Environmental Entomology, XX(XX), 2023, 1–7
https://doi.org/10.1093/ee/nvad086
Research

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Plant-Insect Interactions

The aphid *Myzus persicae* (Hemiptera: Aphididae) acquires chloroplast DNA during feeding on host plants

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Subject Editor: Christopher Ranger

Received on 22 May 2023; revised on 28 July 2023; accepted on 15 August 2023

Aphids (Hemiptera: Aphididae) extract nutrients from host plant phloem via stylets that facilitate salivation and sap uptake. When navigating to the phloem, aphids periodically puncture nonvascular cells and sample cell contents, but rarely cause significant cell damage. As a result, aphids are considered "stealthy" feeders. In contrast, insects that do cause damage, such as chewing herbivores, will take up host cell contents—including DNA—into their guts. Researchers can use molecular barcoding methods to identify recent host use patterns of chewing herbivores. This information is valuable for both pest management and basic ecological studies. Because of their stealthy feeding style, it was assumed that host plant DNA could not be recovered from aphids and other Sternorrhyncha. However, several recent studies document host plant DNA uptake by psyllids, which feed in a similar manner to aphids. Therefore, we hypothesized that aphids may also acquire DNA from host plants. Since aphids puncture and sample cytosol contents from cells, we predicted that aphids would be most likely to acquire DNA from chloroplasts. To test this, we performed host feeding and host transfer experiments with *Myzus persicae* (Sulzer), then used PCR to recover and sequence a region between the *trnT* and *trnF* genes from acquired chloroplast DNA. We found that *M. persicae* readily acquires chloroplast DNA, even prior to phloem contact, and that fragment sizes sufficient for host plant identification can be recovered. Our work suggests that molecular gut content analysis is a viable tool for studying aphid–host interactions.

Keywords: gut content analysis, *Capsicum annuum*, *Brassica napus*, *trnL*

Introduction

Molecular gut content analysis is a well-established technique for identifying trophic links among arthropods and other organisms in the environment. The first studies to apply DNA barcoding approaches to identify ingested material in terrestrial arthropods focused on identifying key predators of prey groups for which taxon-specific targets could be developed (e.g., Agusti et al. 1999, Zaidi et al. 1999, Chen et al. 2000). The focus of terrestrial arthropod gut content analysis remained on predator–prey interactions until genetic barcode options were validated for plants, which facilitated PCR-based gut content analysis of herbivorous insects and molecular analysis of pollen in and on pollinators (Jurado-Rivera et al. 2009, Macgregor et al. 2019). In the last decade, the number of studies applying DNA barcoding to the study of herbivore diets increased steadily (Avanesyan et al. 2021b). Work in this area focused on organisms that cause noticeable physical damage to plant hosts through the breakdown of plant cells, including mobile chewing herbivores (e.g., Coleoptera, Orthoptera) (Avanesyan 2014, Avanesyan et al. 2021b, Palmer et al. 2022). Among sap-feeding organisms, studies have found evidence of plant DNA uptake by Hemiptera in the suborder Auchenorrhyncha, including the potato leafhopper (Hemiptera: Cicadellidae), which has a lacerate-and-flush feeding style (Avanesyan et al. 2021a), and the spotted lanternfly (Hemiptera: Fulgoridae) (Avanesyan and Lamp 2020). In both cases, the plant DNA acquired was from the chloroplast genome.

Hemipteran insects in the suborder Sternorrhyncha make minimal incursions into nonvascular cells and generally avoid destroying cells when they do so (Walling 2008). Because of this, most Sternorrhyncha are excellent vectors of microbes that require living cells to establish infections (plant viruses and phloem-limited bacteria) but were not thought to be able to acquire plastid or genomic DNA from their hosts. However, two studies provide evidence that phylogenetically divergent Sternorrhyncha can acquire plant DNA during feeding activities. Matheson et al. (2008) demonstrated uptake of host chloroplast DNA by an aphid (*Myzus persicae*) through amplification and sequencing of short regions (157 bp) of the chloroplast ribulose bisphosphate carboxylase (*rbcL*) gene. This effort was enough to confirm DNA acquisition, but amplicons of only

