Bulletin of Entomological Research (2015) **105**, 728–735
doi:10.1017/S000748531500067X

Detection of seed DNA in regurgitates of granivorous carabid beetles

C. Wallinger*, D. Sint, F. Baier, C. Schmid, R. Mayer and M. Traugott

Mountain Agriculture Research Unit, Institute of Ecology, University of Innsbruck, Technikerstraße 25, 6020 Innsbruck, Austria

Abstract

Granivory can play a pivotal role in influencing regeneration, colonization as well as abundance and distribution of plants. Due to their high abundance, nutrient content and longevity, seeds are an important food source for many animals. Among insects, carabid beetles consume substantial numbers of seeds and are thought to be responsible for a significant amount of seed loss. However, the processes that govern which seeds are eaten and are therefore prevented from entering the seedbank are poorly understood. Here, we assess if DNA-based diet analysis allows tracking the consumption of seeds by carabids. Adult individuals of *Harpalus rufipes* were fed with seeds of *Taraxacum officinale* and *Lolium perenne* allowing them to digest for up to 3 days. Regurgitates were tested for the DNA of ingested seeds at eight different time points post-feeding using general and species-specific plant primers. The detection of seed DNA decreased with digestion time for both seed species, albeit in a species-specific manner. Significant differences in overall DNA detection rates were found with the general plant primers but not with the species-specific primers. This can have implications for the interpretation of trophic data derived from next-generation sequencing, which is based on the application of general primers. Our findings demonstrate that seed predation by carabids can be tracked, molecularly, on a species-specific level, providing a new way to unravel the mechanisms underlying in-field diet choice in granivores.

Keywords: Carabidae, granivory, spermatophagous species, feeding experiment, *Harpalus rufipes*, *trn*L, seed predation

(Accepted 26 July 2015; First published online 14 August 2015)

Introduction

Granivory describes the consumption of plant seeds by animals. It influences regeneration, colonization as well as abundance and distribution of plants (Janzen, 1971; Crawley, 1997; Hulme & Benkman, 2002; Forget *et al*., 2005). Seed predation occurs in all terrestrial and freshwater habitats populated by higher plants. Compared with other plant tissues, seeds are a food source extremely rich in many nutrients important to the consumers' development and reproduction, often equalling or even exceeding the levels present in animal prey (Hulme & Benkman, 2002; Lundgren, 2009). This may explain why seeds are highly sought after and granivory is widespread. Seed predators come from a diverse range of taxa, including mammals, birds as well as insects such as beetles, bugs, flies, harvester ants, crickets and parasitoids (Janzen, 1971; Heithaus, 1981; Lundgren, 2009). Among beetles, carabids consume substantial amounts of seeds, and are of particular importance in arable land (Tooley & Brust, 2002; Honek *et al*., 2003). According to the large-scale study of Bohan *et al*. (2011), the turnover of the weed seedbank in individual fields is negatively related to the abundance of carabids, indicating their impact of seed predation on the demography of individual weed species (Westerman *et al*., 2003).

However, there is a lack of knowledge on the processes that govern which seed species are actually eaten in the field and thus removed from the seedbank. Feeding experiments indicate that carabids selectively feed on specific seed species

*Author for correspondence
Phone: 0043512-507-51675
Fax: 0043512-507-51799
E-mail: corinna.wallinger@uibk.ac.at

over others, depending on seed size, hardness, mass, coat strength and even density (Lundgren, 2009). As these conclusions stem from observations in the laboratory, they do not necessarily reflect the actual dietary choice of carabids in the field (Boursault, 2013). The conditions of the experiments may distort their choice of specific seeds over others (Lundgren, 2009): In the laboratory, beetles can take what they want, whereas in the field, they do face natural barriers impacting their feeding choice. To date, little direct validation of whether seed species eaten in the laboratory studies are the same as those eaten by a certain predator in the field has been conducted. As such, there is a great need to disentangle the carabids' choice of particular weed seed species from the effects of seed availability in the field. The direct observation of the in-field food choice of granivorous beetles is practically impossible because of the minuteness of the species, their complex environment or their predominantly nocturnal feeding ecology. Over a long period of time, a satisfactory method to examine the food choice of granivorous carabids under field conditions has been missing, leaving us with laboratory feeding studies and field-based seed baiting experiments as the primary methods of observation (Tooley & Brust, 2002). More recently, food-web approaches have been used to predict ecosystem services delivered via seed predation, by inferring trophic links from spatiotemporal analyses of changes in predator and prey (i.e., seed) species abundances, (Pocock *et al*., 2012); with the limitation that they are rarely quantitative or probabilistic.

DNA-based diet analyses have the potential to expand the abilities of these food-web approaches by verifying consumption of certain species and providing information on the strength of the various trophic links. Thus, they can enhance the predictability of food-web-based models. Among the variety of molecular approaches, next generation sequencing (NGS), which has the potential to reveal many consumed species simultaneously (DNA metabarcoding), has become one of the prime methods of choice for answering questions in trophic ecology (Pompanon *et al*., 2012). Continual improvement of NGS technologies, ongoing decreases in costs and current expansion of reference databases make this approach promising. However, because of the short history of NGS, the examination of potential biases associated with this approach has only started. Especially the implicit assumption that frequently consumed species are reflected in a dominant number of their congenial DNA sequences, cannot be taken for granted. Target-specific differences in PCR amplification efficiency using 'general' primers which bind to phylogenetically conserved regions of the DNA, introduce additional variation in sequence numbers (Polz & Cavanaugh, 1998; Sipos *et al*., 2007). Diagnostic PCR employing specific primers offers a well-approved alternative for the molecular identification of specific taxa to unravel trophic interactions, especially when the prey spectrum is known and restricted to a certain number of species. The possibility to screen several hundreds or thousands of samples individually, which is still very costly and usually not affordable on a NGS platform, is one of the biggest pros of diagnostic PCR. Only sufficiently high sample numbers will provide robust and representative diet information for whole populations or species and will be able to answer ecological questions. And other than for general primers, reactions using species-specific primers can be optimized to achieve a balanced sensitivity and amplification efficacy for the different target taxa to minimize methodological bias (Sint *et al*., 2012).

