

Limited detection of secondarily consumed plant food by DNA-based diet analysis of omnivorous carabid beetles

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Abstract

Trophic interactions are monitored by molecular methods based on the identification of prey DNA in feces, regurgitates, or the gut content of consumers. In providing highly resolved taxonomic information on dietary choice, DNA-based analysis has become a key approach to unraveling the structure and dynamics of food webs. It is crucial, however, to correctly assign and evaluate trophic linkages among species. Links might be distorted by secondary predation, such as when the DNA of food items that have been consumed by the prey of the predator is detected and incorrectly assigned to the predator's diet. This may become a particular problem when evaluating the diet spectrum of omnivores. We present the first assessment, to our knowledge, of the potential for trophic link errors resulting from secondary consumption of prey in predator–herbivore–plant interactions. Individuals of *Drosophila suzukii*, fed raspberries (*Rubus idaeus*) as food items, were freeze-killed at 0, 6, and 9 hr post-feeding and then offered to the carabid *Pseudoophonus rufipes*. Post-consumption detection intervals (0–128 hr) for DNA of primary prey (*D. suzukii*) and secondarily consumed raspberry in carabid regurgitates were assessed via PCR assays. Secondary consumption was detected only when *D. suzukii* flies consumed by the carabid had been feeding on raspberry immediately before they were eaten. The maximum detection time for secondarily consumed food was 32 hr post-feeding of the carabid, and only in a single regurgitate out of twelve. No secondary food DNA was detected at all when the consumed *D. suzukii* had digested raspberry for 6 or 9 hr beforehand. These findings would indicate that secondarily consumed plant food is unlikely to contribute to significant error in food web structure. Nevertheless, it is advisable to consider feeding interactions across food webs for estimating the potential of incorrect trophic assignments due to secondary predation events.

KEYWORDS

feeding experiment, *Harpalus rufipes*, herbivory, molecular analysis of trophic interactions, secondary predation

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1 | INTRODUCTION

DNA-based techniques have become a basic tool of trophic ecology. By providing detailed information on dietary interactions between species, molecular trophic ecology improves our understanding of food web structures and dynamics (Clare, 2014). In traditional food web approaches, the number of trophic links that can be examined is often personnel- and time-limited, or identity of the consumed prey remains uncertain due to a lack of identifiable prey structures, as in the case of fluid feeders (spiders, aphids, carabids, etc.). DNA-based methods, in contrast, are highly sensitive (King, Read, Traugott, & Symondson, 2008; Traugott, Kamenova, Ruess, Seiber, & Plantegenest, 2013), detecting very low concentrations of prey DNA, thus facilitating an improved characterization of arthropod food webs. They are applicable to many kinds of dietary samples that can be obtained from consumers such as feces (Ibanez et al., 2013), regurgitates (Thalinger et al., 2016; Wallinger et al., 2015), gut contents (Leray, Meyer, & Mills, 2015; Mollot et al., 2014) or whole-body DNA extracts (Staudacher, Wallinger, Schallhart, & Traugott, 2011). Only a few molecules of prey DNA are sufficient for the molecular identification of trophic links of even very small organisms such as mites (Perez-Sayas et al., 2015) and zooplankton (Durbin, Casas, & Ryneerson, 2011) up to larger invertebrates such as spiders or beetles (Roubinet et al., 2017, 2018; Staudacher et al., 2018; Symondson, 2012). Moreover, high-throughput sequencing of DNA in diet samples allows the cost-effective analysis of large sample sizes, irrespective of the ecosystem sampled (Symondson & Harwood, 2014).

Despite these benefits, an outstanding conceptual problem for the molecular diet analysis is that it cannot be assumed that any detected DNA of a potential prey originates from a direct predation event. DNA in a gut content sample can either come from the animal eaten by the predator, the primary prey, or from a secondary predation event whereby the DNA of prey in the digestive system of the primary prey is detected (Sheppard et al., 2005). Aside from the possibility that secondary predation may interfere with the primary prey by inhibition of DNA amplification (Anita Juen, personal communication), it could also lead to incorrect trophic assignments resulting in food web structural errors (Greenstone, Payton, Weber, & Simmons, 2014). Using monoclonal prey-specific antibodies, Harwood, Phillips, Sunderland, and Symondson (2001) demonstrated in an aphid-spider-carabid system that aphid DNA could only be detected in the guts of carabid beetles that were frozen immediately after having consumed at least two spiders that were, in turn, killed immediately after having fed on the aphid *Sitobion avenae*. Based on this observation, these authors concluded that secondary predation is unlikely to be a source of error in field studies of carabid beetle predation. The study of Sheppard et al. (2005) on the effect of secondary predation for the same aphid-spider-carabid system, but using DNA-based prey detection, resulted in similar to longer detection times of secondary predation when analyzing small DNA amplicons for the secondary prey (120 hr for 110 bp fragments).

Generalist predator carabids have also been observed consuming high numbers of seeds in addition to animal food (Honek, Martinkova, & Jarosik, 2003; Westerman, Wes, Kropff, & Van der Werf, 2003). The results of a recent large scale field study, based on molecular food identification of carabids collected in Central European cereal fields, indicated an unexpectedly high share of seeds in the diet of carabids (Frei et al., 2019). As with animal prey, the detection of directly consumed plant DNA can be overlain with secondary plant DNA material that was eaten by the primary prey. We know that DNA detection rates of plant food are different from those of animal prey (Sint, Guenay, Mayer, Traugott, & Wallinger, 2018; Staudacher et al., 2011) and we can therefore hypothesize that the detectability of this secondary “prey” will also differ. For reasons of clarity, we will henceforth use the terms “secondary prey” and “secondary predation” to refer to secondarily consumed plant material and animal prey.

