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Detecting ingested plant DNA in soil-living insect larvae

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ABSTRACT

Although a significant proportion of plant tissue is located in roots and other below-ground parts of plants, little is known on the dietary choices of root-feeding insects. This is caused by a lack of adequate methodology which would allow tracking below-ground trophic interactions between insects and plants. Here, we present a DNA-based approach to examine this relationship. Feeding experiments were established where either wheat (*Triticum aestivum*) or maize (*Zea mays*) was fed to *Agriotes* larvae (Coleoptera: Elateridae), allowing them to digest for up to 72 h. Due to the very small amount of plant tissue ingested (max = 6.76 mg), DNA extraction procedures and the sensitivity of polymerase chain reaction (PCR) had to be optimized. Whole-body DNA extracts of larvae were tested for the presence of both *rbc*L and *trn*L plastid DNA using universal primers. Moreover, based on cpDNA sequences encoding chloroplast tRNA for leucine (*trn*L), specific primers for maize and wheat were developed. With both, general and specific primers, plant DNA was detectable in the guts of *Agriotes* larvae for up to 72 h post-feeding, the maximum time of digestion in these experiments. No significant effect of time since feeding on plant DNA detection success was observed, except for the specific primers in maize-fed larvae. Here, plant DNA detection was negatively correlated with the duration of digestion. Both, meal size and initial mass of the individual larvae did not affect the rate of larvae testing positive for plant DNA. The outcomes of this study represent a first step towards a specific analysis of the dietary choices of soil-living herbivores to further increase our understanding of animal–plant feeding interactions in the soil.

1. Introduction

Usually, large parts of plant biomass are present in the soil (Gregory, 2006). Hence, roots and other below-ground parts of plants are representing an important food source in below-ground ecosystems and many herbivores, including insects, exploit them. However, only few studies have been focusing on the distribution, abundance and feeding ecology of soil-living herbivorous insects so far (Hunter, 2001). The minuteness of the species involved and the opaque habitat with many co-occurring plant species that are not separated spatially impedes the assessment of feeding interactions in soil ecosystems.

Molecular gut content analysis opens up entirely new approaches to study the trophic relationships in soil-living insects, overcoming these methodological hurdles. Several studies successfully used polymerase chain reaction (PCR)-based methods to detect animal prey in arthropods living in the soil or on the soil surface (e.g. Read et al., 2006; Juen and Traugott, 2007; Lundgren et al., 2009; King et al., 2010). Also root-feeding insects contain a 'molecular record' of their food source in the form of ingested plant material in their guts. But, although most insects are herbivores (May, 1988), there have been no studies carried out using molecular detection for the identification of herbal food of soil-living insects so far. This fact may be partly traced back to the difficulties in finding an appropriate DNA barcode in plants, comparable to what is the cytochrome *c* oxidase subunit 1 (COI) in animals (Hollingsworth et al., 2009; Fazekas et al., 2008).

In plants, different regions have been proposed as a barcode, primarily focusing on the plastid genome (e.g. Hollingsworth et al., 2009). For a molecular analysis of ingested plants, the DNA region used should be short enough to amplify degraded DNA. Here, we chose primer sets which amplify only a short portion of the *rbc*L (Poinar et al., 1998) and the *trn*L (UAA) intron (Taberlet et al., 2007). The latter already proved appropriate in identifying digested plant DNA (Pegard et al., 2009; Valentini et al., 2009; Jurado-Rivera et al., 2009; Junnila et al., 2010; Navarro et al., 2010; Schnell et al., 2010).

For the present investigation, *Agriotes* wireworms, the larvae of click beetles (Coleoptera: Elateridae) were chosen as study species since they feed on roots of a wide range of plants in grasslands and arable fields (Langenbuch, 1932; Parker and Howard, 2001; Traugott et al., 2008). They are counted among the most abundant soil pests

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in agricultural land worldwide. In the present experiments we tested whether it is possible to amplify plant DNA from whole-body extracts of *Agriotes* wireworms, fed with either wheat (*Triticum aestivum*) or maize (*Zea mays*), and for how long post-feeding the plant DNA can be detected. Furthermore, we examined if meal size and initial mass of the wireworms affect the detectability of plant DNA in their guts. The analysis was based on plastid DNA, the *rbc*L and the *trn*L (UAA) intron.

