Journal of Pest Science
https://doi.org/10.1007/s10340-018-0963-3

ORIGINAL PAPER

Identifying plant DNA in the sponging–feeding insect pest *Drosophila suzukii*

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Received: 23 November 2017 / Revised: 1 February 2018 / Accepted: 16 February 2018

Abstract

Drosophila suzukii (Diptera: Drosophilidae) is a highly polyphagous invasive pest threatening fruit production in the Americas and Europe. The current knowledge of its host plants is mainly based on oviposition and larval development in fruits, while little is known on the diet of the adult flies. This information is important for developing effective control strategies. Here, we examine DNA-based techniques to determine food plants of *D. suzukii*. Adult flies were fed with raspberries (*Rubus idaeus*) and allowed to digest up to 72 h after feeding. Raspberry DNA was detected by diagnostic PCR for up to 48 h post-feeding with a significant negative effect of time on DNA detection success but no significant differences between male and female flies in detection probabilities. As *D. suzukii* walks on plants, its body surface can get contaminated with DNA. With a bleaching experiment, we succeeded to remove contaminating external plant DNA, while the DNA in the gut content stayed unaffected. Finally, field-collected flies were subjected to a next-generation sequencing approach, demonstrating that plant DNA from different host plants can be efficiently detected in both bleached and non-bleached specimens. In order to safeguard against erroneous host plant detections, we recommend bleaching flies before they are subjected to DNA extraction. The current findings encourage the use of DNA-based gut content analysis in *D. suzukii* to obtain a better understanding of its feeding ecology which is a prerequisite for developing successful control strategies.

Keywords Chloroplast DNA · Diet metabarcoding · Feeding experiment · Molecular gut content analysis · Spotted Wing Drosophila · High-throughput sequencing · NGS

Key message

- We developed and evaluated PCR-based methods to detect plant DNA ingested by *D. suzukii*.
- DNA of ingested plants could be identified for up to 48 h post-feeding with no significant differences between males and females.
- Flies were successfully decontaminated from external eDNA by a newly adapted bleaching protocol.
- Host plant DNA could be identified within field-collected specimens using a next-generation sequencing approach.
- DNA-based gut content analysis provides a promising approach to unravel *D. suzukii* feeding ecology.

Communicated by A. Biondi.

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Introduction

The invasive and highly polyphagous frugivore pest species *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) is threatening soft and thin-skinned fruit crops in the Americas, Europe and its native Sino-Japanese realm, causing severe economic impacts (Asplen et al. 2015; Farnsworth et al. 2017; Mazzi et al. 2017). Unlike native Drosophilidae that infest rotten fruits, female *D. suzukii* own a serrated

ovipositor enabling them to oviposit directly into ripening and ripe fruits (Atallah et al. 2014). Infested fruits quickly collapse and become unmarketable.

Since its first observation in Southern Europe (Italy and Spain) and North America (California) in 2008 (Calabria et al. 2012; Cini et al. 2012; Hauser 2011), several studies focused on the host plants of this pest (Kenis et al. 2016; Poyet et al. 2015). Accordingly, a wide range of cultivated, ornamental and wild fruits has been examined and more than 80 host plants are described for Europe (Briem et al. 2016; Kenis et al. 2016; Poyet et al. 2015). This knowledge is mainly based on studies dealing with field-collected fruit samples, laboratory feeding and oviposition trials, development of larvae and the emergence of adult flies (Hamby et al. 2016; Mazzetto et al. 2015; Poyet et al. 2014; Tochen et al. 2016). However, studies on infield food choices are rare. This is because identifying food plants is usually difficult: direct observations of feeding activity in the field are problematic in (1) habitats with limited access (e.g. forest canopy) and (2) due to correct identification of small insects such as *D. suzukii*. However, during laboratory feeding trials insects often feed on and/or oviposit into host plants which are not typically used infield (Garcia-Robledo et al. 2013). As a consequence, the diet range may be overestimated by the above-mentioned approaches and we lack information about the plants utilized as food source by *D. suzukii* under natural conditions. This knowledge, however, is crucial for developing more effective biotechnological control strategies such as "attract & kill" or "push & pull" (Alnajjar et al. 2017; Renkema et al. 2016). For these strategies, either the food plants itself or the volatile organic compounds emitted by such plants could be used. In the latter, further research is needed to identify such compounds.

Sponging–feeding insects such as *D. suzukii* are well adapted to feed on fruit or tree sap, extrafloral and floral nectar using a haustellum (Kanzawa 1939; Tochen et al. 2016). Therefore, studying its diet by dissecting the flies is impossible as no microscopically identifiable food remains can be found within the gut content. Several studies showed that DNA-based techniques provide a tool to obtain detailed information on the gut content in such situations, including the detection of DNA from ingested plants (Avanesyan and Culley 2015; De la Cadena et al. 2017; Staudacher et al. 2013; Wallinger et al. 2015). Junnila et al. (2011) and Lima et al. (2016) already showed that plant DNA is identifiable in the guts of blood feeding sand flies (Diptera: Nematocera) which have sucking mouthparts. In Drosophilidae (Diptera: Brachycera) which are sponging feeders that soak up small amounts of food in a liquid form, this has not yet been shown. Here, we test how well the molecular approach is suited to identify plant DNA ingested by *D. suzukii* using a series of laboratory experiments employing diagnostic PCR and next-generation sequencing (NGS). Besides assessing post-feeding food DNA detection intervals for *D. suzukii*, we also considered sex-specific effects on plant food detection probabilities in our experiments.

Field-collected insects can be externally contaminated with DNA (so-called environmental DNA: eDNA) which stems from the environment they live in (Greenstone et al. 2012). This might be especially relevant for species such as *D. suzukii* which walk on plants and fruits they feed on with their typical foraging behavior (Kanzawa 1939; Poyet et al. 2015). Extracting the DNA from whole bodies of those individuals includes the risk of also amplifying eDNA, leading to false-positive assignments of food sources (Greenstone et al. 2012; Wallinger et al. 2013). To check whether such eDNA is a potential source of error and if so, how it can be removed from the flies before DNA extraction, the bleaching method (Greenstone et al. 2012; Wallinger et al. 2013) was adapted and modified for *D. suzukii*. Experiments were conducted where fed flies were externally contaminated with plant DNA and subjected to a bleach cleaning treatment.