To date, secondary predation has not been considered a source of error in the assignment of trophic links in plant-herbivore-omnivore systems. As an example, this would mean that it might be impossible to differentiate between a predator acting as a biological control agent of a weed, via primary predation on the seeds, or a secondary predator of weeds that eats the biological control agents of weeds.

To examine this problem of error in assignment, we used laboratory feeding trials to evaluate the detection of secondarily consumed plant DNA which will be helpful for the interpretation of field-derived data (King et al., 2008). In this study, the post-feeding detection intervals of DNA of a primary prey (here *Drosophila suzukii*) that had consumed plant material (here raspberry, *Rubus idaeus*) at different time intervals prior to being fed to an omnivorous carabid beetle were assessed in a feeding experiment. We tested: (a) for how long the primary animal prey can be detected post-feeding and (b) the maximum detection time of DNA stemming from a secondarily consumed plant meal of the primary prey.

2 | MATERIAL AND METHODS

2.1 | Specimen collection and laboratory conditions

In July 2017, 242 adult carabid beetles of *Pseudoophonus rufipes* (syn. *Harpalus rufipes*) (DeGeer, 1774) were trapped on arable fields around Innsbruck (760 m a.s.l.; Tyrol, Austria). For the collection of carabids in this area, no special permits are needed and access permission was granted by the farmers. To standardize the beetles for the feeding treatments, each beetle was kept in a plastic beaker (71 × 58 mm, h × Ø, screw top lid) with a piece of moistened tissue (5 × 5 cm). They were located in a climate cabinet (L:D 14:10 hr at 22 and 12 °C, respectively). The beakers were opened daily and cleaned as needed. Every second day, the tissues were replaced and half a mealworm (*Tenebrio molitor* L.) was provided as food for the carabids. All carabids were maintained this way for 14 days before starting with the feeding experiment: Four days prior to experimentation,

the mealworm diet was stopped to induce a standard level of beetle starvation.

2.2 | Feeding experiment

We established three experimental feeding treatments with the three different groups (0, 6, or 9 hr post-feeding) of *D. suzukii* flies (Figure 1). Carabid beetles were individually placed into clean feeding beakers (60 × 35 mm, h × Ø), containing a drop of water and two male and one female *D. suzukii* of the same type (killed either 0, 6 or 9 hr post-feeding). Following two hours in the dark climate cabinet, the carabid individuals that had at least consumed one *D. suzukii* fly were put individually into clean beakers in the climate cabinet for regurgitation. Carabids that had consumed fewer than three flies occurred approximately evenly across the post-feeding time points of each treatment (see also Table 1). The number of consumed *D. suzukii* flies (one to three) was recorded for each beetle and will be referred to as meal size henceforth. Carabids were divided into batches of 9–14 individuals. Each batch was stimulated to regurgitate according to the descriptions of Wallinger et al. (2015) at a certain time following the experimental feeding: 0, 32, 64, 96, and 128 hr post-feeding (Table 1). These time points were chosen from experience of earlier feeding experiments with *P. rufipes*, which showed that consumed plant food can be detected up to 128 hr post-feeding (Sint et al., 2018). For the experimental treatment with the *D. suzukii* flies that had been freeze-killed 0 hr post-feeding on raspberry, additional time points at 6, 12, 24, and 40 hr postcarabid consumption were included to achieve a particularly high resolution in the first two days post-consumption. Regurgitates were then stored at –28 °C until DNA extraction. All test carabids were subsequently released into their original habitat.

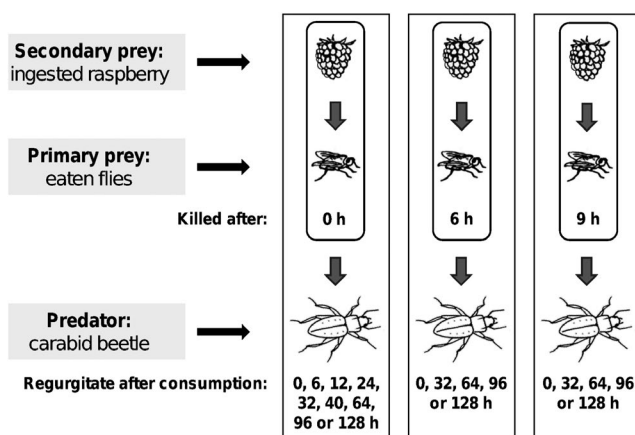


FIGURE 1 Flow diagram of the experimental treatments to assess secondary predation. Beetles (*Pseudoophonus rufipes*) were fed on *Drosophila suzukii* flies that were killed at various time periods (0/6/9 hr) after they had eaten raspberry (*Rubus idaeus*). A minimum of nine carabids was fed on *D. suzukii* flies from each digestion time category (0/6/9 hr)

2.3 | DNA extraction, PCR and electrophoresis

Regurgitates were dissolved in 200 µl 1× TES (0.1 M Tris, 10 mM EDTA, 2% SDS, pH 8) buffer and 5 µl Proteinase K (20 mg/ml). After an overnight incubation at 56 °C, DNA was extracted with the BioSprint® 96 DNA blood kit on a BioSprint® 96 extraction robotic platform (Qiagen) following the manufacturer's instructions except for the elution of each DNA sample in 200 µl 1× TE buffer. This method has been found to provide high-quality DNA for molecular dietary studies (Wallinger et al., 2017). All extractions and PCRs were done in a separate pre-PCR laboratory using a UVC-equipped laminar flow hood and DNA extracts stored at –28°C. The four extraction negative controls (PCR-grade water) of each 96-well plate were checked for cross-sample contamination during the extraction process. All extraction controls were negative. The testing of DNA samples as well as extraction negative controls was conducted with two singleplex PCRs.

