

ORIGINAL ARTICLE

First molecular evidence of an invasive agricultural pest, *Drosophila suzukii*, in the diet of a common bat, *Pipistrellus pipistrellus*, in Belgian orchards

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ABSTRACT

Bats are major consumers of arthropods, including many agricultural pest species, and can thus reduce and prevent crop damage. However, few, if any, data is available on the potential role of bats in pest control in central Europe. Evidence that bats prey upon locally important pest species would be an important first step to demonstrate their value to local farmers and facilitate conservation measures. In this pilot study, we used a DNA metabarcoding approach to investigate the diet composition of common pipistrelles and brown long-eared bats captured in orchards in Belgium. We show that the spotted wing drosophila (*Drosophila suzukii*), one of the most harmful pest species in this region, was part of the diet of common pipistrelles. This pest species was recorded in one of the five samples from common pipistrelles. Our results indicate that bats can be valuable assets for biological pest suppression in West-European orchards, thus setting a path for future studies.

INTRODUCTION

Farmers are increasingly confronted with the need to consider all available pest control techniques and select appropriate measures, while minimizing risks to human health and the environment. Such integrated pest management (FAO 2020) encourages natural pest control. Many bat species, with diverse foraging strategies, forage intensively in agricultural systems and can thus deliver such an ecosystem service (Boyles et al. 2011, Russo et al. 2018). Recent studies using molecular methods to investigate diet composition have shown that bat species prey upon pest species. For instance, a study at several sites across southern Europe shows that the diet of *Miniopterus schreibersii* includes more than 40 agricultural pest species affecting diverse crop types, ranging from rice paddies to corn fields, to olive groves (Aizpurua et al. 2018). Moreover, large-scale field experiments in both tropical and temperate zone agricultural systems show that predation by bats can control herbivorous insect populations, and cascading effects can lead to increased crop yields (Maas et al. 2013, Maine & Boyles 2015). Yet, scarce data is available from central and northern Europe (Russo et al. 2018). Evidence that bats prey upon locally important pest species is the first step to demonstrate their value to local farmers and to stimulate further studies.

One such agricultural pest species is *Drosophila suzukii*, an invasive species of Asian origin that affects a wide range of soft-skinned fruits such as blueberries, blackberries, plums and cherries. Since its introduction in the late 2000's this species spread rapidly across North America and Europe (Asplen et al. 2015). Contrary to many other *Drosophila* species, females of *D. suzukii* deposit their eggs in intact ripening fruits, after which their larvae rapidly destroy the fruits (Rota-Stabelli et al. 2013). For farmers, the use of insecticides is often not effective because the larvae develop deep enough inside the fruit to avoid contact. There are currently no effective and environmentally sustainable pest control methods for this species, resulting in extensive damage to fruit crops in Europe and North America (Schetelig et al. 2018). For instance, in Belgium, since its first record in 2011 (Mortelmans et al. 2012), *D. suzukii* has become the most important pest species in orchards, leading to yearly economic losses of ca. 20 million euro (Belien et al. 2014, Nijland 2015).

Many species of bats forage intensively in European orchards (Stahlschmidt et al. 2017). A recent study recorded *D. suzukii* in the diet of *Rhinolophus hipposideros*, *Pipistrellus pipistrellus* and *P. kuhlii* (Galan et al. 2018) in Western France. By DNA metabarcoding faeces from captured bats, we set out to investigate if bats foraging in Belgian orchards prey upon *D. suzukii*.

MATERIAL AND METHODS

Field sampling

In May and August 2016, bats were captured with mist nets (Ecotone, Poland) whilst they were foraging in orchards in the Limburg region in Belgium (Table 1). In this region, high activity of *Pipistrellus* bats has been observed during acoustic surveys in orchards. Captured bats were kept individually in clean cotton bags for up to 30 min, and droppings were collected from the bag and stored in pure ethanol. At three sites, samples from different captured individuals were pooled (see Table 1). Additionally, two maternity roosts situated in the middle of orchards were visited, and fresh droppings were collected (Table 1). Droppings were stored at room temperature until extraction. Apart from *P. pipistrellus* and *Plecotus auritus*, we also captured *Myotis bechsteinii*, but could not collect faeces of this species within the 30 min time span. Bat captures and handling was carried out under license and guidelines from the Belgian authorities (permit ANB/BL-FF/V15-00095). All bats were released unharmed at the capture site.