~150 bp are insufficient for identifying hosts using public databases. Later, Cooper et al. (2016) showed that potato psyllids (*Bactericera cockerelli* Šulc *[Hemiptera: Triozidae]*) can acquire and retain both chloroplast and genomic DNA (Cooper et al. 2016). Subsequent application of barcoding-based gut content analysis to psyllids via high-throughput sequencing revealed that numerous unexpected hosts are being used by pest psyllid species in addition to known crop hosts (Cooper et al. 2019). This landscape-scale analysis of psyllid movements provides insight into nonhosts that may serve as unexpected sources of psyllids colonizing crops, and even as possible reservoirs for the pathogens they transmit.

The discovery that hemipterans can acquire and retain host plant DNA opens numerous avenues for the expanded study of pest population dynamics. Such efforts would also be beneficial for understanding the ecology of other sternorrhynchan vectors, particularly aphids, which are major vectors of plant viruses. Because of their economic impacts as virus vectors, there are already extensive sampling efforts for aphids (e.g., suction trap networks, grower-based pan trapping and reporting) (Coceano et al. 2009, Schmidt et al. 2012, Steinger et al. 2015, Luquet et al. 2018). Captured aphids are identified and used to forecast the need for near-term controls, for retrospective analyses and modeling of aphid phenology, and, more recently, for virus surveillance (Congdon et al. 2019). Matheson et al. (2008) found that small amplicons of chloroplast DNA (157 bp) can be recovered from aphids that have fed on the same host for several days. However, recovery of larger amplicons of sufficient size to identify unknown host plants has not yet been demonstrated, so aphid monitoring efforts cannot currently be leveraged to derive landscape-scale host use information. Additionally, it is not known whether aphids can acquire chloroplast DNA over shorter time frames, including during the probing activities associated with host assessment prior to phloem contact.

To address this, we used molecular gut content analysis to determine whether *Myzus persicae* (Sulzer) can acquire DNA from host plants over different time frames. *Myzus persicae* is a polyphagous aphid species that transmits numerous economically damaging plant viruses in agricultural environments (Capinera 2020). Like other aphids, *M. persicae* makes brief stylet incursions into nonvascular epidermal and mesophyll cells along the largely apoplastic pathway to the phloem sieve elements. During cellular incursions, aphids salivate and ingest small quantities of plant cell contents (Martín et al. 1997). We hypothesized that these behaviors may facilitate the uptake of chloroplast DNA during normal feeding activities. To determine whether this is the case, we reared *M. persicae* on pepper and screened them for evidence of chloroplast DNA acquisition using primers targeting a small region (180 bp) of the *trnL* locus of the chloroplast genome—a common barcoding gene for identification of plant hosts (Avanesyan et al. 2021b). We then performed a sequential host exposure experiment to determine (i) whether short-term probing activities that occur prior to phloem contact result in chloroplast DNA acquisition, (ii) whether DNA from multiple hosts can be recovered, and (iii) whether both long-term feeding and short probing events yield amplicons of a sufficient size for host identification.

Methods

Host Plants and Aphid Rearing Conditions

Plants used in experiments with aphids included pepper (*Capsicum annuum* L. [Solanales: Solanaceae] cv. California Wonder) and canola (*Brassica napus L. [Brassicales: Brassicaceae]* cv. Dwarf Essex). *Myzus persicae* were originally collected in Riverside, CA and reared on pepper or canola for experiments, as described below. Colonies for all experiments were maintained at 25 ± 2 °C, 55–60% relative humidity, in mesh cages with supplemental lighting on a 16L:8D photoperiod. Insects were transferred to new plants every 10–14 days. For one experiment, we also screened potato psyllids (*B. cockerelli*) reared on pepper. These insects were maintained in colonies under the same conditions as *M. persicae*, with the exception that insect transfers to new colonies occurred every 6–8 weeks instead of biweekly.