Overall, molecular gut content analysis has become a major tool in examining trophic interactions under natural conditions, addressing a wide range of feeding relationships (Traugott *et al*., 2013; Symondson & Harwood, 2014). Besides animal–animal interactions, these methods have been successfully applied for directly assessing herbivory in insects, in aquatic (Nejstgaard *et al*., 2003; Troedsson *et al*., 2007; Garros *et al*., 2008; Händeler *et al*., 2010) and terrestrial systems, aboveground (Jurado-Rivera *et al*., 2009; Pegard *et al*., 2009; Junnila *et al*., 2010; Navarro *et al*., 2010; Wilson *et al*., 2010; Pumarino *et al*., 2011; Hereward & Walter, 2012; García-Robledo *et al*., 2013; Hereward *et al*., 2013; Kitson *et al*., 2013) and belowground (Schallhart *et al*., 2012; Staudacher *et al*., 2013; Wallinger *et al*., 2014), including the detection of scavenged plant food (Wallinger *et al*., 2013). As yet, granivory has not been considered by this work.

Food DNA degrades continuously during the digestion process and digestion rates have a strong influence on the time span during which consumption of a certain food item can be tracked molecularly. Thus, before newly developed PCR assays can be used to study trophic interactions in the field, their applicability needs to be assessed in calibratory feeding trials (King *et al*., 2008). Such experiments in an environment emulating field conditions provide reliable information on DNA detection limits for specific consumers and prey types and they allow investigating the impact of factors such as consumer biomass and meal size on food DNA digestion rates (Hoogendoorn & Heimpel, 2001; Greenstone *et al*., 2007); information that is highly relevant for a correct interpretation of field-derived data.

In the present study, we will test (i) if and for how long post-feeding plant DNA can be amplified from regurgitates of *Harpalus rufipes* (De Geer, 1774) fed with seeds of either dandelion (*Taraxacum officinale* F.H. Wigg) or perennial ryegrass (*Lolium perenne* L.); and (ii) whether there is a difference in DNA detection rates using general and species-specific plant primers, respectively. Furthermore, we examine how the post-feeding seed DNA detection success is affected by beetle biomass, number of seeds consumed and seed species identity.

Material and methods

Species and experimental setup

Both plant species, *T. officinale* and *L. perenne*, exhibit medium sized seeds which have been reported to be preferred by *H. rufipes* over large and small seeded species (Honek & Martinkova, 2003). The two plant species vary significantly in the mass and nutrient composition of their seeds (table 1); with *T. officinale* seeds exhibiting an oil-content 13 times higher than those of *L. perenne* together with much lower seed mass. In many plant species, such as *T. officinale* what is regularly referred to as the 'seed' is actually an achene; a fruit containing a single seed which does not open at maturity. In the case of *L. perenne* the grain resembles an achene, except that it is a 'caryopsis' with a seed coat fused to the seed wall. For reasons of simplicity we will refer to both the achene and the caryopsis as seeds in the text hereafter.

Most carabids are generalist feeders and consume a wide range of food types (Lövei & Sunderland, 1996). With *H. rufipes* we chose a species that is widespread throughout arable systems (Brandmayer, 1990) and usually occupies a dominant position in ground beetle communities (Luff, 2002). According to Honek *et al*. (2007) the species of the genus

https://doi.org/10.1017/S000748531500067X Published online by Cambridge University Press

Table 1. Seed features of the two seed species fed to *H. rufipes* in the feeding experiments. Data source: Seed Information Data Base SID (http://data.kew.org/sid).

Plant species	Mean 1000 seed mass (g)	Oil content (%)	Protein content (%)
L. perenne	2.2	1.8	18.8
T. officinale	0.6	26.7	30.0

Harpalus eat a greater proportion of seed species compared with other carabids of the same size. A large proportion of its diet is made up of seeds, compared with other food types such as plant parts and invertebrates (Holland, 2002; Tooley & Brust, 2002).