DNA extraction

DNA was extracted from the 8 faecal samples (one to three pooled droppings; see Table 1) using ZR-96 Faecal DNA Kit (cat. nr D6011; Zymo Research Corp., Irvine, USA). We used whole droppings as starting material (samples dried briefly on clean paper). DNA was eluted into 100 µL of BE buffer and stored at –20°C until analysis.

PCR and library construction

We used a single primer pair to amplify the mitochondrial *cytochrome c oxidase subunit I* gene (COI) of potential prey (ZBJ-ArtF1c and ZBJ-ArtR2c; Zeale et al. 2011). Despite the potential bias in these primers toward some Orders (Diptera and Lepidoptera; Clarke et al. 2014), we chose COI region because of the well-developed reference library and these specific primers to allow comparison of our results with earlier studies (eg. Krüger et al. 2014, Rydell et al. 2016, Vesterinen et al. 2016, 2018, Aizpurua et al. 2018). The PCR and library construction closely followed Kaunisto et al. (2017). The first-step PCR reactions—prepared in two replicates—included locus-specific primers targeting prey COI gene, and the second PCR followed directly after this including Illumina-specific adapters with a unique dual-index combination for each single reaction. Cycling conditions for COI were 3 min in 95°C, then 16 cycles of 30 s in 95°C, 30 s in 61°C (with the annealing temperature decreased by 0.5°C for each cycle) and 30 s in 72°C, then additional 24 cycles of 30 s in 95°C, 30 s in 53°C and 30 s in 72°C ending with 3 min in 72°C. The samples were pooled by equal volume (2 µL each library) and purified using dual-SPRI (Solid Phase Reversible Immobilisation) beads following the protocol in Vesterinen et al. (2016). Sequencing was performed on an Illumina MiSeq platform at the Functional Genomics Unit (FuGU) of the University of Helsinki, Finland using v2 (300 cycles) 2 × 150 bp paired-end sequencing Bioinformatics and prey list construction.

The Illumina sequencing as a whole yielded 10,161,700 paired-end reads identified to samples with unique dual-index combinations. Trimming and quality control of the sequences were conducted according to Vesterinen et al. (2018). Consequently, paired-end reads were merged and trimmed for quality using program USEARCH (Edgar 2010). Primers were removed using the program cutadapt (Martin 2011). The reads were then collapsed into unique sequences (singletons removed), after which the sequences were clustered into zero-radius operational taxonomical units (ZOTU; Edgar 2016) and mapped back to the original trimmed reads to establish the total number of reads in each sample using USEARCH ‘unnoise3’ algorithm. In short, the UNOISE algorithm allows detection and removal of chimeras (PCR artefacts where two fragments of different origin bind together) and point errors (substitutions and gaps resulting from incorrect or omitted base calls), and results in zero-radius OTUs (ZOTUs). After processing, our dataset consisted of 141,369 prey reads. The data was filtered so that each ZOTU was discarded from samples where only one replicate produced reads (reducing the stochasticity of the PCR) for that specific sample. Furthermore, ZOTU was discarded from the sample if fewer reads were found from that sample compared to any negative control (reducing the effect of so-called tag jumping or sample cross-talk). Finally, we compared our ZOTU sequences to the BOLD reference database, which is by far the most comprehensive curated COI database (Ratnasingham & Hebert 2007). We used the following criteria for including prey species in the final data: (1) sequence similarity with the reference sequence of minimum 96% for the ZOTU to be given any – even higher order taxa – assignment, (2) minimum ten reads of the assigned prey species and (3) assigned prey species recorded in Belgium (<https://observations.be>). After the above trimming, we identified and retained 77.34% of the prey reads.

To visualize the prey use for both bat species, we analyzed the data using package ‘bipartite’ (Dormann et al. 2009) in R v 3.4.3 (R core team 2017) to construct quantitative and semi-quantitative bipartite graphs based on relative read abundance (RRA; number of OTU reads/sum of all OTU reads) and percentage of occurrences (POO; Deagle et al. 2019) in the samples. The most common prey items (relative read abundance of at least 15% for RRA data; percentage of occurrences of at least 5% for POO data) were highlighted in the bipartite graphs.