We chose the carabid–*D. suzukii*–raspberry system to test for the detection of secondary predation, because suitable assays, which have been established and optimized in our laboratory, were available. Using these assays, we have found that the detection of *D. suzukii* requires as little as 0.02 pg DNA still producing strong signals (Wolf et al., 2018), for dilutions of raspberry DNA extracts at 1:1,000 (unpublished data). Primer design, PCR assay and conditions for amplifications of *D. suzukii* using the primer pair Dro-suz-S390 and Dro-suz-A380 followed protocols described by Wolf et al. (2018), and for the *trnL* gene of raspberry chloroplasts the one of Briem et al. (2018) using the forward universal primer c (Taberlet, Gielly, Pautou, & Bouvet, 1991) and reverse primer Rub-ida-A575 (Briem et al., 2018). The fragment lengths of the amplicons were 171 bp for *D. suzukii* and 245 bp for raspberry. Each PCR was run with one positive (*D. suzukii* and raspberry DNA; mixture 1:1) and one negative (PCR-grade water) control per 96-well plate. PCR fragments were separated with the automated capillary electrophoresis system QIAxcel (Qiagen) using the method AL320 and analyzed with the accompanying software QIAxcel ScreenGel (version 1.4.0). This visualization of the PCR products is known to augment the sensitivity compared with classical gel electrophoresis (Sint, Raso, Kaufmann, & Traugott, 2011). Amplicons of predicted fragment length were counted as being positive for signal intensities above 0.08 relative fluorescent units. All PCR negative controls were negative.

2.4 | Statistical analysis

The influence of the digestion period (i.e., time post-feeding) of carabids on the detection of the primary prey *D. suzukii* and secondary prey *R. idaeus* was tested using generalized linear models (GLM, LOGIT link function, quasi-binomial distribution). GLM (I) estimated the detection of *D. suzukii* DNA as a function of time post-feeding of carabids. GLM (II) modeled the detection of raspberry DNA with the time post-feeding of the carabid and the *D. suzukii*, respectively.

TABLE 1 The numbers of regurgitates collected from *Pseudoophonus rufipes* carabids fed with *Drosophila suzukii* flies as primary prey that for their part had consumed raspberry (*Rubus idaeus*, which represents the secondary “prey” of *P. rufipes*)

	Hours post-feeding of fly	Hours post-feeding of carabid	n carabid regurgitates (Total)	n regurgitates of 3 consumed flies	n regurgitates of 2 consumed flies	n regurgitates of 1 consumed fly
Experimental Treatment I	0	0	10	10	-	-
	0	6	14	11	-	3
	0	12	14	11	2	1
	0	24	14	12	1	1
	0	32	12	9	3	-
	0	40	12	8	3	1
	0	64	9	7	1	1
	0	96	10	8	2	-
	0	128	9	8	-	1
Treatment II	6	0	12	10	1	1
	6	32	12	10	1	1
	6	64	12	10	1	1
	6	96	13	11	1	1
	6	128	9	8	1	-
Treatment III	9	0	14	9	2	3
	9	32	14	9	2	3
	9	64	13	8	2	3
	9	96	13	10	2	1
	9	128	13	9	2	2

Note: The offered flies had been freeze-killed at (I) 0 hr, (II) 6 hr, and (III) 9 hr after fruit consumption, and the carabids were provoked to regurgitate at different time points post-feeding (0–128 hr). The last three columns indicate the number of flies that had been consumed by the carabids (pooled for each time point).

Given that not all carabids had consumed all *D. suzukii* flies offered, we tested for potential effects of these differences in meal size regarding the primary prey. We analyzed whether the number of consumed *D. suzukii* flies (1–3) affected the post-feeding detection time of both fly DNA, GLM (III), and raspberry DNA, GLM (IV), in the regurgitates of carabid beetles. For this analysis, the regurgitates were pooled across the three fly treatments (killed 0/6/9 hr post-feeding, Figure 1) according to the numbers provided in Table 1. All calculations were done in R 3.4.4 (R Core Team, 2018).

3 | RESULTS

All extraction and PCR negative controls were clean, indicating that no cross-contamination via DNA carryover had occurred. Overall, most of the carabid beetles consumed three *D. suzukii* flies, with an average of 2.67 (± 0.66 SD) flies within a 2 hr feeding period. In total, 229 carabid regurgitates were DNA extracted and analyzed. The results revealed no influence of the number of consumed *D. suzukii* flies on DNA detection either for *D. suzukii* or *R. idaeus*, respectively (Figure 2). The time post-feeding of the carabid significantly affected detectability of both primary and secondary prey (Figure 2): GLM I: $p < .0001$ for the detection of *D. suzukii* DNA; GLM II: $p < .05$ for detection of raspberry DNA; GLM III: $p < .005$ for *D. suzukii* including

meal size as a covariate; GLM IV: $p < .0001$ for raspberry including meal size as a covariate (Figure 3).