Adult beetles of *H. rufipes* were trapped on arable land near Innsbruck (760 m a.s.l.; Tyrol, Austria) in July and August 2013. The beetles were kept individually in a climate cabinet (L:D 14:10 h at 22 and 12°C, respectively) in plastic beakers (71 × 58 mm^2, *h* × Ø, screw top lid), containing a piece of moistened tissue. Beakers were ventilated and the tissues replaced every second day. The beetles were maintained on a diet of *Tenebrio molitor L.* larvae, which were offered every fifth day. As experimental food, we choose seeds of *T. officinale* and *L. perenne*, that are highly abundant in arable land and which are known to be eaten by *H. rufipes* (Lundgren, 2009). Prior to the feeding experiments, beetles were starved for 5 days. Fresh feeding containers (60 × 35 mm^2, *h* × Ø) were prepared with a drop of water and five plant seeds. The beetles were weighed to the nearest 0.01 mg, put individually into the tubes, and allowed to feed for 2 h in the dark climate cabinet. Afterwards, the seeds were removed and beetles put back individually in clean beakers in the climate cabinet. Beetles were stimulated to regurgitate in batches of 13–15 individuals at 0 h (n = 14), 4 h (n = 14), 8 h (n = 14), 16 h (n = 13), 24 h (n = 14), 32 h (n = 15), 48 h (n = 14) or 72 h (n = 15) post-feeding as follows: each beetle was transferred headfirst into a 2 ml reaction tube, which was then dipped into 65–70°C hot water for 1–2 s following the recommendations of Straube (2013). Only regurgitates from beetles which had consumed at least one seed were considered for the feeding experiment. While the beetles were transferred in clean plastic beakers and put back in the climate cabinet, regurgitates were immediately frozen and stored at −28°C until DNA extraction. All beetles were set free in their original habitat at the end of the experiments.

DNA extraction, PCR and electrophoresis

Regurgitates were dissolved in 200 µl 1 × TES buffer, 5 µl Proteinase K (20 mg ml^{-1}) and 1 mg polyvinylpyrrolidone (PVP) and incubated for 3 h at 58°C. DNA was extracted with the BioSprint 96 DNA Blood Kit (Qiagen, Hilden, Germany) on a Biosprint® 96 extraction robotic platform (Qiagen) following the manufacturer's instruction, with the exception that DNA was finally diluted in 200 µl TES buffer each. DNA extracts were stored at −28°C. All extractions were done in a separate pre-PCR laboratory using a UVC-equipped laminar flow hood and two extraction-negative controls (PCR-grade water instead of regurgitate) were included in each batch of 48 samples to check for cross-sample contamination during the extraction process. None was detected by testing the controls using the diagnostic assays described below.

First, a diagnostic PCR assay with general plant primers targeting chloroplast DNA was used to test the DNA extracts for the presence of plant DNA. Primer c A49325, situated in the *trn*L exon (Taberlet *et al.*, 1991) and primer *trn*L110R located in the *trn*L intron (Borsch *et al.*, 2003) were combined to amplify a fragment of 120 bp length. The assays and PCR conditions followed the description provided in Wallinger *et al.*, (2013). Then the regurgitates were additionally screened with two different multiplex PCR assays specifically targeting DNA of *T. officinale* (multiplex TAT, fragment length 194 bp) and *L. repens* (multiplex FLPS, fragment length 254 bp), respectively, which are described in Wallinger *et al.*, (2012). Both species have a detection limit of 100 template molecules with all assays employed (Wallinger *et al.*, 2012, 2013). Within each PCR, one negative control (PCR water instead of template DNA) and one positive control (seed DNA) was run to check for DNA carry-over contamination and amplification success, respectively.

All PCR products were visualized using QIAxcel, an automated capillary electrophoresis system (Qiagen), with method AL320 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 were deemed to be positive. The DNA extracts of the regurgitates that tested negative in a first run were re-tested in a second PCR to increase the chances of amplification in samples which contained only minute quantities of plant DNA (Seeber *et al.*, 2010).

Statistical analyses

Overall plant DNA detection rates were tested for significant differences between the two seed species and the application of specific and general primers using χ^2 tests. The effect of digestion time (=time post-feeding) on plant DNA detection success was tested for both, the general and the species-specific plant primers, employing a PROBIT regression model as the dependent variables were binomially distributed. To identify those parameters with the highest predictive power for seed DNA detection success, backward logistic regressions were calculated with the following variables: time post-feeding, beetle biomass, number of seeds consumed as well as the interactions seed number × species and seed number × beetle biomass. PROBIT regressions with their 95% confidence intervals were calculated using R 3.1.2 (R Core Team, 2012) and the package 'drc' (Ritz & Streibig, 2005) and non-overlapping confidence intervals for the 50% detection probability were interpreted as significant differences. All other calculations were performed in SPSS 20 (IBM, Armonk, NY, USA).

Results

The mean body mass of *H. rufipes* was 106.13 mg (±27.62 SD; range 31.35–195.41 mg) and 2.42 (±0.79 SD) and 1.87 (±0.80 SD) seeds of *T. officinale* and *L. perenne*, respectively, were consumed on average within the 2 h feeding period. The oil content of *T. officinale* seeds was 13 times higher than for *L. perenne*. While the seeds of *T. officinale* were consumed as a whole, those of *L. perenne* were opened by the beetles and only the inner part was eaten. In total, DNA of 230 regurgitates of *H. rufipes* was extracted and analysed (116 and 114 fed with *T. officinale* and *L. perenne*, respectively). Overall seed DNA detectability was significantly higher for beetles fed with

https://doi.org/10.1017/S000748531500067X Published online by Cambridge University Press