RESULTS

In this study, we analysed five samples of *Pipistrellus pipistrellus* and three of *Plecotus auritus*. We recovered 37 taxa of 22 arthropod families (Table 2; Fig. 1). *D. suzukii* was found in one of the five samples from *P. pipistrellus* (sample ID 4 in Table 1). In this sample of a single individual, *D. suzukii* was the most abundantly recovered taxon, accounting for 29.87% of the reads. Across the sequenced region, these reads were between 97.4 and 100% identical only to sequences of *D. suzukii* in BOLD.

Apart from *D. suzukii*, we recorded several other species that can cause agricultural damage in the investigated samples, namely *Tipula oleracea*, *Delia platura* and *Autographa*

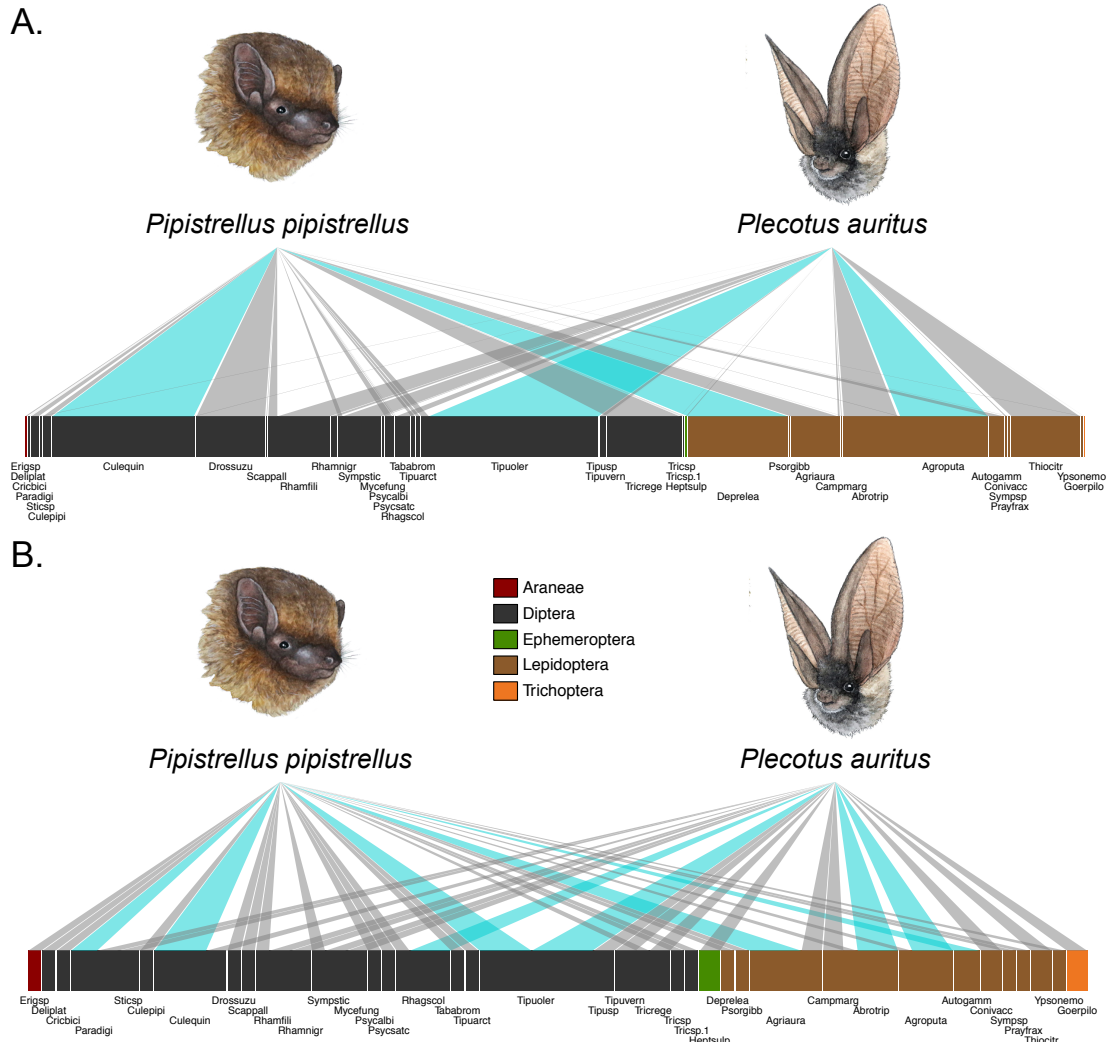


Fig. 1 - Bipartite graphs of the two bat species and their prey species. The upper rows represent predators and the blocks in the lower rows the prey species. A line connecting a predator with a prey represents a detected predation record, and the thickness of the line represents A) the relative read abundance (RRA; see Deagle et al. 2019) or B) the percentage of occurrence (POO; Deagle et al. 2019) in the diet. The abbreviations below the lower blocks correspond to the prey species in Table 2. Bat drawings: Maija Laaksonen.