2. Materials and methods

2.1. Species and feeding experiment

Sixth to eighth instar larvae of *Agriotes* spp. (Eschscholtz 1829) were collected in summer 2008 in arable land in the district of Heilbronn (Baden-Württemberg, Germany) and in the Inn Valley (Tyrol, Austria). They were kept separately in soil-filled transparent plastic tubes (20 ml) at 16 °C constant in climatic chambers. Soil moisture was checked regularly and the larvae were maintained on germinating wheat. Prior to the experiment wireworms were starved for at least two weeks. As the morphological identification of *Agriotes* species in larval stage is far from straightforward, a molecular assay developed by Staudacher et al. (2010) was used to post-mortem identify all specimens used.

Feeding experiments were conducted where the wireworms were allowed to feed on moistened seeds of either wheat (*T. aestivum*) or maize (*Z. mays*). The feeding activity of each larva was checked regularly. Before and after feeding, the mass per larva was determined to the nearest 0.01 mg, to estimate the amount of consumed plant material. Wireworms which left the seeds untouched were excluded from the experiment. After a 24 h feeding period, larvae were transferred to new soil-filled tubes and individually kept without food at 16 °C constant. A minimum of 10 larvae each was individually frozen at −28 °C at 0, 4, 8, 12, 16, 20, 24, 36, 48 and 72 h post-feeding.

2.2. DNA extraction

Because of the minute amounts of ingested plant tissue (see results), much more wireworm-DNA than plant DNA is present in the whole-body DNA extracts. We decided for a CTAB-based protocol described in Juen and Traugott (2005).

Total DNA was extracted from the fed wireworms as a whole, including any plant DNA present within their guts, as well as from wheat and maize plants. By using whole-body extracts, DNA from plant material sticking on the wireworms' surface may be extracted too, potentially leading to false positive results. Therefore, the outer body surface of each larva was carefully cleaned with a tissue prior to extraction. All extractions were done in a pre-PCR lab using a UV-equipped laminar flow hood; extraction negative controls were included in each batch of wireworms to check for cross-contamination of samples.

2.3. PCR primers

The multi-locus approach, targeting two general markers, should enhance the chance of detection of ingested plant DNA: the DNA extracts of the wireworms were screened in a multiplex PCR which included two pairs of general plant primers (Table 1): one primer pair encompassed a short fragment of the cp gene encoding *rbc*L (*rbc*L19 and *rbc*LZ1; Poinar et al., 1998) and another primer pair amplified the p6-loop within the *trn*L (UAA) intron (primers *g* and *h*; Taberlet et al., 2007). Both fragments have been used successfully to barcode degraded plant DNA (Chase et al., 2007; Jurado-Rivera et al., 2009; Valentini et al., 2009; Junnila et al., 2010; Navarro et al., 2010; Schnell et al., 2010). All wireworm extracts which tested negative for one of these two amplicons in a first run were re-tested in a second PCR.

Table 1
General plant primers targeting plastid *rbc*L and *trn*L DNA, as well as specific primers for wheat (*Triticum aestivum*, TA) and maize (*Zea mays*, ZM) developed for this study. Columns show the primer name (forward followed by reverse primer), plastid DNA region, primer sequence and expected product size.

Primer name	Plastid DNA region	Sequence (5′–3′)	Size (bp)
*rbc*L19[a]	*rbc*L gene	AGATTCCGCAGCCACTGCAGCCCCTGCTTC	153
*rbc*LZ1[a]	*rbc*L gene	ATGTCACCACAAACAGAGACTAAAGCAAGT	
*trn*L-*c*[b]	*trn*L exon	CGAAATCGGTAGACGCTACG	647
*trn*L-*d*[b]	*trn*L exon	GGGGATAGAGGGACTTGAAC	
*trn*L-*g*[b]	*trn*L (UAA) intron	GGGCAATCCTGAGCCAA	91 (wheat)
*trn*L-*h*[b]	*trn*L (UAA) intron	CCATTGAGTCTCTGCACCTATC	89 (maize)
TA-s506	*trn*L	CGGGAGGTGGGGGAGAT	228
TA-a508	*trn*L	TTTCCCTCTTCTTTGAGACMAG	
ZM-s510	*trn*L	ATTTGATCATTATATACATTTTTGAGAT	94
ZM-a514	*trn*L	TATGATTCTATCTTCTCCTTAGTGAAC	

[a] From Poinar et al. (1998).
[b] From Taberlet et al. (2007).