T. officinale (55.2%) than with *L. perenne* (33.6%). When testing the regurgitate samples with general plant primers, the ($\chi^2 = 11.01$; $P < 0.001$). Contrastingly, with the species-specific primers there was no significant difference detectable ($\chi^2 = 0.07$; $P > 0.05$): 50.0% of the regurgitates after feeding with *T. officinale* and 48.7% of those with *L. perenne* were tested positive with the respective species-specific primers. DNA of the consumed plant seeds could be detected in regurgitates for up to 72 h post-feeding, the maximum digestion time in our experiments, for both seed species using general and specific plant primers, whereas it was between 20 and 26% of the beetles' regurgitates that tested positive (fig. 1). The detectability of seed DNA in regurgitates decreased significantly with digestion time for both *T. officinale* and *L. perenne*. With the general primers, the digestion time after which a detection probability of 50% was estimated by the PROBIT regressions, was significantly lower for *L. perenne* (1.5 h; <0–19.2 h 95% confidence interval) compared with *T. officinale* (31.0 h; 22.0–40.1 h). With the species-specific primers, no significant difference was present (*L. perenne* 23.2 h; 11.9–34.4 h; *T. officinale* 25.9 h; 15.3–36.4 h). The results of the backward logistic regressions indicate that neither the parameters tested (meal size, beetle biomass) nor the interactions between them affected the post-feeding seed DNA detection success.

Discussion

We were able to amplify chloroplast DNA from the consumed seeds for up to 3 days post-feeding. The extraction procedure implementing a 3 h lysis step and the use of the Biosprint® extraction robot turned out to be simple, fast, and yield high-quality DNA from beetle regurgitates sufficient for diagnostic PCR. The current study demonstrates that DNA of ingested seeds can be detected in carabid regurgitates via diagnostic PCR at extended times post-feeding, by the example of *H. rufipes*. An initial testing with seed fed *Poecilus versicolor* (Sturm, 1824), *Poecilus cupreus* L. and *Amara* spp. confirmed that plant DNA can in principle also be amplified from regurgitates of other carabid species (data not shown). This indicates a general applicability of the present approach, which has to be tested in more detail in the future. Similar to experiments of Monzo *et al*., (2011) and Waldner *et al*., (2013), where *H. rufipes* was fed animal prey, maximum detection times seem to lie beyond the 3 days tested, indicating that it is possible to identify consumed food with the present approach even if the feeding event has taken place a longer time ago. This fact is highly important when it comes to the analysis of field-collected animals, where the exact time of actual consumption is unknown in most cases.

The present study represents the first attempt to use regurgitates of granivores for the molecular identification of their diet. Hitherto, typically whole-body extracts have been used for identifying trophic interactions in herbivorous arthropods (Nejstgaard *et al*., 2003; Jurado-Rivera *et al*., 2009; Pumarino *et al*., 2011; Staudacher *et al*., 2011; Wallinger *et al*., 2013) or their dissected guts (Matheson *et al*., 2008) entailing several disadvantages connected with the overabundance of non-target DNA, degradation, elevated cross-contamination risk of samples during potential dissection processes and others (Paill, 2004; Waldner & Traugott, 2012; Straube, 2013). In regurgitates, detection rates of seed DNA may not always be 100% even immediately after feeding due to overall low DNA concentrations compared with other samples types (Sint *et al*., 2014) which can cause dropouts during PCR (Sint *et al*., 2011). However, with the present approach we avoid killing the insects which is essential for example in surveys of populations small-sized, where replacement after regurgitate sampling minimizes the impact on the population (e.g., in high Alpine carabid pioneer species (Raso *et al*., 2014) or in experimental settings where populations size is controlled. In the specific case, the application of this non-invasive approach offers the opportunity to monitoring the dietary variations during the season, which are believed to be characteristic for many carabid beetles (Holland, 2002). For example, *H. rufipes* and *Harpalus affinis* were found to take mainly plant material early in the season and insect food during summer, which may be ascribed to changes in seed abundance, as well as changes in other food sources and/or granivore densities (Hulme & Benkman, 2002). Besides, seed feeding can also differ ontogenetically. Some granivorous carabid larvae and even adult beetles are known to create seed caches by burying seeds (Thiele, 1977; Lundgren, 2009). However, the function of these caches is not entirely clear (Lundgren, 2009). While some species seem to consume the seeds, others do not. The molecular approach applied here could help to clarify whether the larvae utilize the stored seeds as food.

Overall plant DNA detection rates ranged between 34 and 61% within a 3-day-digestion period, which is comparable with experiments with the herbivorous *Agriotes* click beetle larvae (Staudacher *et al*., 2011; Wallinger *et al*., 2013). For seeds of both species, *T. officinale* and *L. perenne*, detection of plant DNA was negatively correlated with time post-feeding. This was in accordance with the DNA detection rates observed in click beetle larvae, that fed on roots of *T. officinale* or *Pimpinella major* (L.) HUDS (Wallinger *et al*., 2013). However, there is evidence for the importance of plant species identity in this context, since no such correlation was present for larvae that fed on other plant species (Staudacher *et al*., 2011; Wallinger *et al*., 2013). Likewise for the consumption of seeds, further experimentation is needed to clarify how seed species identity affects post-feeding plant DNA detection rates. As in other studies on herbivorous (Staudacher *et al*., 2011) and carnivorous arthropods (Hoogendoorn & Heimpel, 2001; Juen & Traugott, 2005; Hosseini *et al*., 2008), also here consumer (carabid) biomass and meal size, i.e., number of consumed seeds, did not affect plant DNA detection. However, since the beetles consumed similar amounts of seeds for both seed species in the present feeding experiments, we cannot exclude that meal size might have an effect if there would be stronger differences in the quantity of food consumed (*cf*. King *et al*., 2010).