Finally, as *D. suzukii* is highly polyphagous an NGS approach was applied to a set of field-collected adult flies to check how well this technique is suited to identify DNA of consumed plants. The NGS approach includes general plant primers which amplify DNA from virtually all plant species which might be utilized by *D. suzukii*, while a diagnostic approach employing species- or genus-specific primers would only allow assessing the consumption of these particular plant taxa. For example, the NGS approach should be especially useful to unravel the food resources utilized by this pest in winter and spring, which is a bottleneck period for the species. Furthermore, we examined with these field-collected specimens how important eDNA might be as a source of contamination when using NGS to identify food plants under natural conditions.

According to the knowledge gaps identified above, our study had three aims: (1) to develop new protocols to identify plant DNA ingested by *D. suzukii* via diagnostic PCR to determine for how long post-feeding plant DNA can be detected in its gut content and whether post-feeding detection intervals differ between males and females, (2) to develop a bleaching protocol to decontaminate *D. suzukii*'s outer body surface from eDNA without affecting ingested plant DNA and (3) to test how well plant DNA can be identified in field-caught individuals using a NGS-based approach of diet analysis.

Materials and methods

Colony maintenance

Individuals of *D. suzukii* used in this study were obtained from the laboratory colony maintained at the Julius

Kühn-Institut (JKI) in Dossenheim (Germany) since October 2013. The culture has been started with adults that emerged from different fruits (i.e. blackberry, cherry and raspberry) sampled at the experimental fields of the JKI Dossenheim and is yearly refreshed. Flies were kept in an environmental chamber (Vötsch Industrietechnik GmbH, Germany) at constant conditions with 23 °C, 60% RH and a photoperiod of 16L:8D. As nutrition, a mixture of dried brewer's yeast (Diana, Germany) with sucrose (1:1) and a 5% sucrose–water solution was offered. For oviposition, plastic cups (125 ml, Huhtamaki, Finland) filled with 75 ml JKI standard diet (Briem et al. 2016) were placed in the cages for 2–3 days. These oviposition substrates were stored under the same conditions as the colony in a second environmental chamber until adult emergence. All individuals emerged within 8–10 days before the experiments started.

Feeding experiment

Adult *D. suzukii* were starved for at least 24 h to adjust them to the same starving level. Then, mashed defrosted raspberries (*Rubus idaeus*, Luxfrost Sárl, Luxemburg) were offered as experimental food to the flies. After 1 h, the food was removed and individuals were checked for feeding activity, indicated by a red abdomen (Fig. 1). Feeding active individuals ($n = 150$ males and $n = 150$ females) were separated by sex and transferred in batches of 10 individuals per sex to starving containers (2 L, Rotilabo®, Carl Roth + Co. KG, Germany) for each post-feeding time point (0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24, 32, 48 and 72 h). Those containers were prepared with gauze (mesh size 500 µm) for ventilation, and a small petri dish (∅ 35 mm, Greiner Bio-One International GmbH, Austria) at the bottom was filled with a moistened cotton pad. To avoid stress due to starvation every 12 h post-feeding, the JKI standard diet was offered for 1 h. Nevertheless, only 3 males survived for 72 h. After reaching the desired post-feeding time point, individuals were separately stored in 2-ml safe seal reaction tubes (Sarstedt AG & Co., Germany) at − 28 °C until DNA extraction.

Fig. 1 Female *D. suzukii* fed with raspberry for 1 h indicated by the red abdomen (left) and a starved female with a clear abdomen (right); photographs: A. Frank, F. Briem, J. Just; JKI Dossenheim. (Color figure online)

eDNA decontamination of the flies' body surface

A new protocol to remove eDNA contamination sticking on the fly's outer body surface based on previously published studies using similar methods was developed (Greenstone et al. 2012; Remen et al. 2010; Wallinger et al. 2013). To establish this method, 20 *R. idaeus*-fed females were artificially contaminated with mistletoe (*Viscum album*), sampled at the Dossenwald near Mannheim, Germany (Briem et al. 2016). Females were dipped softly with a tibia or wingtip into the mashed *V. album* berries to simulate an "authentic" scenario. A "worst-case" scenario was simulated by dipping another 10 females with a tibia or wingtip AND their abdomen into mashed *V. album* berries. Ten females from the "authentic" scenario served as control ("no-bleach"). For easier handling and to avoid unpredictable fly movement, the females of both scenarios were CO_2 anesthetized before dipping them into the mashed mistletoe berries. In both scenarios, we used sodium hypochlorite ("bleach," Sigma-Aldrich, St. Louis, Missouri, USA) with a concentration of 1–1.5% (incl. 0.02% Tween® 20, Sigma-Aldrich) as bleaching agent for 30 s based on the outcomes of testing varying concentrations of sodium hypochlorite and incubation durations. After bleaching, all individuals were washed with molecular grade water.

Primer design and screening for plant DNA

For primer design, several *trn*L sequences of *R. idaeus* and *V. album* which included the binding site for the universal primer *c* (5′-CGAAATCGGTAGACGCTACG-3′) (Taberlet et al. 1991) were downloaded from Genbank. Sequences were assembled in BioEdit Sequence Alignment Editor v7.1.9 (Hall 1999), and the alignment was extended with further plant sequences such as maize and soy which are components as corn and soy flour of the JKI standard diet for *D. suzukii*. Primer Premier 5 (PREMIER Biosoft International, Palo Alto, USA) was used to design specific reverse primers targeting *R. idaeus* and *V. album*, respectively. We chose primers that can be combined with the universal forward primer *c* to amplify a 245-bp fragment for *R idaeus* (Rub-ida-A575; 5′-GGAAGGATTCCTTTACGAACAC-3′) and a 170-bp fragment for *V. album* (Vis-alb-A576; 5′-TAT TGTTGTTTGCTTGGATAAGCT-3′). The primers' specificity and sensitivity were evaluated in vitro and a final singleplex PCR protocol established: 15 µl PCRs contained 4 µl DNA extract, 7.5 µl 2× Type-it Mutation Detect PCR Kit (Qiagen, Hilden, Germany), each primer at 0.5 µM, 0.5 µg BSA (Sigma-Aldrich) and PCR grade water to adjust the

volume. Amplifications were carried out under the following thermocycling conditions: 5 min at 95 °C, 35 cycles of 20 s at 92 °C, 90 s at 60 °C and 30 s at 70 °C and finally 5 min at 70 °C.