Primer Selection

We selected two primer sets targeting the region between the *trnT* and *trnF* genes in the chloroplast genome. These included the *trnL* c/d primers (Taberlet et al. 1991), which amplify a 500-bp region, and *trnL* 575F/755R primers (Cooper et al. 2016), which amplify a shorter, 180-bp region (Supplementary Table S3). This second set of primers was selected for the initial screening experiment (as in Matheson et al. 2008) to ensure DNA acquisition was not missed due to degradation into smaller fragments not amplified by the *trnL* c/d primers (Steffan and Atlas 1991).

Aphid Rearing Experiment

We created colonies of *M. persicae* on pepper plants and allowed the insects to establish and produce offspring for several weeks (at least three generations) under the rearing conditions cited above. Ten apterous offspring were collected randomly from each plant after reaching adulthood (3–5 days old) and stored in 70% ethanol at –80 °C until DNA extraction as described below. As a positive control during later sample processing, we also included a group of 10 adult *B. cockerelli* (mixed ages, collected and stored as for aphids) reared on pepper under the same conditions as aphids. This insect is known to acquire chloroplast DNA from pepper hosts (Cooper et al. 2016).

Aphid Probing Experiment

To determine whether DNA acquisition by *M. persicae* can occur within short time frames (e.g., during initial host assessment), we performed a transfer experiment using two hosts (pepper and canola) that produce amplicons of distinct sizes using the *trnL* c/d primer set (pepper: 454 bp, canola: 357 bp). Amplicons are distinguishable during gel electrophoresis, allowing for easy identification of individuals that have acquired DNA from the transfer host. *Myzus persicae* reared on pepper for multiple generations were relocated to a canola leaf in a Petri dish and allowed to probe for three different time intervals: 5, 10, and 15 min. These time intervals were selected because we previously performed electrical penetration graphing (EPG) recordings from *M. persicae* feeding on *B. napus* cv. 'Dwarf Essex' for a separate project. Aphids took an average of 69.34 ± 13.5 min to reach the phloem from first stylet contact with the plant, which exceeds our longest time point by at least 40 min. Each time interval was tested three times using 10 aphids (90 aphids total). Aphids were collected directly from the rearing host (pepper) without a starvation interval and transferred to an attached *B. napus* leaf positioned under a dissecting microscope. We monitored aphids for antennal movement indicating initiation of probing (folded-back antennae). The timer began as soon as the aphids were introduced to canola, and we confirmed that aphids collected for each time point engaged in probing behavior prior to collection. At the end of the time allotment, each group of aphids was frozen in separately labeled 1.7-ml tubes of 70% ethanol for later DNA extraction and subsequent PCR assessment. Aphids were also collected from the source colony on pepper and a separate colony reared on canola.

Table 1. Summary of sequencing efforts for recovery of short (180 bp) amplicons from aphids and potato psyllids reared on pepper (gel image, Supplementary Fig. S1)

Sample name	Approximate band size	Sequence length recovered (nt)	Alignment summary[a]
Pepper	180	168	*Capsicum annuum* (MH559327.1: 100% coverage, PI = 98.21)
Aphids on pepper 1	180	141	Targeted alignment to positive control: PI = 100
Aphids on pepper 2	180	168	Targeted alignment to positive control: PI = 95.04
Potato psyllids	180	168	Targeted alignment to positive control: PI = 94.64

[a]Closest trnL region match accession numbers listed. PI, percent identity.

DNA Extractions

We extracted DNA from host plants using the cetryltrimethylammonium bromide (CTAB) method (Li et al. 2006). Small pieces of plant tissue (2–3 mm^2) were placed in Eppendorf tubes equipped with two steel grinding balls. All samples were then submerged in liquid nitrogen and homogenized under cryogenic conditions using a GenoGrinder (1,100 shakes per minute for one min). The full CTAB extraction protocol is provided in Supplementary Materials. After extraction, DNA was transferred to an EconoSpin column (Epoch Life Sciences), washed, and eluted in 40 µl of ultrapure water.