The *trn*L intron was successfully applied to detect the consumption of the two seed species tested. It has been promoted by Taberlet *et al*., (1991) as a plant barcode harbouring its main power in ecological applications, i.e., when working with degraded DNA (Jurado-Rivera *et al*., 2009; Navarro *et al*., 2010; Schnell *et al*., 2010) as in the present case. The *trn*L-region has been used in studies on herbivory using both NGS techniques (Soininen *et al*., 2009, 2013) and diagnostic PCR (Staudacher *et al*., 2011; Wallinger *et al*., 2012). When applying general plant primers, significant differences between *T. officinale* and *L. perenne* were observed in the overall detection rates for seed DNA. These differences disappeared when regurgitates were tested with species-specific plant primers, even though according to sensitivity tests, all primers employed have the same detection limit when tested without consumer DNA being co-present (Wallinger *et al*., 2012, 2013). This finding corresponds with the assumption of Morales &

https://doi.org/10.1017/S000748531500067X Published online by Cambridge University Press

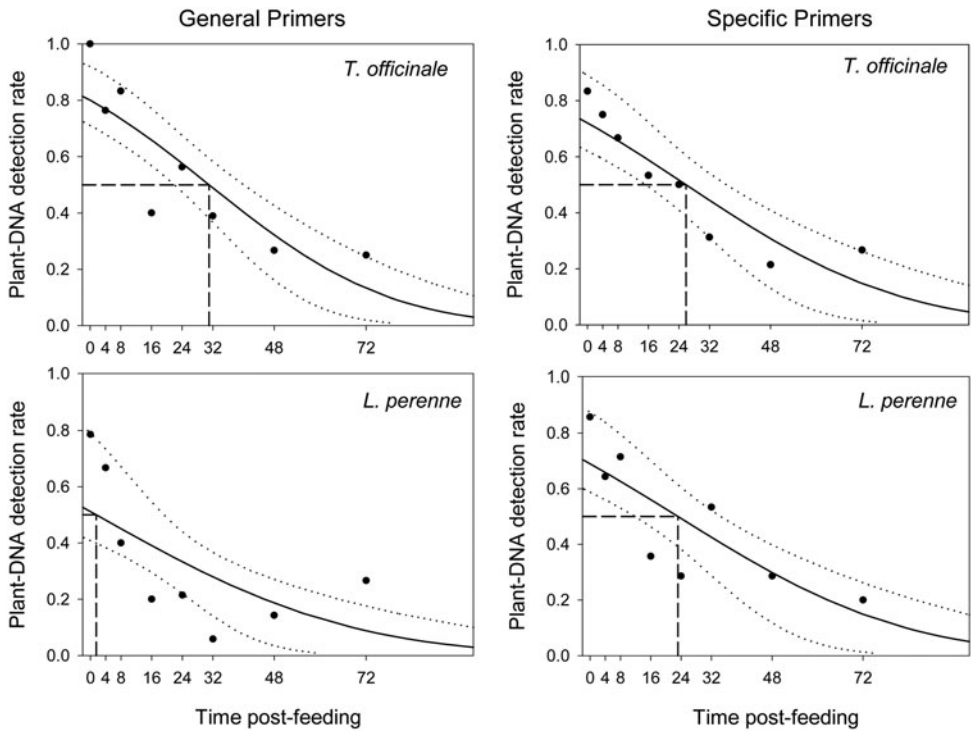

Fig. 1. Detectability of seed DNA in regurgitates of *H. rufipes* fed with seeds of *T. officinale* and *L. perenne* at different time points ranging from 0 to 72 h post-feeding. Detection rates are provided for the different time points as circles along with the fitted PROBIT models for the decrease in seed DNA detection success including the lower and upper 95% confidence intervals (dotted lines) and the time points where the detection probability equals 50% (dashed lines). A minimum of 13 regurgitates per time point post-feeding was tested with general- (left panel) and plant species-specific primers (right panel), respectively.

Holben (2009) that the efficacy of general primers can vary significantly between different targets. It may have implications for the use of NGS techniques which have already been adopted for diet analysis in a wide variety of herbivores, including insects, to unravel feeding interactions on the basis of general primers (Soininen *et al.*, 2009, 2013; Pompanon *et al.*, 2012; Czernik *et al.*, 2013; Srivathsan *et al.*, 2014). As helpful as NGS is in a variety of situations, where only little *a priori* knowledge of the potential prey spectrum is available, the present findings demonstrate that caution is required when NGS-derived trophic data from field-collected samples are interpreted, as detection success might differ between plant species. In cases of a well-known spectrum of potential prey, i.e., when we do know which seed or animal species are available for a given consumer, the development of species or group-specific primers and diagnostic PCR should be taken into account. Especially, when combining them in a multiplex PCR approach, where more than ten species can be identified in a single PCR (Harper *et al.*, 2005); once established this diagnostic approach provides the maximum of validity together with a minimum of time and cost effort and is especially suited for surveys where the screening of large sample numbers is in demand. In the near future, we plan to optimize such diagnostic multiplex PCR approaches for granivorous carabids, based on which we will be able to construct semi-quantitative plant–herbivore trophic links, which depict the relative abundances of both the carabids and the food sources screened for (e.g., weed seeds), as well as the consumption frequencies of these seeds in the field-collected carabids.