To identify consumed plant DNA to species level, specific primers were required. For the design of these specific primers, targeting either maize or wheat, four individuals per plant species were sequenced using the primer pair *c* and *d* (Taberlet et al., 1991), which amplifies the entire *trn*L (UAA) intron plus flanking regions. PCR products were purified using ExoSAP®-IT (GE Healthcare) and subjected to cycle sequencing PCR (BigDye® Terminator v3.1 Cycle Sequencing Kit, Applied Biosystems, Foster City, USA) following the manufacturer's recommendation. After precipitation and cleanup, PCR products were sequenced on a 3130 Genetic Analyzer (Applied Biosystems) in both, forward and reverse directions. Sequences were assembled in BioEdit Sequence Alignment Editor v7.0.9.0 (Hall, 1999) and compared to sequences already present in GenBank. Based on these sequences, several primer pairs for *T. aestivum* (TA) and *Z. mays* (ZM) were designed (Table 1) using Primer Premier 5 (Premier Biosoft International, Palo Alto, USA).

The specificity of the PCR assays which utilized both general- and specific-plant primers was evaluated against *Agriotes* DNA. Concentration-response trials (serial dilution of plant DNA in the presence of wireworm-DNA) were conducted to identify DNA detection limits and to evaluate the primers' efficiency.

All wireworm-DNA extracts were tested for maize or wheat DNA with the respective specific primers in singleplex PCR. Those samples which did not amplify in this first PCR were tested a second time.

2.4. PCR and visualization of PCR products

The 10 μl reactions included 3 μl DNA extract, 5 μl 2× Type-it Mutation™ PCR Master Mix (Qiagen), 0.5 μl Q-Solution (Qiagen), 5 μg bovine serum albumin (BSA, 10 mg/ml) and the primers at their respective concentrations. For the general primers (*rbc*L- and *trn*L-primers) a final concentration of 0.5 μM and 0.7 μM, respectively, was used whereas the specific primers were employed at 0.6 μM each. The cycling protocol included 5 min at 95 °C, 40 cycles of 30 s at 95 °C, 3 min at 65 °C (universal primers) or 55 °C (specific primers), 90 s at 65 °C and a final elongation of 10 min at 68 °C. One positive (DNA of *T. aestivum* and *Z. mays*, respectively) and several negative controls (PCR-grade water instead of DNA) were run within each PCR assay to check for amplification success and DNA carry-over contamination, respectively.

Visualization of PCR products was done by the QIAxcel system (Qiagen), an automatic capillary electrophoresis, using the high QIAxcel DNA High Resolution kit and the OL400 separation protocol. Electropherograms were analyzed and scored using Bio-Calculator Fast Analysis Software version 3.0 (Qiagen); all samples generating >0.2 fluorescent units were deemed to be positive. The length of the PCR products amplified from the fed *Agriotes* larvae was identified by comparing them with PCR fragments from the positive controls.

2.5. Statistical analyses

The influence of digestion (time post-feeding) on plant DNA detection success was tested for each primer pair (*trn*L, *rbc*L, TA, ZM). Additionally, plant DNA detection rates in relation to meal size (percent mass increase during the 24 h feeding period) and wireworms' initial mass (mg) were investigated. We employed a logit regression model as the dependent variables were binomially distributed. Plant DNA detection rates were tested for significant differences between general and specific primers using chi^2 tests. All calculations were done using SPSS 15.0.1 (SPSS Inc., Chicago, USA).

3. Results

In total, 217 feeding-active *Agriotes* larvae were analyzed (n = 114 wheat-fed; n = 103 maize-fed), comprising at least ten individuals per time point. According to the molecular identification, the following *Agriotes* species were included in the feeding experiments: 189 *Agriotes ustulatus* (Schaller 1783) (wheat-fed 102, maize-fed 87), 15 *Agriotes obscurus* (Linnaeus 1758) (wheat-fed 6, maize-fed 9), and 13 *Agriotes sputator* (Linnaeus 1758) (wheat-fed 6, maize-fed 7).