We extracted DNA from insects using the Qiagen DNeasy Blood and Tissue Kit. Insect samples were surface sterilized prior to extraction to ensure that detected plant DNA was from ingested material and not from surface acquisition of DNA. Each insect was submerged in 70% ethanol for 1 min, then 2% bleach for 1 min, and finally rinsed in ddh_2O (similar to methods in Cooper et al. 2016). Surface sterilized aphid and psyllid samples were homogenized under cryogenic conditions as described above for plant samples, and DNA was extracted according to the kit manufacturer's instructions. All resulting insect and plant DNA extracts were evaluated using a Thermo Scientific NanoDrop 2000 Spectrophotometer to confirm successful DNA extraction. To monitor for DNA contamination, we periodically carried out "blank" extractions in the same space as the aphid extractions, using the same reagents and kits. These blank extractions were screened for the presence of contaminating DNA using the *trnL* c/d primer set as described below. Amplicons of the *trnL* region were not detected in any of these blank extractions (e.g., see Supplementary Fig. S3). We also used standard precautions for contamination control, including sterile tubes and reagents, pipettor tips with filters, separate physical spaces, lab coats, and pipettors for extraction, PCR, and electrophoresis, UV irradiation of pipettors, and cleansing of all surfaces with 3% sodium hypochlorite solution.

PCR Reagents and Thermocycler Conditions

PCR conditions and reagent measurements were determined by the optimal temperatures recommended by the original reference reporting each primer set as well as conditions recommended by the manufacturer of reagents (Phusion polymerase, NEB). A sample reaction mixture is shown in Supplementary Table S1, and thermocycler conditions for primers targeting the *trnL* region are shown in Supplementary Table S2 (Supplementary Materials). For all PCR reactions, positive controls of host plant DNA were run along with a non-template control consisting of the reaction mixture used for all samples with water added instead of extracted DNA in solution.

Gel Electrophoresis and Sanger Sequencing

PCR products were mixed with 2 µl of loading dye and pipetted into a 1% agarose gel for visualization using SYBR Safe DNA stain (Invitrogen). A Quick-Load Purple 1 kb Plus DNA ladder (New England Biolabs) was included as a size reference. Gels were photographed and annotated after each trial (see Supplementary Materials). Visible amplicons were excised and purified using a Zymoclean Gel DNA Recovery kit following the manufacturer's instructions, and purified PCR products were quantified to verify DNA recovery. If sufficient material was present, PCR products were sent for Sanger sequencing (Retrogen Inc.) to verify their identity. The resulting DNA sequences were quality checked and trimmed using BioEdit v. 5.0.9 sequence alignment editing software (Hall 2021). We used BLASTn and the NCBI database to confirm the identity of the plant from which amplicons were produced.

Results

Aphids Acquire Chloroplast DNA From a Host Plant During Long-Term Feeding

The screening for plant chloroplast DNA using the *trnL* 575F/755R primer set produced visible amplicons with sufficient DNA for Sanger sequencing for DNA samples from *M. persicae* reared on pepper (Table 1). Additionally, we confirmed pepper chloroplast DNA uptake by *B. cockerelli* reared on the same pepper cultivar, as first reported by Cooper et al. (2016), suggesting that our methods should be sufficient to reveal DNA acquisition by aphids if it is occurring.

Chloroplast DNA Acquisition Can Occur Prior to Phloem Contact

Our timed exposure probing experiment shows that *M. persicae* can acquire chloroplast DNA in as short a time period as 5 min. Aphids reared on pepper and transferred to canola produced visible amplicons, indicating the presence of canola chloroplast DNA in 5 of 9 exposures (trial 1: 5 and 10 min; trial 2: 10 and 15 min; trial 3: 5 min) (Supplementary Fig. S2). Amplicons corresponding in size to pepper chloroplast DNA were present in 9 of 9 time points across the 3 trials (a faint band corresponding in size to the pepper positive control is visible in trial 1: 10 min).