In conclusion, the present data show that seed DNA can be readily detected in regurgitates of carabid beetles for extended times post-feeding via general and species-specific plant primers. The molecular approach presented here represents an appropriate methodology for tracking actual seed consumption of beetles in their natural environment. Moreover, the results confirm the caution recommended when choosing a molecular tool (diagnostic PCR, NGS or any other approach) in regard to its suitability for a specific application. Detection rates of seed DNA were high in general, indicating that regurgitates are suitable for identifying seed consumption in *H. rufipes* and probably other carabid species. The approach outlined here is useful for future studies on trophic interactions of plant species and granivorous insects at various scales, circumventing the need of killing those granivores which can be stimulated to regurgitate. Ultimately, the outcomes of this study represent a first step towards an analysis of the dietary choices of granivorous insects to further increase our understanding of seed predation and its ecological significance in both managed and natural systems.

Acknowledgements

Financial support was provided by the project 'The effect of seed identity and digestion time on molecular detection of ingested seeds in two species of granivorous carabid beetles' funded by the Mountain Agriculture Research Unit of the University of Innsbruck. We thank Karin Staudacher from

https://doi.org/10.1017/S000748531500067X Published online by Cambridge University Press

University of Innsbruck and Dave Bohan from INRA Dijon for valuable discussion and comments on the paper.

References

Bohan, D., Boursault, A., Brooks, D. & Petit, S. (2011) National-scale regulation of the weed seedbank by carabid predators. *Journal of Applied Ecology* **48**, 888–898.

Borsch, T., Hilu, K., Quandt, D., Wilde, V., Neinhuis, C. & Barthlott, W. (2003) Noncoding plastid trnT–trnF sequences reveal a well resolved phylogeny of basal angiosperms. *Journal of Evolutionary Biology* **16**, 558–576.

Boursault, A. (2013) Caractérisation des relations trophiques entre composantes d'un agroécosystème: le cas de la prédation des graines d'adventices par les Carabidae, INRA/Universite de Bourgogne.

Brandmayer, T. (1990) Spermophagous (seed-eating) ground beetles: first comparison of the diet and ecology of the harpaline genera *Harpalus* and *Ophonus* (Col., Carabidae). pp. 307–316 *in* Stork, N. (*Ed.*) *The Role of Ground Beetles in Ecological and Environmental Studies*. Andover, Intercept.

Crawley, M. (1997) Plant–herbivore dynamics. *in* Crawley, M. (*Ed.*) *Plant Ecology*. Oxford, Blackwell Publishing Ltd, 401–474.

Czernik, M., Taberlet, P., Swislocka, M., Czajkowska, M., Duda, N. & Ratkiewicz, M. (2013) Fast and efficient DNA-based method for winter diet analysis from stools of three cervids: moose, red deer, and roe deer. *Acta Theriologica* **58**, 379–386.

Forget, P.-M., Lambert, J., Hulme, P. & Cander Wall, S. (2005) *Seed Fate: Predation, Dispersal and Seedling Establishment*. Wallingford, UK, CABI Publishing.

García-Robledo, C., Erickson, D., Staines, C., Erwin, T. & Kress, W. (2013) Tropical plant–herbivore networks: reconstructing species interactions using DNA barcodes. *PLoS ONE* **8**, e52967.

Garros, C., Ngugi, N., Githeko, A., Tuno, N. & Yan, G. (2008) Gut content identification of larvae of the *Anopheles gambiae* complex in western Kenya using a barcoding approach. *Molecular Ecology Resources* **8**, 512–518.

Greenstone, M., Rowley, D., Weber, D., Payton, M. & Hawthorne, D. (2007) Feeding mode and prey detectability half-lives in molecular gut-content analysis: an example with two predators of the Colorado potato beetle. *Bulletin of Entomological Research* **97**, 201–209.

Händeler, K., Wägele, H., Wahrmund, U., Rüdinger, M. & Knoop, V. (2010) Slugs' last meals: molecular identification of sequestered chloroplasts from different algal origins in Sacoglossa (Opisthobranchia, Gastropoda). *Molecular Ecology Resources* **10**, 968–978.

Harper, G.L., King, R.A., Dodd, C.S., Harwood, J.D., Glen, D.M., Bruford, M.W. & Symondson, W.O. (2005) Rapid screening of invertebrate predators for multiple prey DNA targets. *Molecular Ecology* **14**, 819–827.

Heithaus, E. (1981) Seed predation by rodents on three ant-dispersed plants. *Ecology* **62**, 136–145.

Hereward, J. & Walter, G. (2012) Molecular interrogation of the feeding behaviour of field captured individual insects for interpretation of multiple host plant use. *PLoS ONE* **7**, e44435.

Hereward, J., DeBarro, P. & Walter, G. (2013) Resolving multiple host use of an emergent pest of cotton with microsatellite data and chloroplast markers (Creontiades dilutus Stål; Hemiptera, Miridae). *Bulletin of Entomological Research* **103**, 611–618.

Holland, J. (2002) *The Agroecology of Carabid Beetles Andover*. Intercept Limited.

Honek, A. & Martinkova, Z. (2003) Seed consumption by ground beetles. *BCPC International Congress: Crop Science and Technology* **1**, 451–456.

Honek, A., Martinkova, Z. & Jarosik, V. (2003) Ground beetles (Carabidae) as seed predators. *European Journal of Entomology* **100**, 531–544.

Honek, A., Zdenka, M., Saska, P. & Pekar, S. (2007) Size and taxonomic constraints determine the seed preferences of Carabidae (Coleoptera). *Basic and Applied Ecology* **8**, 343–353.