DNA was extracted from whole *D. suzukii* specimens. Each individual was submerged in 200 µl TES buffer (0.1 M TRIS, 10 mM EDTA, 2% SDS, pH 8) and 5 µl proteinase K (20 mg/ml). Then, they were homogenized with three to four 3-mm glass beads in a Precellys® 24 tissue homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France) for 120 s at 5000 rpm. The samples were incubated overnight at 56 °C. DNA was extracted with the Biosprint 96 DNA blood kit on a Biosprint 96 extraction robotic platform (both: Qiagen) following the manufacturer's instructions except for the modification of the lysis described above and elution in 200 µl 1× TE buffer. Each 96-well plate contained four extraction negative controls. All extractions were done in a separate pre-PCR laboratory using an UVC-equipped laminar flow hood.

PCRs were run with two positive (raspberry and mistletoe DNA, respectively) and two negative (molecular grade water) controls per 96-well. No cross-sample contamination was detected by testing the extraction and the PCR negative controls using the diagnostic assays described above.

All PCR products were visualized using the automated capillary electrophoresis system QIAxcel (Qiagen), and the results were scored with BioCalculator "Fast Analysis software version 3.0" (Qiagen). Samples showing the expected fragment length with a signal above 0.1 relative fluorescent units (RFUs) were assigned as positive. It is important to note that the diagnostic assays developed here were not tested for their specificity beyond the species involved in this feeding experiment, also including ingredients of the JKI standard diet and that further specificity testing would be required if these assays are to be applied in systems which contain other (plant) species.

Field sampling and sample preparation for NGS

For NGS analysis, 40 (20 male and 20 female) *D. suzukii* were collected from the raspberry plantation (variety: Himbotop) of the JKI Dossenheim and checked for feeding activity (Fig. 1). Half of the individuals were washed with bleach as described above (eDNA decontamination) prior to DNA extraction. The remaining 20 flies were processed without this cleaning step, and all 40 flies were DNA-extracted as described above. Additionally to these field-collected flies, those 5 samples of the eDNA decontamination experiment ("worst-case" contamination scenario) that were still positive for DNA of *V. album* were also processed for NGS to test whether sequences of the contaminating *V. album* DNA can be obtained.

To analyze a broad spectrum of ingested plant species from the whole-body extracts of *D. suzukii* without a priori decision on focal groups, we used the universal primers B49317 and B49466 (Taberlet et al. 1991, 2007) that amplify a fragment of the *trn*L intron and modified them in terms of adding the binding region for the Illumina Nextera adaptors. The PCR was performed with the Type-it Mutation Detect PCR Kit (Qiagen), using additional BSA (Sigma-Aldrich) under the following PCR conditions: 5 min at 95 °C followed by 40 cycles of 20 s at 92 °C, 90 s at 55 °C, 60 s at 70 °C and 5 min at 70 °C once. Positive and negative controls (as described above) were included in these PCRs as well. The resulting PCR products were checked with a QIAxcel as described above for appropriate size and purified using the magnetic bead capture kit SPRIselect (left side selection) (Beckman Coulter, Inc., Brea, USA) as recommended by the manufacturer.

NGS of field-collected individuals

The NGS was conducted at the Department of Genomic and Applied Microbiology (University of Göttingen, Germany). Purified PCR products were used to attach indices and Illumina sequencing adapters using the Nextera XT Index kit (Illumina, San Diego, USA). Index PCR was performed using 5 µl of template PCR product, 2.5 µl of each index primer, 12.5 µl of 2× KAPA HiFi HotStart ReadyMix and 2.5 µl of molecular grade water. Thermal cycling scheme was as follows: 95 °C for 3 min, 8 cycles of 30 s at 95 °C, 30 s at 55 °C and 30 s at 72 °C and a final extension at 72 °C for 5 min. Quantification of the products was performed using the Quant-iT dsDNA HS assay kit and a Qubit fluorometer (Invitrogen GmbH, Karlsruhe, Germany) following the manufacturer's instructions. MagSi-NGSPREP Plus Magnetic beads (Steinbrenner Laborsysteme GmbH, Wiesenbach, Germany) were used for purification of the indexed products as recommended by the manufacturer, and normalization was performed using the Janus Automated Workstation (Perkin-Elmer, Waltham Massachusetts, USA). Sequencing was conducted at an Illumina MiSeq platform using dual indexing and MiSeq reagent kit v3 (600 cycles) according to the manufacturer's protocol.

NGS data processing

Quality control checks of the paired-end sequencing raw data files were run with the software FastQC-0.11.4 (Andrews 2010). Due to detected Nextera transposase sequences in each R1 (1.7–3.4%) and R2 file (11.5–20. 9%) of the 45 samples, reads were 3′ end trimmed using cutadapt-1.14 (Martin 2011) and afterward merged with PEAR-0.9.10 (Zhang et al. 2014). As universal chloroplast *trn*L primers for targeted sequencing were utilized, these primer sequences were

removed with cutadapt before doing local BLAST search. Therefore, all BLAST+ (Camacho et al. 2009) searches on the downloaded nt database (ftp://ncbi.nlm.nih.gov/blast/db/, status: 25/7/2017) were performed on the high-performance compute cluster LEO3e of the University of Innsbruck. Each sample file was split into file pieces containing 4000 or 8000 sequences using Linux split command and then executed separately, and for evaluation their output files joined together per sample with the Linux cat command.

Statistical analysis

Generalized linear models (GLMs) were used to analyze the influence of ingested *R. idaeus* DNA detection success over time using the package "MASS" (Venables and Ripley 2002), "VGAM" (Yee 2010) and "car" (Fox and Weisberg 2011). The GLM was performed using binomial family due to the binary type of the data (detection/no detection). Significance of terms was tested using Chi-square test and the function drop1. Models were simplified by removing nonsignificant interactions ($P > 0.05$) and nonsignificant factors. Factors that figured in significant interactions were kept in the model (Crawley 2002). The time point for food DNA detection probability of 25, 50 and 75% and their 95% confidence intervals were calculated using the package "drc" (Ritz et al. 2015). *T* tests were performed to test for significant differences in RFUs measured. Significance level was set at $P < 0.05$. All analyses were performed using R (R Development Core Team 2016).