Table 1 - Overview of the samples, with bat species, type of sample, capture date, latitude & longitude (WGS84), and fruit type of the orchards.

ID	Bat species	Type of sample	Date	Lat	Lon	Fruit types at the study sites
1	<i>P. pipistrellus</i>	Sample from a maternity roost	17/08/2016	50.773	5.159	Building: Surrounded by a mix of orchards of Sweet Cherry, Apple, Pear, Strawberry, Blackberry, Raspberry
2	<i>P. pipistrellus</i>	Combined sample of 3 adult females	04/08/2016	50.899	5.222	Orchard: Mix of Apple & Pear
3	<i>P. pipistrellus</i>	Combined sample of 3 adult females	08/08/2016	50.730	5.243	Forest nearby orchards of Cherry, Apple & Pear
4	<i>P. pipistrellus</i>	Sex and age unknown	24/08/2016	50.898	5.402	Mix of Sweet Cherry, Apple & Pear
5	<i>P. pipistrellus</i>	Combined sample of 3 adult females	08/08/2016	50.730	5.243	Forest edge near orchard of Cherry, Apple & Pear
6	<i>P. auritus</i>	Sample from a maternity roost	17/08/2016	50.773	5.159	Building: Surrounded by a mix of orchards of Sweet Cherry, Apple, Pear, Apricot, Strawberry, Blackberry, Raspberry
7	<i>P. auritus</i>	Adult female	21/05/2016	50.913	5.391	Sweet Cherry
8	<i>P. auritus</i>	Adult female	21/05/2016	50.913	5.391	Sweet Cherry

Table 2 - The prey taxa identified in the study, with the relative proportion of reads in each bat species' samples. The abbreviations in the first column correspond to Fig. 1

	Higher taxa	Species	Host and pest status	<i>P. pipistrellus</i>	<i>P. auritus</i>
	ARACHNIDA				
	Araneae				
Erigsp	Linyphiidae	<i>Erigone sp.</i>		0.41	0
	INSECTA				
	Diptera				
Deliplat	Anthomyiidae	<i>Delia platura</i>	Phaseolus vulgaris, Zea mays; EPPO 2020	0.04	0
Cricbici		<i>Cricotopus bicinctus</i>		0.08	0
Paradigi	Chironomidae	<i>Parachironomus digitalis</i>		2.35	0.02
Sticsp		<i>Stictochironomus sp.1</i>		0	0.45
Culepipi	Culicidae	<i>Culex pipiens</i>		1.87	0
Culequin		<i>Culex sp.</i>		9.7	0.35
Drossuzu	Drosophilidae	<i>Drosophila suzukii</i>	Fruit crops; Rota-Stabelli et al. 2013	2.71	0
Scappall		<i>Scaptomyza pallida</i>		0.21	0
Rhamfili	Empididae	<i>Rhamphomyia sp.1</i>		2.1	0.24
Rhamnigr		<i>Rhamphomyia sp.2</i>		0	0.03
Sympstic	Limoniidae	<i>Symplecta stictica</i>		1.02	0.18
Mycefung	Mycetophilidae	<i>Mycetophila fungorum</i>		0	0.56
Psycalbi	Psychodidae	<i>Psychoda albipennis</i>		2.33	0
Psycsatc		<i>Psychoda satchelli</i>		0.23	0
Rhagscol	Rhagionidae	<i>Rhagio scolopaceus</i>		0.08	3.49
Tababrom	Tabanidae	<i>Tabanus bromius</i>		0.87	0
Tipuarct		<i>Tipula sp.1</i>		0.86	0
Tipuoler	Tipulidae	<i>Tipula oleracea</i>	Fruit crops; Carter 1984, Hill 2002, Bailey 2007, Alford 2012, 2014	0.18	56.39
Tipusp		<i>Tipula sp.2</i>		0	1.18
Tipuvern		<i>Tipula vernalis</i>		0	0.03
Tricrege		<i>Trichocera regelationis</i>		23.5	0.1
Tricsp	Trichoceridae	<i>TrichoceridaeIntGen1 sp.</i>		0.18	0
Tricsp.1		<i>TrichoceridaeIntGen2 sp.</i>		0.32	0
Heptsulp	Ephemeroptera				
Deprelea	Heptageniidae	<i>Heptagenia sulphurea</i>		0	0.27
	Lepidoptera				
Psorgibb	Depressariidae	<i>Depressaria sp.</i>		38.75	0
Agriaura	Gelechiidae	<i>Psoricoptera gibbosella</i>		0.04	0