Hoogendoorn, M. & Heimpel, G. (2001) PCR-based gut content analysis of insect predators: using ribosomal ITS-1 fragments from prey to estimate predation frequency. *Molecular Ecology* **10**, 2059–2067.

Hosseini, R., Schmidt, O. & Keller, M. (2008) Factors affecting detectability of prey DNA in the gut contents of invertebrate predators: a polymerase chain reaction-based method. *Entomologia Experimentalis et Applicata* **126**, 194–202.

Hulme, P. & Benkman, C. (2002) Granivory. *in* Herrera, C. and Pellmyr, O. (*Eds*) *Plant-animal Interactions: an Evolutionary Approach*. Oxford, UK, Blackwell Science, 132–154.

Janzen, D. (1971) Seed predation by animals. *Annual Review of Ecology and Systematics* **2**, 465–492.

Juen, A. & Traugott, M. (2005) Detecting predation and scavenging by DNA gut-content analysis: a case study using a soil insect predator-prey system. *Oecologia* **142**, 344–352.

Junnila, A., Müller, G. & Schlein, Y. (2010) Species identification of plant tissues from the gut of *Anopheles sergentii* by DNA analysis. *Acta Tropica* **115**(3), 227–233.

Jurado-Rivera, J., Vogler, A., Reid, C., Petitpierre, E. & Gómez-Zurita, J. (2009) DNA barcoding insect–host plant associations. *Proceedings of the Royal Society B* **276**, 639–648.

King, R., Read, D., Traugott, M. & Symondson, W. (2008) Molecular analysis of predation: a review of best practice for DNA-based approaches. *Molecular Ecology* **17**, 947–963.

King, R., Moreno-Ripoll, R., Agusti, N., Simon, P., Shayler, S., Bell, J., Bohan, D. & Symondson, W. (2010) Multiplex reactions for the molecular detection of predation on pest and nonpest invertebrates in agroecosystems. *Molecular Ecology Resources* **11**, 370–373.

Kitson, J., Warren, B., FFB, V., Baider, C., Strasberg, D. & Emerson, B. (2013) Molecular characterization of trophic ecology within an island radiation of insect herbivores (Curculionidae: Entiminae: *Cratopus*). *Molecular Ecology* **22**, 5441–5455.

Lövei, G. & Sunderland, K. (1996) Ecology and behaviour of ground beetles (Coleoptera: Carabidae). *Annual Review of Entomology* **41**, 231–256.

Luff, M. (2002) Carabid assemblage organization and species composition. pp. 41–79 *in* Holland, J. (*Ed.*) *The Agroecology of Carabid Beetles*. Andover, UK, Intercept.

Lundgren, J. (2009) *Relationships of Natural Enemies and Non-prey Foods*. UK, Springer Science & Business Media.

Matheson, C., Müller, G., Junnila, A., Vernon, K., Hausmann, A., Miller, M., Greenblatt, C. & Schlein, Y. (2008) A PCR method for detection of plant meals from the gut of insects. *Organisms Diversity and Evolution* **7**, 294–303.

Monzo, C., Sabater-Muñoz, B., Urbaneja, A. & Castañera, P. (2011) The ground beetle *Pseudophonus rufipes* revealed as predator of *Ceratitis capitata* in citrus orchards. *Biological Control* **56**, 17–21.

Morales, S. & Holben, W. (2009) Empirical testing of 16S rRNA gene PCR primer pairs reveals variance in target specificity and efficacy not suggested by *in silico* analysis. *Applied and Environmental Microbiology* **75**, 2677–2683.

Navarro, S., Jurado-Rivera, J., Gomez-Zurita, J., Lyal, C. & Vogler, A. (2010) DNA profiling of host-herbivore interactions in tropical forests. *Ecological Entomology* **35**, 18–32.

Nejstgaard, J., Frischer, M., Raule, C., Gruebel, R., Kohlberg, K. & Verity, P. (2003) Molecular detection of algal prey in copepod guts and fecal pellets. *Limnology and Oceanography: Methods* **1**, 29–38.

Paill, W. (2004) Slug feeding in the carabid beetle *Pterostichus melanarius*: seasonality and dependence on prey size. *Journal of Molluscan Studies* **70**, 203–205.

Pegard, A., Miquel, C., Valentini, A., Coissac, E., Bouvier, F., Franois, D., Taberlet, P., Engel, E. & Pompanon, F. (2009) Universal DNA-Based methods for assessing the diet of grazing livestock and wildlife from feces. *Journal of Agriculture and Food Chemistry* **57**, 5700–5706.

Pocock, M., Evans, D. & Memmott, J. (2012) The robustness and restoration of a network of ecological networks. *Science* **335**, 973–977.

Polz, M. & Cavanaugh, C. (1998) Bias in template-to-product ratios in multitemplate PCR. *Applied and Environmental Microbiology*, **64**(10), 3724–3730.

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.

Pumarino, L., Alomar, O. & Agusti, N. (2011) Development of specific ITS markers for plant DNA identification within herbivorous insects. *Bulletin of Entomological Research* **101**, 271–276.

Raso, L., Sint, D., Mayer, R., Plangg, S., Recheis, R., Kaufmann, R. & Traugott, M. (2014) Intraguild predation in pioneer predator communities of Alpine glacier forelands. *Molecular Ecology* **23**, 3744–3754.

R Core Team (2012) R: A Language and Environment for Statistical Computing Vienna, Austria, R Foundation for Statistical Computing.