In all three experimental treatments, the detection rates of *D. suzukii* DNA decreased with postcarabidfeeding time: When all samples of the three treatments were considered together ($n = 229$), the primary prey was detected in 92% of the regurgitates collected at 0 hr ($n = 36$), 86% at 6 hr ($n = 14$), 71% at 12 hr ($n = 14$), 79% at 24 hr ($n = 14$), 29% at 32 hr ($n = 38$), 50% at 40 hr ($n = 12$), and 12% at 64 hr ($n = 34$). At 96 hr ($n = 36$) and 128 hr post-feeding ($n = 31$), no DNA of the consumed *D. suzukii* flies was detected in any of the three experimental treatments. Secondly consumed raspberry DNA was only detected in regurgitates of carabids that had eaten flies that had been freeze-killed immediately after the consumption of raspberry (0 hr, Figure 2a). In this case, detection rates were 50% ($n = 10$) for 0 hr and 8% for 32 hr ($n = 12$), respectively (Figure 3).

4 | DISCUSSION

Our results provide the first evidence, to our knowledge, of potential link assignment errors resulting from secondary predation in predator–herbivore–plant interactions. Comparison with studies using aphid–spider–carabid systems (Harwood et al., 2001; Sheppard et al., 2005) indicates a longer time span of detection of

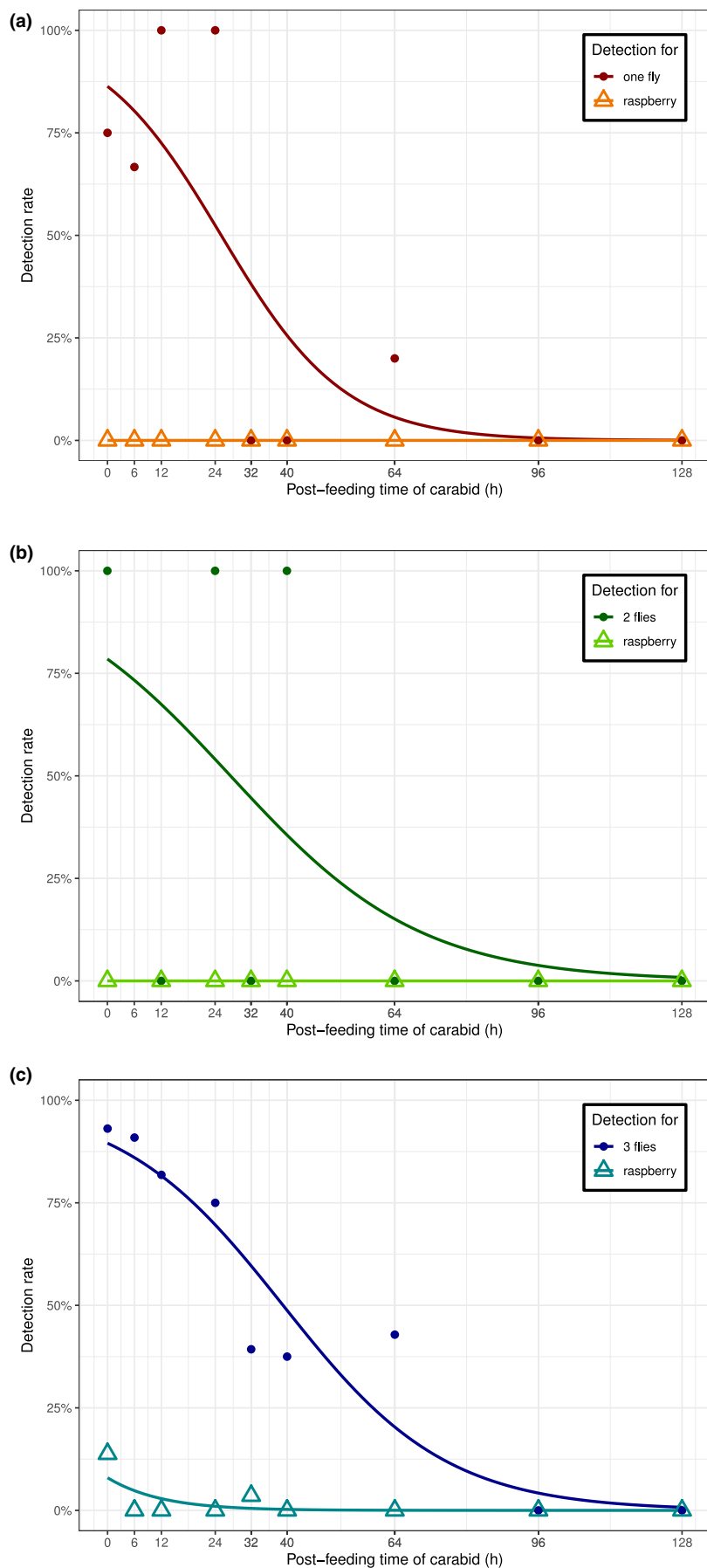
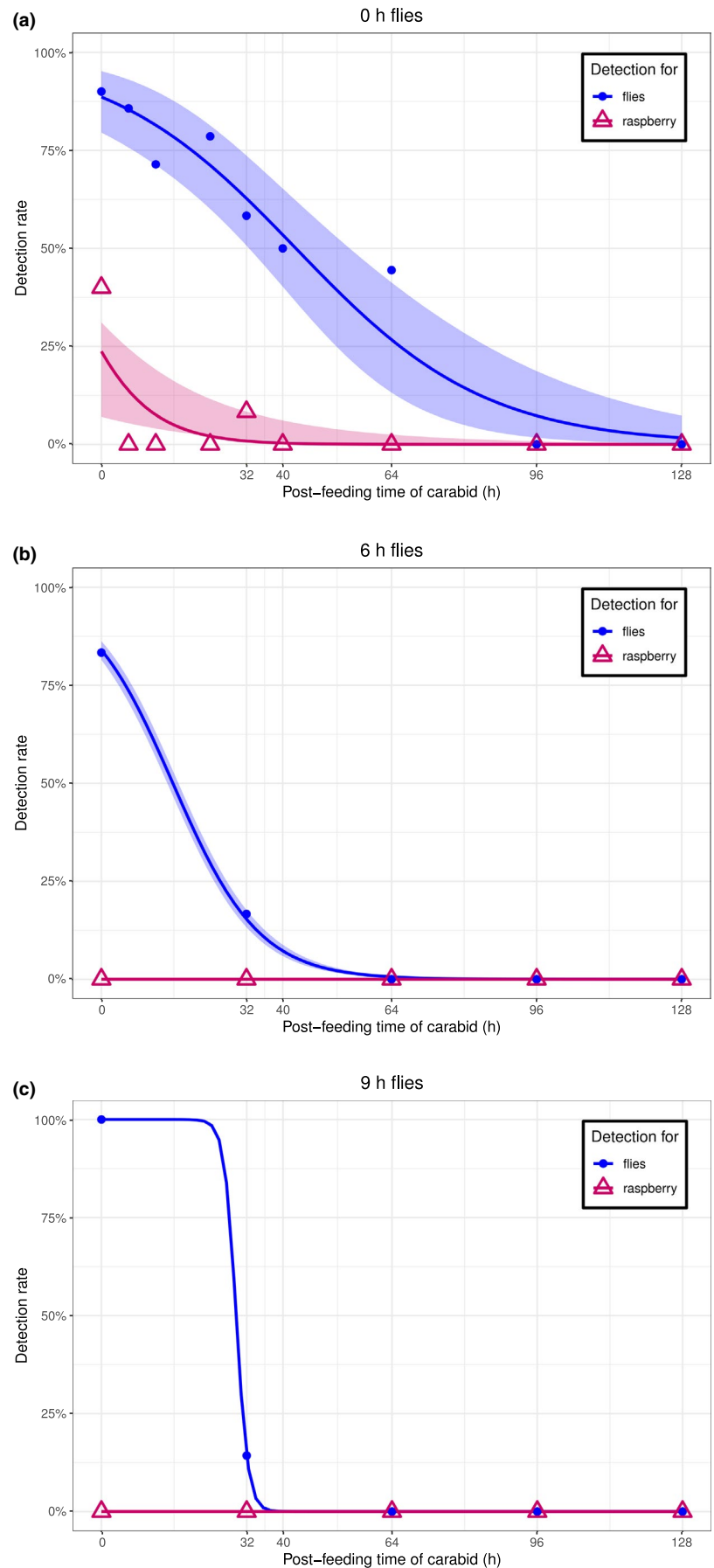