The initial mean mass was 28.6 mg (±10.9 SD) and 28.5 mg (±11.9 SD) for wheat- and maize-fed wireworms, respectively. After 24 h food availability, the average change in mass (meal size) was 1.32 mg (±1.15 SD; range −1.05–6.67 mg) and 0.41 mg (±1.41 SD; range −6.84–3.85 mg) for wheat- and maize-fed individuals, respectively. This equals an average mass increase of 4.6% (±3.3 SD) in the wheat- and 1.5% (±4.5 SD) in the maize-feeding experiment. However, neither the initial mass of the wireworms nor meal size affected plant DNA detection rates.

DNA of ingested plants could be detected in the guts of wireworms for up to 72 h post-feeding, the maximum digestion time in our experiments, with both general- and specific-plant primers (Figs. 1 and 2). In all of the wireworms frozen immediately after feeding, the *trn*L-fragment could be amplified, whereas the *rbc*L-fragment was detected in approximately 80% of maize-fed and 40% of wheat-fed larvae only.

Considering all time points, detection rates differed significantly between the two general primer pairs used in wheat-fed larvae (Fig. 1): 91% tested positive for the *trn*L-fragment (90 bp) compared to only 48% where the *rbc*L-fragment (153 bp) could be amplified (chi^2 = 49.9, $P < 0.001$). No significant differences were present in detection rates of maize-fed wireworms between the *trn*L- (82.5%) and the *rbc*L-fragment (73.0%) (Fig. 2; chi^2 = 2.8, $P < 0.1$). Using specific primers, significantly fewer wheat-fed larvae (46.5%; TA product length: 228 bp; Fig. 1) than wireworms fed with maize (77%; ZM product length: 94 bp; Fig. 2) tested positive (chi^2 = 20.7, $P < 0.001$).

Detection success of plant DNA was independent from the length of digestion time. The only exceptions were the maize-fed larvae tested with the ZM primer pair, where food detectability decreased significantly with time (P = 0.013, R^2 = 0.058 [Cox & Snell], R^2 = 0.088 [Nagelkerkes]).

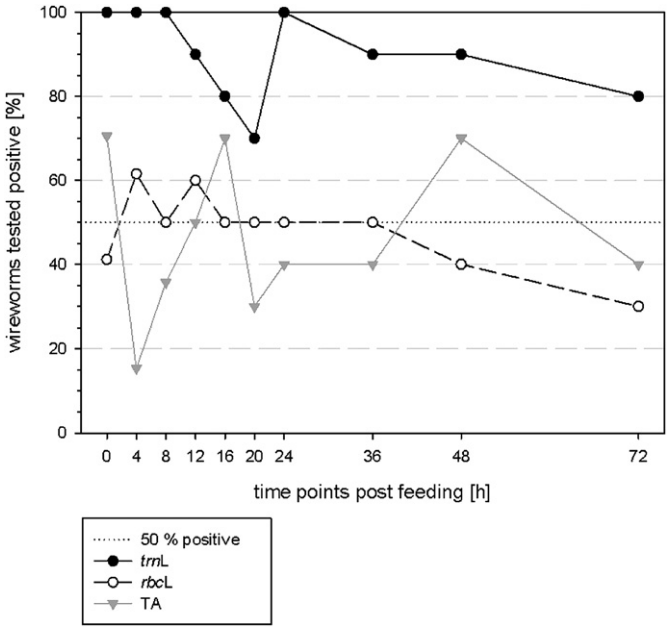

Fig. 1. Detectability of plant DNA in the guts of *Agriotes* spp. larvae fed with wheat (*Triticum aestivum*, TA) for up to 72 h using general primers for the *rbc*L (●) and the *trn*L (○) as well as specific primers for wheat (▼). A minimum of 10 larvae were tested at each time point (17 at 0 h, 13 at 4 h, 14 at 8 h).