For the analysis of the NGS sequence data, *t* tests for independent samples were performed using SPSS 24.0 to test for significant differences in the mean number of generated sequences per sample between bleached and non-bleached individuals. This comparison was conducted because the bleach treatment might negatively affect the DNA quality and/or the subsequent molecular downstream analysis.

Results

Detection of ingested plant DNA by diagnostic PCR

DNA of ingested *R. idaeus* was detected by diagnostic PCR in the guts of males and females for up to 24 and 48 h, respectively (Fig. 2). Over all time points tested in our experiment, *R. idaeus* DNA was detected in 38.2% of all analyzed specimens (males 39.9%; females 36.7%). The detection success of plant DNA was negatively related to digestion time (pseudo-$R^2 = 43.184$, SE = 0.143, $P < 0.005$) (Fig. 2). No significant differences were present in *R. idaeus* detection rates between males and females. Directly after the feeding period, the RFUs measured for ingested plant DNA, providing a proxy for the food plant DNA concentrations in the samples, showed no significant differences between males (3.3 ± 0.85 SD) and females (3.9 ± 1.11 SD) ($t = 1.4658$, $df = 16.835$, $P = 0.1611$).

Removal of eDNA via bleaching

All individuals were decontaminated successfully of eDNA (*V. album*) within the "authentic" scenario, whereas in 25% of females (5 out of 20 samples) tested in the "worst-case"

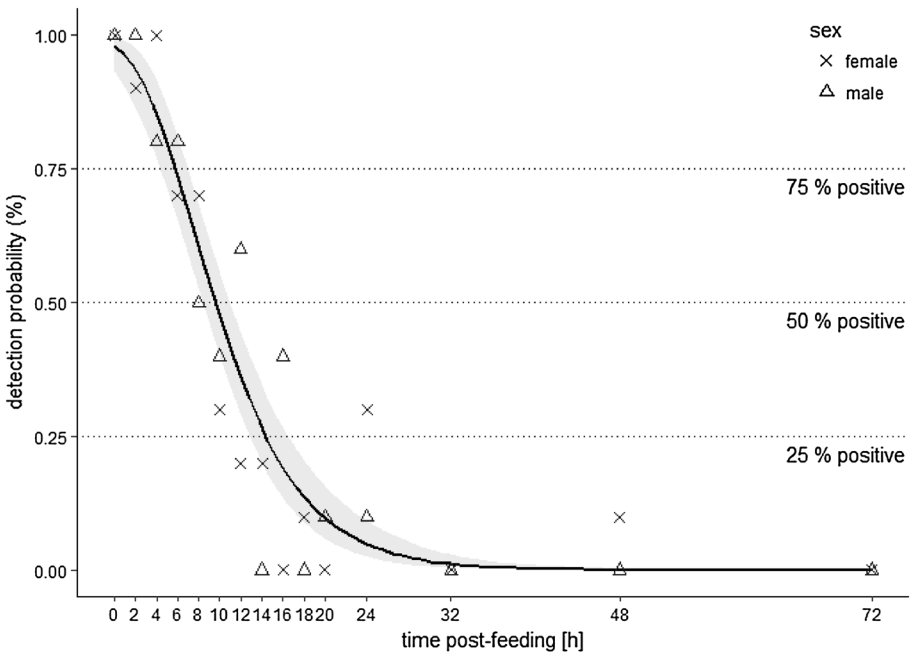

Fig. 2 Plant DNA detection success in whole-body extracts of female and male *D. suzukii* fed with raspberry (*R. idaeus*) at different time points ranging from 0 to 72 h post-feeding. Detection rates measured are provided as Δ = males and *x* = females and a fitted CLOGLOG model including the lower and upper 95% confidence intervals. Time points where the detection probability equals 25, 50 and 75% are given as dotted lines. Ten individuals were tested at each time point for each sex, except for 72 h (only 3 males survived)

scenario eDNA was still amplified. In the control groups ("no-bleach"), 79 and 100% of the females were tested positive for eDNA of *V. album* in the "authentic" and the "worst-case" scenario, respectively. Ingested DNA of *R. idaeus* stayed unaffected in both scenarios (Fig. 3). In four out of the five individuals which tested positive for mistletoe DNA, sequences of *V. album* were generated with NGS accounting for 2.3–37.2% of all sequences obtained from these samples.

Detection of ingested plant DNA in field-collected *D. suzukii* using NGS

In total, 6,282,930 sequences for 45 *D. suzukii* samples were generated, with a mean sequence number of 139,620 sequences per sample (range 39,264–396,573). After read trimming and merging, still 99.25% of the sequence data were appropriate for BLAST search.

All sequences with the minimum number of 1% of all sequences found within a specific sample were taken into account. Thus, we identified three plant species in field-collected *D. suzukii*: *R. idaeus*, *Urtica dioica* and *Polygonum humifusum*. Additionally, BLAST hits for *Prunus* spp., *Pinus* spp. or Rosaceae species other than *R. idaeus* were found but could not be identified at species level due to identical sequences for *trn*L amplicons within each genus (26 different *Prunus* and 69 *Pinus* species) or family (*Comarum salesovianum*, 2 *Sibbaldianthe*, 5 *Rosa*, 9 *Potentilla*, 11 *Fragaria* and 33 *Rubus* species with the same sequence).

In all except one of the field-collected individuals, the proportion of *R. idaeus* (or other unidentified Rosaceae) sequences made up for more than 90% of the reads obtained. DNA of *R. idaeus* was the most frequently found type of DNA as in 85% of the field-collected individuals only sequences of this species were identified. While in one sample the unidentified Rosaceae made up > 85% of all sequences, the other two samples where this taxon was detected yielded 7.4 and 1.4% of the total sequences per sample. Sequences from *Pinus* spp. were detected in one sample accounting for 14.2% of the reads, while the single sample detections of *Prunus* spp., *U. dioica* and *P. humifusum* stayed under 2% of the sequences generated (Table 1).