We excised individual bands from the gel (Supplementary Fig. S2) and purified each amplicon for Sanger sequencing to confirm the source of the acquired DNA. Sufficient DNA was recovered for most of the visible bands (Table 2). For all bands, sequencing confirmed the initial host plant identifications that had been assigned based on amplicon size (Table 2). Trials with multiple bands present sometimes did not yield sufficient material for sequencing. However, we did recover sequences matching both hosts from the two bands visible for the trial 1 five-minute time point sample. A BLASTn search against the sequences of the positive controls and the respective predicted organisms determined that the sequence of the larger amplicon matched the pepper *trnL* sequence and the sequence of the smaller

amplicon matched the canola *trnL* sequence (Table 2). Several other trials resulted in chloroplast DNA acquisition from canola, and these amplicons were also confirmed to match the canola *trnL* sequence with high percent identity (99.21–100%). All sequences matching pepper also had a percent identity value of 100. In addition to our finding that chloroplast DNA can be acquired rapidly, these results show that it is possible to amplify larger fragments suitable for host identification using public database information. Representative pepper and canola sequences were deposited in NCBI GenBank under the following accession numbers: OR359201, OR359200.

Discussion

Our study demonstrates that aphids ingest chloroplast DNA from their host plants during pre-phloem contact probing events as well as long-term host colonization. *Myzus persicae* acquired chloroplast DNA most readily from pepper, which was used as a host for aphid rearing. This suggests that DNA can be acquired even during longer term feeding on reproductive hosts, allowing possible identification of previously unknown crop and noncrop hosts that are colonized by *M. persicae* for extended durations. Building on the initial findings of Matheson et al. (2008), we also amplified larger fragments of sufficient size for taxonomic identification and recovered most amplicons for subsequent sequencing. Through a sequential acquisition experiment with two hosts producing large amplicons of distinct sizes, we demonstrated that aphids could acquire chloroplast DNA from host plants in as little as five minutes, and throughout time periods corresponding to the typical early- to mid-pathway phase (pre-phloem contact). Acquisition of chloroplast DNA during these short-term interactions, and especially the first five minutes of host contact, could provide information about transient host use. This is particularly valuable for understanding the movement of vectors that spread nonpersistently transmitted plant viruses, which are acquired and inoculated during short probes lasting less than five minutes.

Exactly how chloroplast DNA is acquired from host cells by *M. persicae* remains unclear. Previous studies on the morphology of both the organelle and aphid stylets allow us to generate some hypotheses. Aphids ingest plant sap through a food canal, which in *M. persicae* has a diameter of approximately 0.7 µm (Forbes 1964). Chloroplasts are significantly larger than the food canal, with diameters ranging from 3 to 10 µm depending on plant species (Flindt 2006). Therefore, it is reasonable to surmise that DNA is acquired *from* chloroplasts, but not by uptake of the entire chloroplast itself. Aphid damage to

Table 2. Summary of sequencing efforts for the aphid probing experiment (gel image, Supplementary Fig. S2)