FIGURE 2 Differences in DNA detection rates of the primary prey (*Drosophila suzukii*) and the secondary prey (*Rubus idaeus*) depending on the number of *D. suzukii* flies (i.e., meal size) consumed by the carabid *Pseudoophonus rufipes*: (a) one fly, (b) two and (c) three flies. Circles represent the detection rates of primary prey DNA and triangles those of secondary prey DNA, along with GLM models for the decrease in food DNA detectability including the lower and upper 95% confidence intervals. The regurgitates for this analysis were pooled per time point (0–128 hr) and across treatments according to the numbers provided in Table 1

FIGURE 3 Differences in DNA detection rates of the primary prey (*Drosophila suzukii* flies) and the secondary prey (*Rubus idaeus*, raspberry) in regurgitates of carabids (*Pseudoophonus rufipes*) that had consumed *D. suzukii* flies that were killed at (a) 0 hr, (b) 6 hr (c) 9 hr after raspberry consumption. A minimum of 9 regurgitates per time point post-feeding was tested (0–128 hr) using specific primers for *D. suzukii* and *R. idaeus*, respectively. Circles represent the detection rates of primary prey DNA and triangles those of secondary prey DNA, along with GLM models for the decrease in food DNA detectability including the lower and upper 95% confidence intervals: The blue line indicates the GLM (I) of the detection of *D. suzukii* DNA, and the pink line represents GLM (II) of the detection of raspberry DNA, both depending on the time post-feeding of the carabid



the secondarily consumed plant food. In the current study, no secondary predation was detected when the primary prey had digested a plant meal for 6–9 hr. Plant DNA could only be amplified from carabids that had consumed raspberry-fed *D. suzukii* flies that had been killed immediately after their meal, with a maximum time span of 32 hr post-feeding. Although, in this specific case, it was only one out of twelve regurgitates that tested positive for raspberry DNA, the confidence interval of the GLM (II) indicates that the detectability of this secondary prey could be even longer.

It is well known that false negatives caused by bad DNA quality and/or low assay efficiency can become an issue in molecular trophic analysis and we therefore used optimized protocols for PCR, DNA extraction, and visualization. Thus, we are confident that we can exclude false negatives due to a lack of efficiency of either the PCR assay or the visualization of the products. That not all regurgitates collected 0 hr post-feeding tested positive for *D. suzukii* DNA can most likely be ascribed to sampling stochasticity, which plays a significant role at very low DNA concentrations (Sint et al., 2011). Conceptually, if one of the few target DNA molecules present in the regurgitate is not “caught” when pipetting the sample, there will be no PCR product. It may be therefore advisable to include the DNA signal strength instead of using presence/absence data, which would allow for a better characterization of the decrease in prey detectability in dietary samples. In a similar feeding experiment (Sint et al., 2011), variability in detection success by repeated testing identified as sampling stochasticity did not change the outcome of their study. Nevertheless, it may be advisable to include the DNA signal strength instead of using presence/absence data, which would allow for a better characterization of the decrease in prey detectability in dietary samples.