Assuming that the unknown Rosaceae sequences are likely to be *R. idaeus,* only raspberry sequences were found in bleached specimens, while in non-bleached flies three out of 20 flies provided sequences of four additional plant taxa. Also regarding the total number of sequences generated per sample showed no significant difference between bleached (149,348.9 ± 87,680.48 SD) and non-bleached (123,055 ± 80,294.03 SD) individuals ($t = 0.989$, $P = 0.329$).

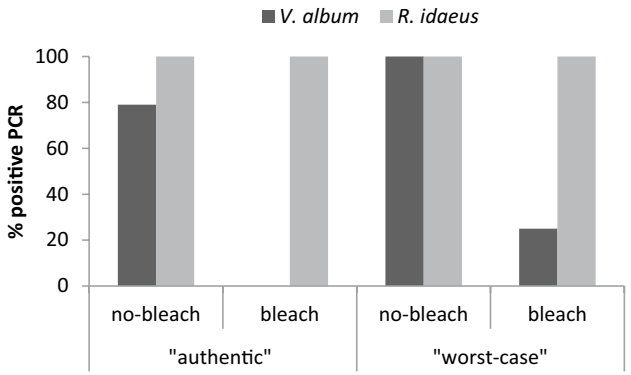

Fig. 3 Plant DNA detection rates (%) of mistletoe (*V. album)*, sticking on the outer body surface and raspberry (*R. idaeus)* in the gut content of *D. suzukii* in the "authentic" and the "worst-case" scenario. For each scenario, the percentage of positive non-decontaminated (no-bleach) and decontaminated (bleach) individuals is shown

Discussion

In this study, we developed a PCR-based method to detect plant DNA ingested by *D. suzukii*. The current results demonstrate that ingested plant DNA is amplifiable with both diagnostic PCR and NGS within whole-body extracts of *D. suzukii.* Additionally, eDNA was successfully removed from the flies' outer body surface using the developed bleaching protocol.

Post-feeding plant DNA detection in *D. suzukii* was possible for a maximum of 48 h post-feeding which is longer than in mirids such as *Apolygus lucorum* (~ 16–20 h) (Wang et al. 2017), but shorter than in caterpillars of *Helicoverpa armigera* or *Tuta absoluta* (50% detection after 24 h) (Pumarino

Table 1 Percentages of total reads per sample from gut content analysis of field-collected individuals of *D. suzukii* that showed more than one plant species in the NGS run. Provided are the sample name (#), the bleach treatment (bleach) followed by the plant taxa identified using BLAST

#	Bleach	*Rubus idaeus*	*Rosaceae*	*Prunus* spp.	*Pinus* spp.	*Urtica dioica*	*Polygonum humifusum*
a14	Yes	14.14	85.12				
a26	Yes	92.40	7.39				
a22	No	98.48	1.40				
a23	No	84.86			14.22		
a39	No	95.71				1.09	1.92
a41	No	96.63		1.29			

et al. 2011) and soil-living *Agriotes* larvae (> 72 h) (Staudacher et al. 2011). *Agriotes* larvae, *T. absoluta* and *H. armigera* are chewing plant tissue, whereas *A. lucorum* and *M. pigmaeus* have sucking mouthparts to take up plant sap, while *D. suzukii* is a sponging–feeding insect ingesting plant fluids. Considering the difference between chewing and liquid feeding insects, plant fluids are probably broken down faster by enzymatic processes than chewed plant tissue (Pumarino et al. 2011; Staudacher et al. 2011), explaining the relatively shorter post-feeding detection intervals found for *D. suzukii*. Besides the feeding mode, the ambient temperature might also affect food DNA detection success: this is because digestion, as any other enzymatic processes, is temperature dependent and the detection interval of food DNA is generally shortened by higher temperatures (von Berg et al. 2008; Hoogendoorn and Heimpel 2001; Hosseini et al. 2008). The extended detection rates post-feeding in *Agriotes* larvae may therefore also be ascribed to a lower ambient temperature of 16 °C during the experiments compared to 25 °C used for *A. lucosum* and *M. pigmaeus* (Pumarino et al. 2011; Wang et al. 2017). The latter are more similar to our study (23 °C) and thus might also contribute to the more similar post-feeding plant DNA detection intervals.

The sex of the flies might have possibly affected food DNA detection periods as, for example, in insects females are often bigger than conspecific males and thus have larger meal sizes (Hosseini et al. 2008). However, Sheppard et al. (2005), Hosseini et al. (2008) and Hoogendoorn and Heimpel (2001) did not find any influence by sex, weight or even the developmental stage on post-feeding food DNA detection periods, corroborating the current findings. Still, in our study, ingested DNA in females could be detected for longer post-feeding periods than in males. Directly after feeding, we found no significantly higher RFUs in females than in males; however, a tendency toward more food intake (higher RFUs) of females was identifiable, perhaps contributing to the longer post-feeding detection intervals in females compared to males.

Comparable to previous studies on herbivorous insects (Jurado-Rivera et al. 2009; Pumarino et al. 2011; Staudacher et al. 2011; Wallinger et al. 2013), we used whole-body DNA extracts to detect ingested plant DNA. To remove unwanted DNA which might be present on the body surface of the flies, we tested several bleach protocols and chose the best one to apply to the flies of our "authentic" and the "worst-case" contamination scenario. None of the individuals of the "authentic" scenario showed any contaminating DNA of *V. album* as indicated by the negative results obtained by diagnostic PCR. Thus, the newly adopted bleaching protocol for *D. suzukii* seems to be feasible for removing potential eDNA contamination without destroying the DNA of the ingested plants. Moreover, we showed that the detection of ingested plant DNA via NGS in whole-body extracts and the reads generated per sample are not negatively affected by the bleach treatment. In 25% of the individuals of the "worst-case" scenario, however, we were still able to amplify DNA of *V. album* after bleaching, indicating that the DNA in heavily contaminated specimens might not always be removed successfully. In all but one of these *V. album*-positive flies eDNA of mistletoe was also obtained by subjecting these specimens to NGS, highlighting the importance of the bleach cleaning step when analyzing food plant DNA via high-throughput sequencing.