Sample name	Approximate band size	Sequence length recovered (nt)	Alignment summary[a]
Pepper	450	428	*Capsicum annuum* (GU595139.1: 100% coverage, PI = 100)
Canola	350	328	*Brassica napus* (EF426775.1: 99% coverage, PI = 100)
Trial 1 5 min	450	445	Targeted alignment to positive control: PI = 100 Top BLASTn hit: *Capsicum annuum* (GU595139.1: 100% coverage, PI = 100)
Trial 1 5 min	350	334	Targeted alignment to positive control: PI = 96.01 Top BLASTn hit: *Brassica napus* (AY752716.1: 100% coverage, PI = 96.21)
Trial 1 10 min	350	339	Targeted alignment to positive control: PI = 99.7 Top BLASTn hit: *Brassica napus* (EF426775.1: 100% coverage, PI = 99.12)
Trial 1 15 min	450	445	Targeted alignment to positive control: PI = 100 Top BLASTn hit: *Capsicum annuum* (trnL GU595139.1: 100% coverage, PI = 100)
Trial 2 5 min	450	435	Targeted alignment to positive control: PI = 100 Top BLASTn hit: *Capsicum annuum* (trnL GU595139.1: 100% coverage, PI = 100)
Trial 2 10 min	350	341	Targeted alignment to positive control: PI = 100 Top BLASTn hit: *Brassica napus* (EF426775.1: 100% coverage, PI = 99.71)
Trial 2 15 min	350	339	Targeted alignment to positive control: PI = 100 Top BLASTn hit: *Brassica napus* (EF426775.1: 100% coverage, PI = 100)
Trial 3 5 min	450	452	Targeted alignment to positive control: PI = 100 Top BLASTn hit: *Capsicum annuum* (trnL GU595139.1: 100% coverage, PI = 100)
Trial 3 10 min	450	445	Targeted alignment to positive control: PI = 100 Top BLASTn hit: *Capsicum annuum* (trnL GU595139.1: 100% coverage, PI = 100)
Trial 3 15 min	450	444	Targeted alignment to positive control: PI = 100 Top BLASTn hit: *Capsicum annuum* (trnL GU595139.1: 100% coverage, PI = 100)

[a]Closest *trnL* region match accession numbers listed. PI, percent identity.

chloroplasts is not well documented, and the few micrograph studies on aphid incursions into plant cells suggest that chloroplasts and mitochondria remain intact (Tjallingii and Esch 1993). However, aphids routinely puncture vacuoles to sample metabolites present in plant cells, presumably to aid in host identification and/or navigation to the phloem (Pettersson et al. 2007). Vacuoles play a key role in the degradation and recycling of chloroplast components, including chloroplast DNA (Otegui 2018). Therefore, a more likely scenario is that aphids may be acquiring chloroplast DNA from vacuoles.

The presence of chloroplast DNA in vacuoles varies with the physiological status of the cell (Otegui 2018). In our study, we extracted DNA from pools of 10 aphids that had fed or probed on specific hosts. The sequential feeding experiment showed that DNA could be acquired in only five minutes of probing, but even with pooling 10 individuals per probing time point (thus increasing the likelihood of host DNA detection), larger amplicons of canola chloroplast DNA were not recovered from all samples. We also did not recover DNA amplicons from all samples extracted from aphids that had fed on a host for extended durations (Supplementary Figs. S2 and S3). This suggests that there is a certain probability of DNA acquisition, which may depend on whether cells with active chloroplast degradation are encountered and whether DNA is sufficiently intact to facilitate amplification of the target locus. Chloroplast breakdown in vacuoles is more active in older tissues undergoing senescence. A possible outcome of this may be that aphids are less likely to acquire DNA from younger host tissues. Chloroplast DNA uptake may also be influenced by host–aphid compatibility (e.g., whether the aphid can ultimately colonize and reproduce on the host). In a small follow-up experiment (Supplementary Fig. S3), we checked for chloroplast DNA acquisition in *M. persicae* that were adapted to feeding on pepper and allowed to probe on cantaloupe (*Cucumis melo*), which aphids from our pepper colony could not colonize. We did not detect *C. melo* chloroplast DNA in these aphids. Exploring the influence of host–aphid compatibility on chloroplast DNA acquisition should be a priority for future work on aphid gut content analysis.

Beyond host plant identity, it is likely that the probability of DNA uptake will also vary depending on other biotic or abiotic factors that affect the frequency of probing into vacuoles, as well as chloroplast function and recycling. For example, many plant viruses transmitted by aphids and other vectors cause changes in cellular processes that directly affect chloroplast architecture, integrity, and recycling in ways that could increase aphid access to chloroplast DNA (Shand et al. 2009, Zhao et al. 2016). Plant viruses can also increase host attractiveness to aphids, resulting in preferential visitation to infected hosts (reviewed in Mauck et al. 2018), and modify the frequency and duration of probing into parenchyma cells with vacuoles (e.g., Carmo-Sousa et al. 2014, 2016). These factors suggest that DNA uptake probability could be modified by virus infections, as well as other environmental factors that alter probing or chloroplast degradation. Future work could test this hypothesis by manipulating host infection status in laboratory feeding experiments or by monitoring infection status and host DNA in field-collected insects (similar to Cooper et al. 2023). It will also be useful to perform experiments to quantify how long DNA is retained, and to complete more reciprocal transfer experiments, as the first feeding host might influence the probability of DNA acquisition from subsequent hosts.