In cereal fields, carabids are feeding on various animal prey such as aphids, collembolans, earthworms and to some extent smaller granivorous carabids such as members of the genus *Amara* (Roubinet et al., 2017, 2018; Staudacher et al., 2018) that consume fresh or decayed plant tissue. Digestion rates of carabids that have consumed fruit flies such as *D. suzukii* are likely to be comparable to those of carabids consuming aphids. Moreover, both prey groups are fluid feeders, that is, fruit juice for fruit flies versus plant sap for aphids. The same applies for collembola prey that has fed on soil algae and decaying plant tissue as primary food. Therefore, the present results would indicate that the trophic link assignment error due to secondarily consumed plant food of aphids or collembolans may be minor, similar to findings for secondarily consumed animal prey (Harwood et al., 2001; Sheppard et al., 2005). The available evidence suggests that smaller granivorous ground beetles, which have been eaten by larger carabids, may be digested more slowly. As a result, the DNA from the ingested seeds of these herbivores may be detected over longer time spans. The maximum detection time of different seeds in regurgitates of *P. rufipes* can be up to five days post-feeding (Sint et al., 2018; Wallinger et al., 2015). Hence, we do not rule out the possibility that the detection rate for secondary prey is prey specific, such as for plant fluids and seeds. Future experiments should investigate the detectability of secondarily consumed weed seeds in

a carabid–granivore–seed system and thus shed light onto this specific part of carabid food webs.

Screening could also help here. The use of primers in a multiplex PCR targeting the major herbivores and granivores that are present in the same habitat as the omnivorous carabids of interest could overcome this uncertainty. Individuals that do not test positive for the DNA of any herbivore/granivore are likely to have eaten plants directly. In contrast, those that test positive for herbivores/granivores can be excluded from analyses of primary predation (Sheppard et al., 2005). Such a screening approach has already been successfully employed for field-collected omnivorous carabid beetles (Frei et al. 2019). Alternatively, one could apply DNA metabarcoding (Pompanon et al., 2012) of the regurgitates, simultaneously assessing consumed species (animals and plants). Once again, one could exclude dietary samples providing DNA of both plants and herbivores/granivores to be on the safe side. Metabarcoding has the merit of providing comprehensive information on the broad spectrum of trophic interactions regarding the variety of the food types of omnivorous arthropods (Harper et al., 2005). It may thus facilitate the assessment of feeding interactions across food chains with much higher precision than has been achieved to date. It should be noted, however, that the detection of food DNA via regurgitate metabarcoding may also be influenced by differences in digestibility and biases for particular groups of organisms. Moreover, using this method, the detection of DNA present in the environment also can become an issue (Taberlet, Coissac, Hajibabaei, & Rieseberg, 2012). Therefore, caution is required during field sampling to avoid DNA cross-contamination. Still, some of the DNA that may stick to the body surface of the carabid can be dropped during regurgitation in the reaction tube and will then be included in the DNA extract of regurgitates. To avoid false positives when testing regurgitates from field-collected carabids, it may be therefore necessary to establish thresholds of DNA detection (Frei et al. 2019). Moreover, positive controls can help to define nonarbitrary thresholds below which sequences cannot be differentiated from deep noise (De Barba et al., 2014; Corse et al., 2017).

Direct application of the results of the present experiment to carabid–herbivore/granivore–plant food webs may be considered with caution. For an accurate assessment, all parts of a given predator–primary prey–secondary prey food chain that are present in the same habitat need to be considered. We expect that the DNA of seeds of different plant species will vary in their detection times post-feeding and differ depending upon the composition of the carabids' diet (Sint et al., 2018). Moreover, carabid species identity (Lövei, Sopp, & Sunderland, 1987), sex (Kulkarni, Dosdall, Spence, & Willenborg, 2017), life stage (larvae versus adult beetles), reproductive activity, and diet range (Sint et al., 2018) are known to impact metabolic rates and thereby DNA digestion. Digestion times of a specific food type even depend upon the specific trophic status of a given carabid (Lövei, Monostori, & Andó, 1985). This needs to be investigated in the near future.

In a study by Frei et al. (2019), the dietary choice of the three most common carabid species in Central European cereal fields

was investigated molecularly. The detection probability of plant DNA was much higher in carabid regurgitates than for the co-existing “herbivorous” animal prey (i.e., aphids, collembolans, and earthworms; Frei et al., 2019). This indicated to Frei et al. (2019) that the majority of detected plant DNA would have come from direct seed consumption. Even when taking into consideration that these findings are limited to three carabid species, this suggests that the potential for food web errors via secondary predation in carabid-herbivore/granivore-plant food chains is likely minor. This is in accordance with the results of the present experiment indicating that secondarily predated plant food is unlikely to distort DNA-derived food webs of omnivorous carabids in agroecosystems.

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CONFLICT OF INTERESTS

None declared.

AUTHOR CONTRIBUTION

M.T., Y.G., and C.W. conceived and designed the study. Y.G., I.G., H.T., and C.W. performed the feeding experiments. Y.G. and H.T. were responsible for DNA extractions and the PCR screening. Y.G. performed the data analysis and created the first draft. C.W. wrote the manuscript, which all authors revised and finally approved to be published.