4. Discussion

This study demonstrates that plants ingested by herbivorous soil-living insects can be detected and identified by PCR using both, general- and specific-plant primers. Although diagnostic PCR has been widely used for analyzing predator–prey interactions (King et al., 2008), this approach has not been applied for root-feeding insects so far. Recently, a universal method for herbivorous diet

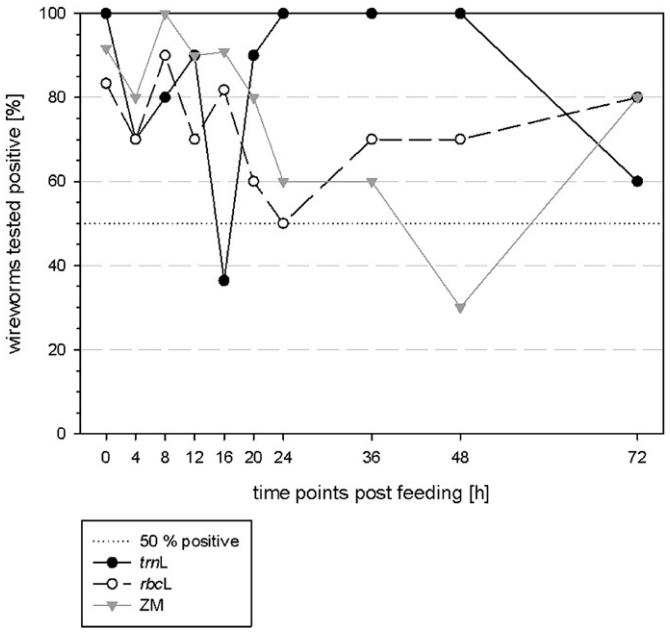

Fig. 2. Detectability of plant DNA in the guts of *Agriotes* spp. larvae fed with maize (*Zea mays*, ZM) for up to 72 h using general primers for the *rbc*L (●) and the *trn*L (○) as well as specific primers for maize (▼). A minimum of 10 larvae were tested at each time point (12 at 0 h, 11 at 16 h).

analysis has been proposed (Valentini et al., 2009), comprising the amplification of the chloroplast *trn*L (UAA) intron (Taberlet et al., 2007) and pyrosequencing of the PCR products. This method proved adequate for analyzing the diet of a wide range of phytophagous species including insects. However, it requires the use of new generation genome sequencers which is costly, especially when large numbers of samples need to be processed. The approach outlined here, on the contrary, can be executed with standard molecular equipment. Nevertheless, a laboratory which is set up for conducting molecular diagnostics is advisable (Mifflin, 2003) as DNA contamination is a serious issue with PCR using general plant primers. Once specific primers are established, the consumer's diet can be specifically analyzed for the respective targets, circumventing the need for DNA sequencing. However, with this approach only plant taxa will be accessible for which primers already have been developed, whereas DNA sequencing will allow an examination without *a priori* knowledge of the dietary choice. If only little is known on the diet of a given herbivore, the pyrosequencing approach provides a first step on identifying the spectrum of the plant species consumed. In a second step, specific trophic links between plants and herbivores can be investigated using the present methodology, which is cost-effective and fast. Hence, the current study represents a first step towards a specific analysis of the dietary choices of soil-living insects on a broader scale.

Both plastid loci selected proved to be suitable for the general detection of ingested plant DNA. The cpDNA sequence encoding *trn*L was used to design primers amplifying differently sized fragments for wheat and maize, enabling an identification of the respective ingested plant species. The *trn*L is highly variable in size and sequence and currently the most widely represented marker in GenBank with 101,508 entries (accessed 4 July 2010). But, no single region employed so far exhibits the high level of resolution seen in most animal COI barcodes. Hence, for some plant species, a multi-locus approach may be inevitable for an unambiguous identification (Chase et al., 2007; Kress et al., 2005; Kress and Erickson, 2007; Fazekas et al., 2008).

Albeit less variable, the plastid locus *rbc*L represents a frequent component of the best performing multi-locus approaches for plant species discrimination (Chase et al., 2005; Newmaster et al., 2006; Hollingsworth et al., 2009). In the present case, the combination of general plant primers targeting *rbc*L and *trn*L did enhance the chance of detecting plant DNA. In fact, 45% of the DNA extracts tested positive either for the *rbc*L or the *trn*L. The regarding wireworms would, by mistake, have been considered as containing no plant DNA in a single-locus approach.