Diagnostic PCR employing species-specific primers represents an efficient approach to test for a limited number of specific DNA types such as consumers from feeding experiments to determine post-feeding detection intervals. However, this approach becomes impractical in situations when many different food sources need to be identified. Here, NGS-based techniques provide a much better way to examine the broad spectrum of host plants which might be consumed by herbivores under field conditions (Valentini et al. 2009). In contrast to NGS assays with universal plant primers, a limited range of plant species is selected a priori when species- or genus-specific primers are used (Wallinger et al. 2012). However, this selection implies two disadvantages: (1) it is not adequate to detect unknown food sources and (2) it reflects only a small range of the plants that can be used as food source by polyphagous herbivores. Besides the *trn*L locus, the two other alternatives would be the chloroplast *rbc*L and *mat*K loci (Hollingsworth et al. 2016). We are not aware of a locus which would allow identifying all species from all plant families. Based on the description of the wide range of several plant families by Taberlet et al. (1991, 2007), the universal plant primer with the locus *trn*L was chosen in this study. Further studies have to consider that one particular DNA locus does most likely not enable an unambiguous identification of all plant species. Thus, when the identification on species level is required, especially without a detailed record of the vegetation, several universal plant primers might be employed.

By testing field-collected *D. suzukii* samples with the NGS approach, DNA of *R. idaeus* was detected in all individuals. Further, we identified additional plant species and genera in 6 of the 40 individuals tested. The BLAST hits for the family Rosaceae or *Prunus* spp. resulted in multiple species hits. *Prunus* spp. is known as host/food plant (Kanzawa 1939; Poyet et al. 2014; Uchino 2005), whereas this is not the case for *U. dioica*, *P. humifusum* and *Pinus* species. It is also important to note that the presented BLAST results only illustrate which of the DNA sequences is closest related to one species within the NCBI nt database. *Polygonum humifusum* is an Asian and North American species and to our knowledge not present in Europe. However, a related European species, *P. aviculare,* is very common. Since Tochen et al. (2016) found a higher survivorship of *D. suzukii* provided with flowers, we assume that

the collected individuals might have fed on nectar or pollen of flowering *P. aviculare* and *U. dioica* and/or on pollen of these plants which was wind-drifted to the raspberry bushes where the flies were collected from. *Pinus sylvestris* is cultivated at the JKI in Dossenheim and is also growing in the surroundings of the experimental fields. As *P. sylvestris* was not flowering during the sampling period, but made up a big proportion in the sequences of the respective sample, we suggest that this particular individual of *D. suzukii* may have fed on *Pinus* spp. tree sap as described by Kanzawa (1939) for *Quercus* spp. Another possibility explaining these finding might be the consumption of honeydew which has been mentioned as possible food source for *D. suzukii* (Lee et al. 2015; Walsh et al. 2011). As we do not know for field-collected specimens when and how much they fed, we may find host plant DNA in many different digestion stages. To minimize the influence on the variability on plant DNA detection success between individual samples, it is recommended to analyze a large number of samples. Moreover, there are studies which showed species-specific differences in the detection probabilities of plant DNA within the gut content of plant-eating insects (Wallinger et al. 2013), which suggests that such feeding experiments could be employed for important host plants to allow for a better interpretation of the field-derived molecular data.

Nevertheless, the detection of these other plant taxa might not depict feeding but external contamination with eDNA. This highlights the need to apply bleach cleaning procedures before DNA extraction. Moreover, the sampling in this study was conducted only in raspberries, and as the levels of eDNA contamination might vary between locations and habitats, bleaching field-collected specimens is strongly recommended. Furthermore, with increasing sequencing depth, minor amounts of contaminating eDNA might become visible (Oskar Rennstam Rubbmark personal communication).

In conclusion, our study demonstrates that the DNA of ingested plants can be identified from the gut contents of the polyphagous pest *D. suzukii* for several hours after the feeding event, allowing for a meaningful assessment of the diet of this important pest using DNA-based techniques. Moreover, it is recommended to decontaminate flies before they are subjected to DNA extraction to safeguard against erroneous host plant detections due to external eDNA. As such, our findings encourage the use of DNA-based gut content analysis in *D. suzukii* and other sponging–feeding dipterans to obtain a better understanding of their feeding ecology under natural conditions.

Author contribution statement

FB, KS, HV and MT conceived and designed the study. KS designed the primers. KS, CZ and FB established the molecular assays and performed the laboratory work. Flies were starved, fed and field-caught by FB at the JKI in Dossenheim, Germany. YG performed the bioinformatic analyses. CZ, YG and MT interpreted the NGS output and BLAST results. FB analyzed the data and compiled tables and figures. FB wrote the manuscript. CZ, YG, KS, MT and HV revised and improved the manuscript.

Acknowledgements We thank the Federal Ministry for Food and Agriculture (BMEL) of Germany for financial funding. We also acknowledge financial support through the Mountain Agriculture Research Unit ("Call for Projects 2016") for KS, D. Swarovski KG (Aktion D. Swarovski KG 2016) and a PhD scholarship (University of Innsbruck) for CZ. We thank Michael Fink for providing access to the *high-performance computing (HPC)* cluster systems as the computational (bioinformatics) results presented have been achieved (in part) using the HPC infrastructure LEO of the University of Innsbruck. Finally, we thank Anto Dominic Raja and Doreen Gabriel for statistical support and Juergen Just and Anja Frank (all JKI) for laboratory assistance and rearing *D. suzukii*.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Alnajjar G, Collins J, Drummond FA (2017) Behavioral and preventative management of *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) in Maine wild blueberry (*Vaccinium angustifolium* Aiton) through attract and kill trapping and insect exclusion-netting. Int J Entomol Nematol 3:51–61

Andrews S (2010) FastQC: a quality control tool for high throughput sequence data. Babraham Institute. http://www.bioinformatics.babraham.ac.uk/projects/fastqc

Asplen MK, Anfora G, Biondi A, Choi DS, Chu D, Daane KM, Gibert P, Gutierrez AP, Hoelmer KA, Hutchison WD, Isaacs R, Jiang ZL, Karpati Z, Kimura MT, Pascual M, Philips CR, Plantamp C, Ponti L, Vetek G, Vogt H, Walton VM, Yu Y, Zappala L, Desneux N (2015) Invasion biology of spotted wing Drosophila (*Drosophila suzukii*): a global perspective and future priorities. J Pest Sci 88:469–494. https://doi.org/10.1007/s10340-015-0681-z