Chloroplast DNA uptake by aphids during intracellular punctures and vacuole sampling is just one possible pathway of acquisition. Studies with psyllids, which can also acquire chloroplast DNA from hosts (Cooper et al. 2016, 2019), suggest that there may be other, nonexclusive mechanisms of DNA uptake by Sternorrhyncha. EPG recordings to date show that psyllids do not perform the same brief intracellular punctures typical of aphid stylet pathways (during which vacuole sampling would occur) (Bonani et al. 2010, Pearson et al. 2014, Antolínez et al. 2017). Furthermore, chloroplast DNA acquisition by psyllids has only been demonstrated with insects that fed on target hosts for several days (Cooper et al. 2016) or field-collected insects that fed for unknown durations (Cooper et al. 2019, 2023, Barthel et al. 2020). Since phloem sieve tube elements do not contain chloroplasts, psyllids must be acquiring chloroplast DNA from a source other than mesophyll cells or sieve elements. One hypothesis is that psyllids acquire DNA from cells near the phloem (e.g., companion cells that can contain chloroplasts), possibly during a unique feeding behavior termed "waveform D" in EPG studies. Waveform D immediately precedes phloem contact and has some features in common with the waveforms typical of aphid intracellular punctures into mesophyll cells (Pearson et al. 2014, Antolínez et al. 2017). Interestingly, a recent series of studies showed that the aphids *Rhopalosiphum padi* and *M. persicae* also perform a unique "pre-phloem" intracellular puncture behavior within sieve element companion cells just prior to sieve element puncture (Jiménez et al. 2020a, 2020b). These pre-phloem interactions with host cells could be another pathway for chloroplast DNA acquisition by aphids, and possibly psyllids. Comparative EPG studies with both aphids and psyllids could explore this hypothesis as well as possible connections between distinct waveforms and chloroplast DNA uptake, as has been done to study behaviors responsible for pathogen acquisition and inoculation (e.g., Antolínez et al. 2017).

In summary, our finding that aphids can acquire chloroplast DNA from hosts through stylet activities occurring prior to phloem contact is consistent with known probing activities of aphids in nonvascular cells, which include at least one pathway for contact between chloroplast DNA and aphid stylets (puncture of vacuoles). Chloroplast DNA contains useful molecular barcoding regions that should allow researchers to learn about prior host use patterns of aphids. Our probing experiment demonstrates that this information could include the identification of hosts on which aphids have probed but not made phloem contact. However, the most consistent recovery was from the rearing host (pepper), not the probing host (canola) or a nonhost (cantaloupe), so researchers may be more likely to recover chloroplast DNA in aphids from plants that were colonized rather than transient hosts that were only briefly probed. As field-collected insects will potentially contain chloroplast DNA from multiple host species, application of aphid gut content analysis to research questions will necessarily include a high-throughput amplicon sequencing step and downstream bioinformatic analyses (Cooper et al. 2019).

Acknowledgments

We are thankful to Dr. Marco Gebiola and Dr. Penglin Sun for their assistance in training students in molecular techniques. Fellowship support for D.B. and M.T. was provided by the U-SPARC program, funded by USDA NIFA award 2017-38422-27135. J.R.K.'s contributions were supported by USDA NIFA award 2022-67014-36271. Funding for supplies was provided by Hatch funds awarded to K.E.M. (CA-R-ENT-5144-H).

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Supplementary Material

Supplementary material is available at *Environmental Entomology* online.

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