The present results show that detecting chloroplast DNA from whole-body extracts of *Agriotes* larvae by PCR is feasible. Comparable to Nejstgaard et al. (2003), Jurado-Rivera et al. (2009) and Pumarino et al. (2010), we used the whole specimen, thus avoiding elaborate preparations of invertebrate guts (Miller et al., 2006; Matheson et al., 2008). Such dissections are delicate and tedious, especially when the herbivores are small. Besides, they bear a contamination risk or can lead to the degradation of the minute amounts of target-DNA present in the consumer's intestine through enzymatic processes (King et al., 2008).

For all but the *ZM*-primers, the detectability of ingested plant DNA did not negatively correlate with time since feeding, as up to 72 h no significant decrease in detection success was observed. Maximum detection limits in *Agriotes* larvae should be established in future experiments. *Agriotes* larvae consume their food in a liquid state (Langenbuch, 1932). The extended post-feeding intervals observed are comparable to other fluid-feeders such as spiders and hemipterans (Sheppard et al., 2005; Greenstone et al., 2007; Traugott and Symondson, 2008), although potential effects of different ambient temperatures need to be considered when comparing between feeding experiments. Moreover, wireworms are physiologically well adapted to starvation (Traugott et al., 2007), probably allowing them to radically shut down their metabolism. This trait has been discussed as one of the reasons for exceptional long post-feeding prey DNA detection intervals which they share, for example, with spiders.

Based on our results, meal size had no effect on plant DNA detection success in *Agriotes* larvae. This is in accordance to other studies (Hoogendoorn and Heimpel, 2001; Juen and Traugott, 2005; Hosseini et al., 2008). Contrary to carnivorous arthropods where meal size can increase up to 50% of the predators' initial mass (Traugott et al., 2005), the wireworms consumed small portions within the 24 h feeding period. Nevertheless, plant DNA detection ranged between 35 and 80% after a three-day-digestion period, indicating that ingested plant tissue is slowly broken down enzymatically. This could be either due to the fact that plant tissue generally is harder to digest (e.g. thick cell walls which encapsulate and protect the DNA) and/or caused by the physiology and metabolic rates of these insect larvae. Further experimentation is needed to answer these questions.

As any enzymatic processes, DNA digestion rates are likely to be temperature dependent. In fact, high ambient temperatures have been found to effectively decrease detection rates of prey DNA (Hoogendoorn and Heimpel, 2001; Hosseini et al., 2008; von Berg et al., 2008). Wireworms, however, as most other soil-living organisms, are usually not subjected to strong daily fluctuations in temperature as above-ground species are. Thus, the effect of temperature on DNA detection rates probably is less important for interpreting molecular gut content data in soil food webs. According to the findings of McMillan et al. (2007), amplicon size rather than temperature influences detection rates. Generally, shorter fragments seem to improve detection rates (Hoogendoorn and Heimpel, 2001; von Berg et al., 2008; but see Juen and Traugott, 2006; Cassel-Lundhagen et al., 2009). In the present study, the short fragments amplified likely boosted detection success and allowed food detection for a comparable long time period.

In conclusion, our study demonstrates that PCR-based detection of herbivory in invertebrates offers an exciting approach to further increase our understanding of food webs in the soil. Further work, however, is needed to unravel how abiotic and biotic factors affect the detection and degradation of plant DNA in the guts of invertebrates in the field and to examine whether general patterns exist. These data will provide a significant step towards exploiting the full potential of the DNA-approach to specifically analyze the dietary choices of soil-living herbivores.

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References

Cassel-Lundhagen, A., Öberg, S., Högfeldt, C., Ekbom, B., 2009. Species-specific primers for predation studies of the pollen beetle, *Meligethes aeneus* (Coleoptera, Nitidulidae). Molecular Ecology Resources 9, 1132–1134.

Chase, M.W., Cowan, R.S., Hollingsworth, P.M., van den Berg, C., Madriñán, S., Petersen, G., Seberg, O., Jµrgsensen, T., Cameron, K.M., Mark, C.S., Pedersen, N., Hedderson, T.A.J., Conrad, F., Salazar, G.A., Richardson, J.E., Hollingsworth, M.L., Barraclough, T.G., Kelly, L., Wilkinson, M., 2007. A proposal for a standardised protocol to barcode all land plants. Taxon 56, 295–299.