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REFERENCES

- Briem, F., Zeisler, C., Guenay, Y., Staudacher, K., Vogt, H., & Traugott, M. (2018). Identifying plant DNA in the sponging-feeding insect pest *Drosophila suzukii*. *Journal of Pest Science*, 91, 985–994. <https://doi.org/10.1007/s10340-018-0963-3>
- Clare, E. L. (2014). Molecular detection of trophic interactions: Emerging trends, distinct advantages, significant considerations and conservation applications. *Evolutionary Applications*, 7, 1144–1157. <https://doi.org/10.1111/eva.12225>
- Corse, E., Meglecz, E., Archambaud, G., Ardisson, M., Martin, J. F., Tougaard, C., ... Dubut, V. (2017). A from-benchtop-to-desktop workflow for validating HTS data and for taxonomic identification in diet metabarcoding studies. *Molecular Ecology Resources*, 17, e146–e159. <https://doi.org/10.1111/1755-0998.12703>
- De Barba, M., Miquel, C., Boyer, F., Mercier, C., Rioux, D., Coissac, E., & Taberlet, P. (2014). DNA metabarcoding multiplexing and validation of data accuracy for diet assessment: Application to omnivorous diet. *Molecular Ecology Resources*, 14, 306–323. <https://doi.org/10.1111/1755-0998.12188>
- Durbin, E. G., Casas, M. C., & Ryneearson, T. A. (2011). Copepod feeding and digestion rates using prey DNA and qPCR. *Journal of Plankton Research*, 34, 72–82. <https://doi.org/10.1093/plankt/fbr082>
- Frei, B., Guenay, Y., Bohan, D. A., Traugott, M., & Wallinger, C. (2019). Molecular analysis indicates high levels of carabid weed seed consumption in cereal fields across Central Europe. *Journal of Pest Science*, 92, 935–942. <https://doi.org/10.1007/s10340-019-01109-5>
- Greenstone, M. H., Payton, M. E., Weber, D. C., & Simmons, A. M. (2014). The detectability half-life in arthropod predator-prey research: What it is, why we need it, how to measure it, and how to use it. *Molecular Ecology*, 23, 3799–3813. <https://doi.org/10.1111/mec.12552>
- Harper, G. L., King, R. A., Dodd, C. S., Harwood, J. D., Glen, D. M., Bruford, M. W., & Symondson, W. O. C. (2005). Rapid screening of invertebrate predators for multiple prey DNA targets. *Molecular Ecology*, 14, 819–827. <https://doi.org/10.1111/j.1365-294X.2005.02442.x>
- Harwood, J. D., Phillips, S. W., Sunderland, K. D., & Symondson, W. O. C. (2001). Secondary predation: Quantification of food chain errors in an aphid-spider-carabid system using monoclonal antibodies. *Molecular Ecology*, 10, 2049–2057. <https://doi.org/10.1046/j.0962-1083.2001.01349.x>
- Honek, A., Martinkova, Z., & Jarosik, V. (2003). Ground beetles (Carabidae) as seed predators. *European Journal of Entomology*, 100, 531–544. <https://doi.org/10.14411/eje.2003.081>
- Ibanez, S., Manneville, O., Miquel, C., Taberlet, P., Valentini, A., Aubert, S., ... Moretti, M. (2013). Plant functional traits reveal the relative contribution of habitat and food preferences to the diet of grasshoppers. *Oecologia*, 173, 1459–1470. <https://doi.org/10.1007/s00442-013-2738-0>
- King, R. A., Read, D. S., Traugott, M., & Symondson, W. O. (2008). Molecular analysis of predation: A review of best practice for DNA-based approaches. *Molecular Ecology*, 17, 947–963.
- Kulkarni, S., Dosdall, L., Spence, J., & Willenborg, C. (2017). Depth of seed burial and gender influence weed seed predation by three species of ground beetle (Coleoptera: Carabidae). *Weed Science*, 63, 910–915. <https://doi.org/10.1614/WS-D-15-00080.1>
- Leray, M., Meyer, C., & Mills, S. (2015). Metabarcoding dietary analysis of coral dwelling predatory fish demonstrates the minor contribution of coral mutualists to their highly partitioned, generalist diet. *PeerJ*, 3, e1047. <https://doi.org/10.7717/peerj.1047>
- Lövei, G., Monostori, É., & Andó, I. (1985). Digestion rate in relation to starvation in the larva of a carabid predator, *Poecilus cupreus*. *Entomologia Experimentalis Et Applicata*, 37, 123–127. <https://doi.org/10.1111/j.1570-7458.1985.tb03462.x>
- Lövei, G., Sopp, P., & Sunderland, K. (1987). The effect of mixed feeding on the digestion of the carabid *Bembidion lampros*. *Acta Pathologica Entomologica Hungarica*, 22, 403–407.
- Mollot, G., Duyck, P. F., Lefeuvre, P., Lescouret, F., Martin, J. F., Piry, S., ... Tixier, P. (2014). Cover cropping alters the diet of arthropods in a banana plantation: A metabarcoding approach. *PLoS One*, 9, e93740. <https://doi.org/10.1371/journal.pone.0093740>
- Perez-Sayas, C., Pina, T., Gomez-Martinez, M. A., Camanes, G., Ibanez-Gual, M. V., Jaques, J. A., & Hurtado, M. A. (2015). Disentangling mite predator-prey relationships by multiplex PCR. *Molecular Ecology Resources*, 15, 1330–1345. <https://doi.org/10.1111/1755-0998.12409>
- Pompanon, F., Deagle, B., Symondson, W., Brown, D., Jarman, S., & Taberlet, P. (2012). Who is eating what: Diet assessment using next

