cytochrome c oxidase subunit I (CO1) (LCO1490 F: GGTCAACAAATCATAAAGATATTG, HCO2198 R: TAAACTTCAGGGTGACCAAAAAATCA) (Folmer et al., 1994) as control for successful nucleic acid extraction. PCR conditions were as followed for both primer pairs: 30 s at 95 °C, 35 cycles of 30 s at 95 °C, 30 s at 55 °C and 1 minute at 68 °C, followed by 300 s at 68 °C. Commercial Sanger sequencing of PCR products was performed by Eurofins Genomics Europe (Ebersberg, Germany). Sequences were analyzed with Geneious (v2023.1.1). The phylogenetic tree was established by aligning 28S rRNA sequences from several related parasitoids (supplemental datafile S1) with MUSCLE (v3.8) (Madeira et al., 2022) and IQ-TREE 2 (v2.2.0) (Minh et al., 2020). The tree was visualized with TreeViewer (v2.1.0) (Bianchini & Sánchez-Baracaldo, 2023). The obtained 28S rRNA sequences were identical to sequences that resulted from our analysis of individual wasps of the respective species. All sequences were deposited at Genbank (*P. vindemiae*: OR246966.1, *T. drosophilae*: OR221.1).

Results

We successfully amplified a PCR product with the hymenopteran specific 28S rRNA primers in SWD pupae infested with either *P. vindemiae* or *T. drosophilae* reared under laboratory conditions. The amplicon was present when either utilizing genomic DNA (not shown) or cDNA from total RNA extractions (Fig 1). There was no 28S rRNA amplicon in samples SWD from non-infested individuals. Performing a nucleotide blast of the obtained 28S rRNA sequence for *P. vindemiae* retrieved a ~98% identical sequence (AJ005242.1) as top hit in the NCBI nr database, belonging to *P. vindemiae*. For the 28S rRNA sequence from *T. drosophilae* we retrieved a ~88% identical sequence in the NCBI nr database originating from *Belythinae* sp. (FJ407363.1). To visualize the relationship of our new sequences we generated a phylogenetic tree with a number of 28S rRNA sequences from related species (Fig 2, datafile S1). As expected, our *P. vindemiae* sequence indeed identified as *P. vindemiae* and grouped into the family Ptermalidae. There was no 28S rRNA reference sequence for *T. drosophilae* in the NCBI nr database. Our original *T. drosophilae* sample

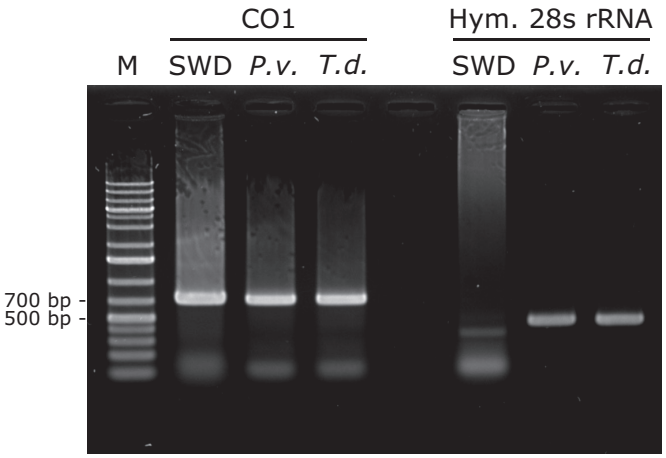

Fig. 1. Detection of *Pachycrepoideus vindemiae* and *Trichopria drosophilae* in pupae of *Drosophila suzukii*. Primers for hymenopteran 28S rRNA amplify a product in the expected size from cDNA of SWD pupae infested with either *P. vindemiae* or *T. drosophilae*, but not from not infested pupae. Primers for mitochondrial cytochrome c oxidase subunit I (CO1) were used as control for successful cDNA synthesis.

was collected at the field station of the Julius Kühn Institute, Dossenheim, in 2015, and was taxonomically identified by David Notton (Senior Curator for Hymenoptera, National Museum of Scotland, Edinburgh). Our sequence groups into the family of Diapriidae as expected, and both sequences were added to Genbank (*P. vindemiae*: OR246966.1, *T. drosophilae*: OR246921.1).