Chase, M.W., Salamin, N., Wilkinson, M., Dunwell, J.M., Kesanakurthi, R.P., Haidar, N., Savolainen, N., 2005. Land plants and DNA barcodes: short-term and long-term goals. Philosophical Transactions of the Royal Society B: Biological Sciences 360, 1889–1895.

Fazekas, A.J., Burgess, K.S., Kesanakurti, P.R., Graham, S.W., Newmaster, S.G., Husband, B.C., Percy, D.M., Hajibabaei, M., Barrett, S.C.H., 2008. Multiple multilocus DNA barcodes from the plastid genome discriminate plant species equally well. PLoS ONE 3, e2802.

Greenstone, M.H., Rowley, D.L., Weber, D.C., Payton, M.E., Hawthorne, D.J., 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.

Gregory, P., 2006. Plant Roots. Growth, Activity and Interaction with Soils. Blackwell Publishing, Oxford, UK, pp. 318.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows. Nucleic Acids Symposium Series 41, 95–98.

Hollingsworth, M.L., Clark, A.A., Forrest, L.L., Richardson, J., Pennington, R.T., Long, D.G., Cowan, R., Chase, M.W., Gaudeul, M., Hollingsworth, P.M., 2009. Selecting barcoding loci for plants: evaluation of seven candidate loci with species-level sampling in three divergent groups of land plants. Molecular Ecology Resources 9, 439–457.

Hoogendoorn, M., Heimpel, G.E., 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.A., 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.

Hunter, M.D., 2001. Out of sight, out of mind: the impacts on root feeding insects in natural and managed systems. Issues in Agricultural and Forest Entomology 3, 3–9.

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.

Juen, A., Traugott, M., 2006. Amplification facilitators and multiplex PCR: tools to overcome PCR-inhibition in DNA-gut content analysis of soil-living invertebrates. Soil Biology & Biochemistry 38, 1872–1879.

Juen, A., Traugott, M., 2007. Revealing species-specific trophic links in soil food webs: molecular identification of scarab predators. Molecular Ecology 16, 1545–1557.

Junnila, A., Müller, G.C., 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.A., Vogler, A.P., Reid, C.A.M., Petitpierre, E., Gómez-Zurita, J., 2009. DNA barcoding insect–host plant associations. Proceedings of the Royal Society B 276, 639–648.

King, R.A., Read, D.S., Traugott, M., Symondson, W.O.C., 2008. Molecular analysis of predation: a review of best practice for DNA-based approaches. Molecular Ecology 17, 947–963.

King, R.A., Vaughan, I.P., Bell, J.R., Bohan, D.A., Symondson, W.O.C., 2010. Prey choice by carabid beetles feeding on an earthworm community analysed using species- and lineage-specific PCR primers. Molecular Ecology 19, 1721–1732.

Kress, W.J., Erickson, D.L., 2007. A two-locus global DNA barcode for land plants: the coding rbcL gene complements the non-coding trnH-psbA spacer region. Plosone, 1–10.

Kress, W.J., Wurdack, K.J., Zimmer, E.A., Janzen, D.H., 2005. Use of DNA barcodes to identify flowering plants. Proceedings National Academic of Science 102, 8369–8374.

Langenbuch, R., 1932. Beiträge zur Kenntnis der Biologie von *Agriotes lineatus* L. und *Agriotes obscurus* L. Zeitschrift für angewandte Entomologie 19, 278–300.

Lundgren, J.G., Ellsbury, M.E., Prischmann, D.A., 2009. Analysis of the predator community of a subterranean herbivorous insect based on polymerase chain reaction. Ecological Applications 19, 2157–2166.

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

May, R.M., 1988. How many species are there on earth? Science 241, 1441–1449.

McMillan, S., Kuusk, A.K., Cassel-Lundhagen, A., Ekbom, B., 2007. The influence of time and temperature on molecular gut content analysis: *Adalia bipunctata* fed with *Rhopalosiphum padi*. Insect Science 14, 353–358.

Mifflin, T.E., 2003. Setting up a PCR laboratory. In: Dieffenbach, C.W., Dveksler, G.S. (Eds.), PCR Primer: a Laboratory Manual, second ed. Cold Spring Harbor Laboratory Press, New York, pp. 5–14.