gamma in the diet of *P. pipistrellus* and *T. oleracea* and *Campaea margaritaria* in the diet of *P. auritus* (Table 2). Some of these were rather common in the diet, as can be seen from the web (Fig. 1). Most other identified taxa in the diet of *P. pipistrellus* included typical prey for aerial-hawking bats such as Chironomidae, Culicidae and other small flies, and several families of Lepidoptera and Trichoptera. Most identified prey taxa in the samples of *P. auritus* were Lepidoptera.

DISCUSSION

We recovered 37 taxa of 22 arthropod families in our samples. Despite the limited sample size, *D. suzukii* was recorded in one out of the five samples from *P. pipistrellus*. These results indicate that *P. pipistrellus*, the most common species in Belgium, potentially acts as a biological pest controller in orchards. In our study the sample size was low, but in light of earlier findings the pattern becomes more interesting. A

recent study from France found *D. suzukii* in faecal samples of *R. hipposideros* (1/11), *P. pipistrellus* (1/31) and *P. kuhlii* (1/3) (Galan et al. 2018). Moreover, other Drosophilidae species were recorded in the diet of *P. pygmaeus*, *Myotis daubentonii*, *M. brandtii*, *Eptesicus nilssonii* and *P. auritus* in Finland and Sweden (Vesterinen et al. 2013, 2018, Rydell et al. 2016), indicating that other bat species could potentially forage on this species. We recorded four additional pest species that, albeit to a lesser extent, cause damage in agricultural systems. *T. oleracea* was observed in the samples of both species, while *C. margaritata* was found in the diet of *P. auritus* and *A. gamma* and *D. platura* in the diet of *P. pipistrellus*.

High numbers of *D. suzukii* are active at dawn and dusk, but not during the night (Evans et al. 2017). Pipistrelle bats mainly catch their prey in the air, so they likely feed on flying *D. suzukii* individuals at dawn and dusk. *P. pipistrellus* emerges relatively early from their roosts (15 to 30 minutes after sunset), and forages close to their roosts (average distance 1.5 km; Dietz & Kiefer 2016). Especially bats roosting in or near orchards may thus predate on *D. suzukii*. Monitoring during the whole year shows that *D. suzukii* populations in orchards rapidly grow in August and reach their peak in autumn. During mild winters and in the subsequent spring months, the species remains present (Belien et al. 2014). We recorded *D. suzukii* in a sample collected in late August. It would be of great interest to investigate to what extent the species is preyed upon by bats across the entire season. Future studies should investigate to what extent predation by bats can indeed decrease the population numbers of *D. suzukii* or other pest species. In this context, experimentally excluding bats from crops, and subsequently comparing crop damage or pest numbers with those in control plots would be highly interesting (e.g. Maas et al. 2013, Maine & Boyles 2015).

Nevertheless, the evidence that a common bat species consumes economically important agricultural pests could already provide incentives for bat conservation in agricultural landscapes (Kross et al. 2018). Many bats are threatened, among other factors due to roost destruction, agricultural intensification and pesticide use (Dietz & Kiefer 2016). Management actions for bats could thus not only increase potential ecosystem services provided to farmers, but also be vital to conserve bat populations in agricultural landscapes (Russo et al. 2018). Such actions should include the creation and preservation of ponds, hedgerows and treelines both locally and at the landscape scale (Heim et al. 2018), protecting existing roost sites (Russo et al. 2018), avoiding artificial light at night (Stone et al. 2015) and moderating pesticide use (Stahlschmidt & Brühl 2012).

To conclude, we show that a common bat species preys upon an economically important agricultural pest in Belgian orchards. This indicates that bats could act as biological pest suppressors, and can stimulate future studies.

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