Ritz, C. & Streibig, J.C. (2005) Bioassay analysis using R. *Journal of Statistical Software* **12**, 1–18.

Schallhart, N., Tusch, M., Wallinger, C., Staudacher, K. & Traugott, M. (2012) Effects of plant identity and diversity on the dietary choice of a soil-living insect herbivore. *Ecology* **93**, 2650–2657.

Schnell, B., Fraser, M., Willerslev, E. & Gilbert, M. (2010) Characterisation of insect and plant origins using DNA extracted from small volumes of bee honey. *Arthropod-Plant Interactions* **4**, 107–116.

Seeber, J., Rief, A., Seeber, G., Meyer, E. & Traugott, M. (2010) Molecular identification of detritivorous soil invertebrates from their faecal pellets. *Soil Biology and Biochemistry* **42**, 1263–1267.

Sint, D., Raso, L., Kaufmann, R. & Traugott, M. (2011) Optimizing methods for PCR-based analysis of predation. *Molecular Ecology Resources* **11**, 795–801.

Sint, D., Raso, L. & Traugott, M. (2012) Advances in multiplex PCR: balancing primer efficiencies and improving detection success. *Methods in Ecology and Evolution* **3**, 898–905.

Sint, D., Niederklapfer, B., Kaufmann, R. & Traugott, M. (2014) Group-specific multiplex PCR detection systems for the identification of flying insect prey. *PLoS ONE* **9**, e115501.

Sipos, R., Szekely, A., Palatinsky, M., Révész, S., Márialigeti, K. & Nikolausz, M. (2007) Effect of primer mismatch, annealing temperature and PCR cycle number on 16S rRNA gene-targetting bacterial community analysis. *FEMS Microbiology Ecology* **60**, 341–350.

Soininen, E., Valentini, A., Coissac, E., Miquel, C., Gielly, L., Brochmann, C., Brysting, A., Sonstebo, J., Ims, R., Yoccoz, N. & Taberlet, P. (2009) Analysing diet of small herbivores: the efficiency of DNA barcoding coupled with high-throughput pyrosequencing for deciphering the composition of complex plant mixtures. *Frontiers in Zoology* **6**, 1–9.

Soininen, E., Zinger, L., Gielly, L., Bellemain, E., Bråthen, K., Brochmann, C., Epp, L., Gussarova, G., Hassel, K. & Henden, J. (2013) Shedding new light on the diet of Norwegian lemmings: DNA metabarcoding of stomach content. *Polar Biology* **36**, 1069–1076.

Srivathsan, A., Sha, J., Vogler, A. & Meier, R. (2014) Comparing the effectiveness of metagenomics and metabarcoding for diet analysis of a leaf-feeding monkey (*Pygathrix nemaeus*) *Molecular Ecology Resources* **15**(2), 250–261.

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.

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. *Ecological Applications* **23**, 1135–1145.

Straube, D. (2013) Development and application of molecular techniques to assess feeding interactions in a forest invaded by the Asian earthworm *Amynthas agrestis*, PhD Thesis, pp. 102, University of Innsbruck.

Symondson, W. & Harwood, J. (2014) Special issue on molecular detection of trophic interactions: Unpicking the tangled bank. *Molecular Ecology* **23**, 3601–3604.

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

Thiele, H.-U. (1977) *Carabid Beetles in their Environments: A Study on Habitat Selection by Adaptation in Physiology and Behaviour*. Berlin, Heidelberg, New York, Springer.

Tooley, J. & Brust, G. (2002) Weed seed predation by carabid beetles. pp. 215–229 in Holland, J.M. (Ed.) *The Agroecology of Carabid Beetles*. Andover, Intercept.

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. pp. 177–224 in Woodward, G. and Bohan, D.A. (*Eds*) *Ecological Networks in an Agricultural World*. Academic Press, San Diego

Troedsson, C., Frischer, M., Neistgaard, J. & Thompson, E. (2007) Molecular quantification of differential ingestion and particle trapping rates by the appendicularian *Oikopleura dioica* as a function of prey size and shape. *Limnology and Oceanography* **52**, 416–427.

Waldner, T. & Traugott, M. (2012) DNA-based analysis of regurgitates: a noninvasive approach to examine the diet of invertebrate consumers. *Molecular Ecology Resources* **12**, 669–675.

Waldner, T., Sint, D., Juen, A. & Traugott, M. (2013) The effect of predator identity on post-feeding prey DNA detection success in soil-dwelling macro-invertebrates. *Soil Biology and Biochemistry* **63**, 116–123.

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. *PLOS ONE* **7**, e29473.

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. *Molecular Ecology Resources* **13**, 75–83.

Wallinger, C., Staudacher, K., Schallhart, N., Mitterrutzner, E., Steiner, E.-M., Juen, A. & Traugott, M. (2014) How generalist herbivores exploit below-ground plant diversity in temperate grasslands. *Molecular Ecology* **23**, 3826–3837.

Westerman, P., Wes, J., Kropff, M. & Van der Werf, W. (2003) Annual losses of weed seeds due to predation in organic cereal fields. *Journal of Applied Ecology* **40**, 824–836.

Wilson, E., Sheena, S., LeVan, K. & Holway, D. (2010) Pollen foraging behaviour of solitary Hawaiian bees revealed through molecular pollen analysis. *Molecular Ecology* **19**, 4823–4829.

https://doi.org/10.1017/S000748531500067X Published online by Cambridge University Press