Discussion

The invasion of new pest species regularly interrupts the functionality of natural and agricultural ecosystems (Kenis et al., 2009). In the latter, this requires the rapid adaptation of pest management strategies to control those foreign, invasive pests below economic damage level. Parasitoids can be key factors for sustainable regulation of invasive agricultural pests (Heraty, 2017). To monitor and measure the role of parasitoids as biocontrol agents, it is crucial to obtain reliable data of "what parasitizes what". Here we present a rapid, accurate and low-cost method to detect parasitoids in SWD. Our method can contribute not only to detect the success of native parasitoid species by evaluating the parasitism rate in local populations, but also by timely tracking the arrival of foreign parasitoids following SWD's path. Data about the latter point is especially important, because often the adventive parasitoids from the same native habitat as their original host have better success rates in controlling their host population (Daane et al., 2016; Giorgini et al., 2019; Girod et al., 2018). Our method allows rapid detection and monitoring of the presence and effect of larval and pupal parasitoids without the need to incubate pupae from the field, and it is transferrable to other host-parasitoid communities. Additionally, our approach does not only work with genomic DNA as template, but also on cDNA derived from RNA. This allows further downstream analysis (e.g. quantitative real-time PCR) of samples originating from the same individuals.

A further possibility to use the presented method is the evaluation of the success of various parasitoid species under natural conditions. *Drosophila spp*. can defend themselves from parasitoid wasps by mounting an immune response. In larvae, the eggs of parasitoids can be recognized, encapsulated and subsequently melanized by specialized enzymes, resulting in a visible black capsule inside their hemocoel (Cerenius et al., 2008; Dudzic et al., 2015). These capsules can be easily seen in adult flies, identifying these individuals as "survivors". It is impossible, however, to draw any conclusions on which parasitoid was unsuccessful in developing in this individual fly. With our method, it might be possible to extract genetic material from these "survivors" to obtain information on the respective parasitoid species and their failure to develop. Additional studies will test this hypothesis.

Supplementary information

The supplementary datafile S1 (excel file) is available online at https://doi.org/10.5073.JfK.2023.09-10.05.

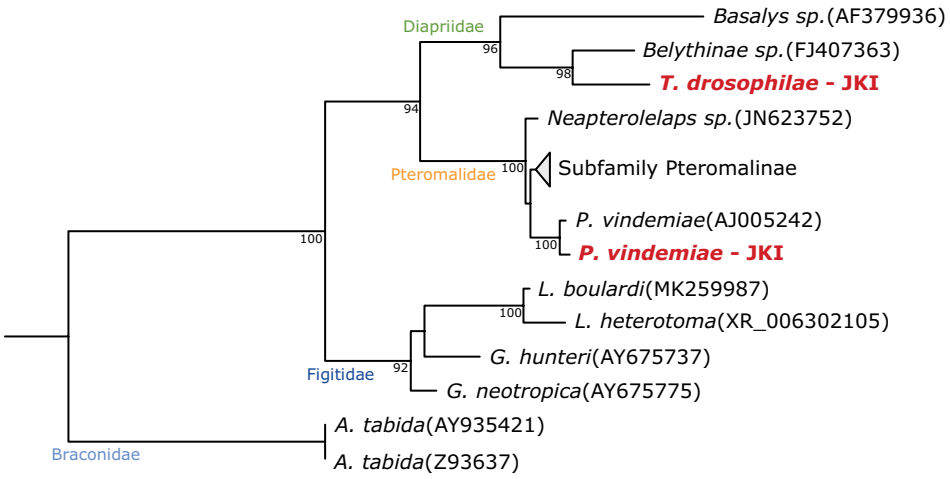

Fig. 2. Phylogenetic analysis of the two hymenopteran 28S rRNA PCR amplicons. The phylogenetic tree was generated using maximum likelihood method implemented by IQ-Tree, with 1000 bootstrap replicates. Bootstrap values ≥90 are indicated. Obtained sequences from both *P. vindemiae* and *T. drosophilae* show the same systematic placement as previously known (Burks et al., 2022).

Conflicts of interest

The authors declare that they do not have any conflicts of interest.

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