Miller, M.A., Müller, G.C., Kravchenko, V.D., Junnila, A., Vernon, K.K., Matheson, C.D., Hausmann, A., 2006. DNA-based identification of Lepidoptera larvae and plant meals from their gut content. Russian Journal of Entomology 15, 427–432.

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

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

Newmaster, S.G., Fazekas, A.J., Ragupathy, S., 2006. DNA barcoding in land plants: evaluation of *rbcL* in a multigene tiered approach. Canadian Journal of Botany 84, 335–341.

Parker, W.E., Howard, J.J., 2001. The biology and management of wireworms (*Agriotes* spp.) on potato with particular reference to the U.K. Agricultural and Forest Entomology 3, 85–98.

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 faeces. Journal of Agricultural Food Chemistry 57, 5700–5706.

Poinar, H.N., Hofreiter, M., Spaulding, W.G., Martin, P.S., Stankiewicz, B.A., Bland, H., Evershed, R.P., Possnert, G., Pääbo, S., 1998. Molecular Coproscopy: dung and diet of the extinct ground Sloth *Nothrotheriops shastensis*. Science 281, 402–406.

Pumarino, L., Alomar, O., Agusti, N., 2010. Development of specific ITS markers for plant DNA identification within herbivorous insects. Bulletin of Entomological Research doi:10.1017/S0007485310000465.

Read, D.S., Sheppard, S.K., Bruford, M.W., Glen, D.M., Symondson, W.O.C., 2006. Molecular detection of predation by soil micro-arthropods on nematodes. Molecular Ecology 15, 1963–1972.

Schnell, B.I., Fraser, M., Willerslev, E., Gilbert, M.P.T., 2010. Characterisation of insect and plant origins using DNA extracted from small volumes of bee honey. Arthropod-Plant Interactions 2, 107–116.

Sheppard, S.K., Bell, J.R., Sunderland, K.D., Fenlon, J., Skervin, D., Symondson, W.O.C., 2005. Detection of secondary predation by PCR analyses of the gut contents of invertebrate generalist predators. Molecular Ecology 14, 4461–4468.

Staudacher, K., Pitterl, P., Furlan, L., Cate, P.C., Traugott, M., 2010. PCR-based identification of *Agriotes* wireworms. Bulletin of Entomological Research doi:1017/S0007485310000337.

Taberlet, P., Coissac, E., Pompanon, F., Gielly, L., Miquel, C., Valentini, A., Vermat, T., Corthier, G., Brochmann, C., Willerslev, E., 2007. Power and limitations of the chloroplast *trn*L (UAA) intron for plant DNA barcoding. Nucleic Acids Research 35, 14–21.

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.

Traugott, M., Weissteiner, S., Strasser, H., 2005. Effects of entomopathogenic fungus *Beauveria brongniartii* on the non-target predator *Poecilus versicolor* (Coleoptera: Carabidae). Biological Control 33, 107–112.

Traugott, M., Pazmandi, C., Kaufmann, R., Juen, A., 2007. Evaluating $^{15}N/^{14}N$ and $^{13}C/^{12}C$ isotope ratio analysis to investigate trophic relationships of elaterid larvae (Coleoptera: Elateridae). Soil Biology & Biochemistry 39, 1023–1030.

Traugott, M., Schallhart, N., Kaufmann, R., Juen, A., 2008. The feeding ecology of elaterid larvae in central European arable land: new perspectives based on naturally occurring stable isotopes. Soil Biology & Biochemistry 40, 342–349.

Traugott, M., Symondson, W.O.C., 2008. Molecular analysis of predation on parasitized hosts. Bulletin of Entomological Research 98, 223–231.

Valentini, A., Miquel, C., Nawaz, M.A., Bellemain, E., Cossiac, E., Pompanon, F., Gielly, L., Crudaud, C., Nascetti, G., Wincker, P., Swenson, E., Taberlet, P., 2009. New perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: the *trn*L approach. Molecular Ecology Resources 9, 51–60.

von Berg, K., Traugott, M., Symondson, W.O.C., Scheu, S., 2008. The effects of temperature on detection of prey DNA in two species of carabid beetle. Bulletin of Entomological Research 98, 263–269.