- generation sequencing. *Molecular Ecology*, 21, 1931–1950. <https://doi.org/10.1111/j.1365-294X.2011.05403.x>
- Roubinet, E., Birkhofer, K., Malsher, G., Staudacher, K., Ekbom, B., Traugott, M., & Jonsson, M. (2017). Diet of generalist predators reflects effects of cropping period and farming system on extra- and intraguild prey. *Ecological Applications*, 27, 1167–1177. <https://doi.org/10.1002/eap.1510>
- Roubinet, E., Jonsson, T., Malsher, G., Staudacher, K., Traugott, M., Ekbom, B., & Jonsson, M. (2018). High redundancy as well as complementary prey choice characterize generalist predator food webs in agroecosystems. *Scientific Reports*, 8, 10. <https://doi.org/10.1038/s41598-018-26191-0>
- Sheppard, S. K., Bell, J., Sunderland, K. D., Fenlon, J., Skervin, D., & Symondson, W. O. (2005). Detection of secondary predation by PCR analyses of the gut contents of invertebrate generalist predators. *Molecular Ecology*, 14, 4461–4468. <https://doi.org/10.1111/j.1365-294X.2005.02742.x>
- Sint, D., Guenay, Y., Mayer, R., Traugott, M., & Wallinger, C. (2018). The effect of plant identity and mixed feeding on the detection of seed DNA in regurgitates of carabid beetles. *Ecology and Evolution*, 8, 10834–10846. <https://doi.org/10.1002/ece3.4536>
- Sint, D., Raso, L., Kaufmann, R., & Traugott, M. (2011). Optimizing methods for PCR-based analysis of predation. *Molecular Ecology Resources*, 11, 795–801. <https://doi.org/10.1111/j.1755-0998.2011.03018.x>
- Staudacher, K., Rennstam Rubbmark, O., Birkhofer, K., Malsher, G., Sint, D., Jonsson, M., & Traugott, M. (2018). Habitat heterogeneity induces rapid changes in the feeding behaviour of generalist arthropod predators. *Functional Ecology*, 32, 809–819. <https://doi.org/10.1111/1365-2435.13028>
- Staudacher, K., Wallinger, C., Schallhart, N., & Traugott, M. (2011). Detecting ingested plant DNA in soil-living insect larvae. *Soil Biology and Biochemistry*, 43, 346–350. <https://doi.org/10.1016/j.soilbio.2010.10.022>
- Symondson, W. O. C. (2012). The molecular revolution: Using polymerase chain reaction based methods to explore the role of predators in terrestrial food webs. In G. M. Gurr, S. D. Wratten, & W. E. Snyder (Eds.), *Biodiversity and insect pests* (pp. 166–184). Hoboken, NJ: John Wiley & Sons.
- Symondson, W. O. C., & Harwood, J. D. (2014). Special issue on molecular detection of trophic interactions: Unpicking the tangled bank. *Molecular Ecology*, 23, 3601–3604. <https://doi.org/10.1111/mec.12831>
- Taberlet, P., Coissac, E., Hajibabaei, M., & Rieseberg, L. (2012). Environmental DNA. *Molecular Ecology*, 21, 1789–1793. <https://doi.org/10.1111/j.1365-294X.2012.05542.x>
- Taberlet, P., Gielly, L., Pautou, G., & Bouvet, J. (1991). Universal primers for amplification of 3 noncoding regions of chloroplast DNA. *Plant Molecular Biology*, 17, 1105–1109.
- R Core Team (2018). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <https://www.R-project.org/>
- Thalinger, B., Oehm, J., Mayr, H., Obwexer, A., Zeisler, C., & Traugott, M. (2016). Molecular prey identification in Central European piscivores. *Molecular Ecology Resources*, 16, 123–137. <https://doi.org/10.1111/1755-0998.12436>
- Traugott, M., Kamenova, S., Ruess, L., Seeber, J., & Plantegenest, M. (2013). Empirically characterising trophic networks: What emerging DNA-based methods, stable isotope and fatty acid analyses can offer. *Advances in Ecological Research*, 49, 177–224. *Ecological Networks in an Agricultural World*.
- Wallinger, C., Sint, D., Baier, F., Schmid, C., Mayer, R., & Traugott, M. (2015). Detection of seed DNA in regurgitates of granivorous carabid beetles. *Bulletin of Entomological Research*, 105, 728–735. <https://doi.org/10.1017/S000748531500067X>
- Wallinger, C., Staudacher, K., Sint, D., Thalinger, B., Oehm, J., Juen, A., & Traugott, M. (2017). Evaluation of an automated protocol for efficient and reliable DNA extraction of dietary samples. *Ecology and Evolution*, 7(16), 6382–6389. <https://doi.org/10.1002/ece3.3197>
- Westerman, P. R., Wes, J. S., Kropff, M. J., & Van der Werf, W. (2003). Annual losses of weed seeds due to predation in organic cereal fields. *Journal of Applied Ecology*, 40, 824–836. <https://doi.org/10.1046/j.1365-2664.2003.00850.x>
- Wolf, S., Zeisler, C., Sint, D., Romeis, J., Traugott, M., & Collatz, J. (2018). A simple and cost-effective molecular method to track predation on *Drosophila suzukii* in the field. *Journal of Pest Science*, 91, 927–935. <https://doi.org/10.1007/s10340-017-0948-7>

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