Atallah J, Teixeira L, Salazar R, Zaragoza G, Kopp A (2014) The making of a pest: the evolution of a fruit-penetrating ovipositor in *Drosophila suzukii* and related species. Proc Biol Sci 281:20132840. https://doi.org/10.1098/rspb.2013.2840

Avanesyan A, Culley TM (2015) Feeding preferences of *Melanoplus femurrubrum* grasshoppers on native and exotic grasses: behavioral and molecular approaches. Entomol Exp Appl 157:152–163. https://doi.org/10.1111/eea.12356

Briem F, Eben A, Gross J, Vogt H (2016) An invader supported by a parasite: mistletoe berries as a host for food and reproduction of spotted wing Drosophila in early spring. J Pest Sci 89:749–759. https://doi.org/10.1007/s10340-016-0739-6

Calabria G, Maca J, Bachli G, Serra L, Pascual M (2012) First records of the potential pest species *Drosophila suzukii* (Diptera: Drosophilidae) in Europe. J Appl Entomol 136:139–147. https://doi.org/10.1111/j.1439-0418.2010.01583.x

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL (2009) BLAST+: architecture and applications BMC. Bioinformatics 10:421. https://doi.org/10.1186/1471-2105-10-421

Cini A, Ioriatti C, Anfora G (2012) A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. Bull Insectol 65:149–160

Crawley MJ (2002) Statistical computing: an introduction to data analysis using S-Plus. Wiley, New York

De la Cadena G, Papadopoulou A, Maes JM, Gomez-Zurita J (2017) Evaluation of bias on the assessment of diet breadth of herbivorous insects using molecular methods. Insect Sci 24:194–209. https://doi.org/10.1111/1744-7917.12303

Farnsworth D, Hamby KA, Bolda M, Goodhue RE, Williams JC, Zalom FG (2017) Economic analysis of revenue losses and control costs associated with the spotted wing Drosophila, *Drosophila suzukii* (Matsumura), in the California raspberry industry. Pest Manag Sci 73:1083–1090. https://doi.org/10.1002/ps.4497

Fox J, Weisberg S (2011) An R companion to applied regression, 2nd edn. Sage, Thousans Oaks CA

Garcia-Robledo C, Erickson DL, Staines CL, Erwin TL, Kress WJ (2013) Tropical plant–herbivore networks: reconstructing species interactions using DNA barcodes. PLoS ONE 8:e52967. https://doi.org/10.1371/journal.pone.0052967

Greenstone MH, Weber DC, Coudron TA, Payton ME, Hu JS (2012) Removing external DNA contamination from arthropod predators destined for molecular gut-content analysis. Mol Ecol Resour 12:464–469. https://doi.org/10.1111/j.1755-0998.2012.03112.x

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Hamby KA, Bellamy DE, Chiu JA, Lee JC, Walton VM, Wiman NG, York RM, Biondi A (2016) Biotic and abiotic factors impacting development, behavior, phenology, and reproductive biology of *Drosophila suzukii*. J Pest Sci 89:605–619. https://doi.org/10.1007/s10340-016-0756-5

Hauser M (2011) A historic account of the invasion of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in the continental United States, with remarks on their identification. Pest Manag Sci 67:1352–1357. https://doi.org/10.1002/ps.2265

Hollingsworth PM, Li DZ, van der Bank M, Twyford AD (2016) Telling plant species apart with DNA: from barcodes to genomes. Philos Trans R Soc Lond B Biol Sci. https://doi.org/10.1098/rstb.2015.0338

Hoogendoorn M, Heimpel GE (2001) PCR-based gut content analysis of insect predators: using ribosomal ITS-1 fragments from prey to estimate predation frequency. Mol Ecol 10:2059–2067. https://doi.org/10.1046/j.1365-294X.2001.01316.x

Hosseini R, Schmidt O, Keller MA (2008) Factors affecting detectability of prey DNA in the gut contents of invertebrate predators: a polymerase chain reaction-based method. Entomol Exp Appl 126:194–202. https://doi.org/10.1111/j.1570-7458.2007.00657.x

Junnila A, Muller GC, Schlein Y (2011) Identification of plant tissues from the gut of *Phlebotomus papatasi* by DNA analysis. Acta Trop 117:14–18. https://doi.org/10.1016/j.actatropica.2010.08.019

Jurado-Rivera JA, Vogler AP, Reid CA, Petitpierre E, Gomez-Zurita J (2009) DNA barcoding insect-host plant associations. Proc Biol Sci 276:639–648. https://doi.org/10.1098/rspb.2008.1264

Kanzawa T (1939) Studies on *Drosophila suzukii* Mats. Kofu, Yamanashi agricultural experiment station 49 (abstr.). Rev Appl Ent 29:622

Kenis M, Tonina L, Eschen R, van der Sluis B, Sancassani M, Mori N, Haye T, Helsen H (2016) Non-crop plants used as hosts by *Drosophila suzukii* in Europe. J Pest Sci (2004) 89:735–748. https://doi.org/10.1007/s10340-016-0755-6

Lee JC, Dreves AJ, Cave AM, Kawai S, Isaacs R, Miller JC, Van timmeren S, Bruck DJ (2015) Infestation of wild and ornamental noncrop fruits by *Drosophila suzukii* (Diptera: Drosophilidae). Ann Entomol Soc Am 108:117–129. https://doi.org/10.1093/aesa/sau014

Lima LH, Mesquita MR, Skrip L, de Souza Freitas MT, Silva VC, Kirstein OD, Abassi I, Warburg A, Balbino Vde Q, Costa CH (2016) DNA barcode for the identification of the sand fly *Lutzomyia longipalpis* plant feeding preferences in a tropical urban environment. Sci Rep 6:29742. https://doi.org/10.1038/srep29742

Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnetJournal. https://doi.org/10.14806/ej.17.1.200

Mazzetto F, Pansa MG, Ingegno BL, Tavella L, Alma A (2015) Monitoring of the exotic fly *Drosophila suzukii* in stone, pome and soft fruit orchards in NW Italy. J Asia Pac Entomol 18:321–329. https://doi.org/10.1016/j.aspen.2015.04.001

Mazzi D, Bravin E, Meraner M, Finger R, Kuske S (2017) Economic impact of the introduction and establishment of *Drosophila suzukii* on sweet cherry production in Switzerland. Insects. https://doi.org/10.3390/insects8010018

Poyet M, Eslin P, Heraude M, Le Roux V, Prevost G, Gibert P, Chabrerie O (2014) Invasive host for invasive pest: when the Asiatic cherry fly (*Drosophila suzukii*) meets the American black cherry (*Prunus serotina*) in Europe. Agric For Entomol 16:251–259. https://doi.org/10.1111/afe.12052

Poyet M, Le Roux V, Gibert P, Meirland A, Prevost G, Eslin P, Chabrerie O (2015) The wide potential trophic niche of the Asiatic fruit fly *Drosophila suzukii*: the key of its invasion success in temperate Europe? PLoS ONE 10:e0142785. https://doi.org/10.1371/journal.pone.0142785

Pumarino L, Alomar O, Agusti N (2011) Development of specific ITS markers for plant DNA identification within herbivorous insects. Bull Entomol Res 101:271–276. https://doi.org/10.1017/S0007485310000465

R Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/

Remen C, Kruger M, Cassel-Lundhagen A (2010) Successful analysis of gut contents in fungal-feeding oribatid mites by combining body-surface washing and PCR. Soil Biol Biochem 42:1952–1957. https://doi.org/10.1016/j.soilbio.2010.07.007

Renkema JM, Wright D, Buitenhuis R, Hallett RH (2016) Plant essential oils and potassium metabisulfite as repellents for *Drosophila suzukii* (Diptera: Drosophilidae). Sci Rep 6:21432. https://doi.org/10.1038/srep21432

Ritz C, Baty F, Streibig JC, Gerhard D (2015) Dose-response analysis using R. PLoS ONE 10:e0146021. https://doi.org/10.1371/journal.pone.0146021

Sheppard SK, Bell J, Sunderland KD, Fenlon J, Skervin D, Symondson WO (2005) Detection of secondary predation by PCR analyses of the gut contents of invertebrate generalist predators. Mol Ecol 14:4461–4468. https://doi.org/10.1111/j.1365-294X.2005.02742.x

Staudacher K, Wallinger C, Schallhart N, Traugott M (2011) Detecting ingested plant DNA in soil-living insect larvae. Soil Biol Biochem 43:346–350. https://doi.org/10.1016/j.soilbio.2010.10.022

Staudacher K, Schallhart N, Thalinger B, Wallinger C, Juen A, Traugott M (2013) Plant diversity affects behavior of generalist root herbivores, reduces crop damage, and enhances crop yield. Ecol Appl 23:1135–1145

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol Biol 17:1105–1109

Taberlet P, Coissac E, Pompanon F, Gielly L, Miquel C, Valentini A, Vermat T, Corthier G, Brochmann C, Willerslev E (2007) Power and limitations of the chloroplast *trn*L (UAA) intron for plant DNA barcoding. Nucleic Acids Res 35:e14. https://doi.org/10.1093/nar/gkl938

Tochen S, Walton VM, Lee JC (2016) Impact of floral feeding on adult *Drosophila suzukii* survival and nutrient status. J Pest Sci 89:793–802. https://doi.org/10.1007/s10340-016-0762-7

Uchino K (2005) Distribution and seasonal occurrence of cherry *Drosophila suzukii* (Diptera: Drosophilidae) injurious to blueberry in Chiba Prefecture. Ann Rep Kanto Tosan Plant Prot Soc 52:95–97

Valentini A, Miquel C, Nawaz MA, Bellemain E, Coissac C, Pompanon F, Gielly L, Cruaud C, Nascetti G, Wincker P, Swenson JE, Taberlet P (2009) New perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: the *trn*L approach. Mol Ecol Resour 9:51–60. https://doi.org/10.1111/j.1755-0998.2008.02352.x

Venables WN, Ripley BD (2002) Modern applied statistics with S, 4th edn. Springer, New York

von Berg K, Traugott M, Symondson WO, Scheu S (2008) The effects of temperature on detection of prey DNA in two species of carabid beetle. Bull Entomol Res 98:263–269. https://doi.org/10.1017/S0007485308006020

Wallinger C, Juen A, Staudacher K, Schallhart N, Mitterrutzner E, Steiner E-M, Thalinger B, Traugott M (2012) Rapid plant identification using species- and group-specific primers targeting chloroplast DNA. PlosOne 7(1):e29473. https://doi.org/10.1371/journal.pone.0029473

Wallinger C, Staudacher K, Schallhart N, Peter E, Dresch P, Juen A, Traugott M (2013) The effect of plant identity and the level of plant decay on molecular gut content analysis in a herbivorous soil insect. Mol Ecol Resour 13:75–83. https://doi.org/10.1111/1755-0998.12032

Wallinger C, Sint D, Baier F, Schmid C, Mayer R, Traugott M (2015) Detection of seed DNA in regurgitates of granivorous carabid beetles. Bull Entomol Res 105:728–735. https://doi.org/10.1017/S000748531500067X

Walsh DB, Bolda MP, Goodhue RE, Dreves AJ, Lee J, Bruck DJ, Walton VM, O'Neal SD, Zalom FG (2011) *Drosophila suzukii* (Diptera: Drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and damage potential. J Integr Pest Manag 2:G1–G7. https://doi.org/10.1603/ipm10010

Wang Q, Bao WF, Yang F, Xu B, Yang YZ (2017) The specific host plant DNA detection suggests a potential migration of *Apolygus lucorum* from cotton to mungbean fields. PLoS ONE 12:e0177789. https://doi.org/10.1371/journal.pone.0177789

Yee TW (2010) The VGAM package for categorical data analysis. J Stat Softw 32:1–34. https://doi.org/10.18637/jss.v032.i10

Zhang JJ, Kobert K, Flouri T, Stamatakis A (2014) PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics 30:614–620. https://doi.org/10.1093/bioinformatics/btt593