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Detecting arthropod intraguild predation in the field

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Abstract The process of biological control carries a distinct risk that an alien biological control agent (BCA) will become established as an invasive alien species with an associated threat to the local ecosystem biodiversity. It is imperative that a wide-ranging environmental risk assessment (ERA) is performed before the release of any BCA. This should include considering various potential but difficult to observe ecological interactions between the BCA and members of the native community, including disruption of intraguild relationships. Detection of intraguild predation (IGP) events involving predatory arthropods in the field can be done by analyzing their gut contents. Polymerase chain reaction (PCR) is a sensitive and specific tool to identify target prey DNA within a predator's gut. This paper reviews the efficiency of a DNA based approach for detecting IGP in the field, compared with detection by the use of monoclonal antibodies or gas chromatography. Prey specificity, detection times after prey consumption, capacity for quantification, multiple prey targeting and the time and costs involved in developing and using the different methods are considered.

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Introduction

Biological invasions threaten the biodiversity of many ecosystems. Invasive alien species can affect ecosystems at the species, population or community level (Kenis et al. 2009). For example the red imported fire ant *Solenopsis invicta* (Hymenoptera: Formicidae) invaded parts of North America from South America. Since its arrival it has been shown to negatively affect arthropods, molluscs, reptiles, birds, amphibians and mammals (Holway et al. 2002). Invasive alien species were found to be the primary factor causing species extinction on isolated islands (Wilcove et al. 1998). Human activities are considered as the major driver of biological invasions (Roy and Wajnberg 2008a; Leibhold et al. 2006). In biological control, natural enemies are introduced to regulate the population of a detrimental species. When the natural enemy is alien to the area of release, it may pose a risk of biological invasion. In the context of augmentative biological control, exotic biological control agents (BCA) may be mass-reared and released on greenhouse crops to control pests. Introduced BCAs may escape from greenhouses, establish in new habitats and negatively affect non-target native species. Classical biological control (where the establishment of the BCA is intended) can be viewed as a controlled biological invasion. In both situations, the use of an alien BCA must follow rigorous environmental risk assessment (ERA) procedures in order to identify, measure and weigh risks to the environment (Bigler et al. 2006). An overview of the current ERA procedure is given in Fig. 1 of Gibbs et al. (2011).

The field of ERA faces many challenges. Firstly, providing data on all aspects of an ERA is time consuming and costly. Secondly, direct and indirect effects of a BCA on the environment (see Gibbs et al. 2011 for a definition) embrace complex aspects of its own biology and interactions with native non-target species. Thirdly, and perhaps the biggest challenge, is the lack of a globally harmonized regulation for the release of BCAs among countries.

Considering the second area of concern, there are many major ecological processes potentially taking place between the BCA and members of the native community such as intraguild predation (IGP), interspecific competition, hybridization and pathogen transmission (van Lenteren et al. 2003). In current ERA schemes all these major ecological processes are handled as a single question in the procedure, in a generic "black-box" approach (see Fig. 1 in Gibbs et al. 2011). There is an urgent need to develop strategies for assessing these important different issues. Paralleling the situation in the field of invasion biology (see Lawson Handley et al. 2011), despite continuous advances in methodology (Harwood and Obrycki 2005; Kasper et al. 2004), molecular techniques are seldom used in the field of ERA to answer biological control questions such as those concerning trophic interactions. This review evaluates the use of immunological, chemical and molecular approaches to determine levels of intraguild predation by BCAs, in the field.

Intraguild predation

Intraguild predation is the killing and eating of a species that uses similar, often limiting, resources. Thus the intraguild prey is also a competitor. IGP differs from classical predation in that the predation event reduces potential exploitation competition (Polis et al. 1989). IGP is widespread in many trophic systems (Polis and Holt, 1992; Arim and Marquet 2004) and can be regarded as important in maintaining the ecological balance between species. BCAs can disrupt this balance and seriously impact various species through IGP (Rosenheim et al. 1995). Efficient methods are needed to assess the potential IGP impact of BCAs. The recent establishment of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) in North America, South America and Europe illustrates this point.

Harmonia axyridis is a large, voracious coccinellid with high fecundity and fertility and a wide dietary niche (van Lenteren et al. 2003; Berkvens et al. 2008). Whilst primarily an aphid-feeder, it can also feed on many other insects, including coccinellids and other aphidophagous insects (Yasuda and Shinya 1997; Gardiner and Landis 2007; Ware and Majerus 2008). It was released as a BCA for classical

biological control in the USA and several European countries (see De Clercq and Bale 2011 and Brown et al. 2011b for a review). After an apparent lag phase it has established in various countries (Roy and Wajnberg 2008a) where it has been shown to negatively affect native insect communities, the North American wine industry (Pickering et al. 2004; Galvan et al. 2007) and human well-being (Roy and Wajnberg 2008b).

Harmonia axyridis has become the most common ladybird in several countries where various non-target effects have already been reported (Brown et al. 2011b). Ecological studies in a number of European countries have shown that certain aphidophagous species may be harmed by *H. axyridis*. Brown et al. (2011) showed evidence of a decline in the abundance of *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) in England and there has been a drop in frequency of *A. bipunctata* and *Adalia decempunctata* (L.) (Coleoptera: Coccinellidae) in Belgium (Adriaens et al. 2010). Studies on intraguild interactions between *H. axyridis* and other aphid predators have been conducted under controlled conditions in laboratories and reveal predation of these other species by *H. axyridis* (Pell et al. 2008). Although these laboratory bioassays enhance our understanding of possible IGP among aphidophagous species, it remains unclear whether the success of *H. axyridis* in the field can be attributed to a direct (e.g. intraguild predation) and/or an indirect (e.g. competition for resources with native species) effect. There is a need for a reliable procedure that can directly assess IGP in the field. The "*H. axyridis* case" is felt by many as the biggest recent failure of biological control with invertebrate species (Roy and Wajnberg 2008b). Following its establishment in different parts of the world, many research groups are trying to understand the reason for its invasive success and its impact on the environment (Roy and Wajnberg 2008a), hoping to prevent similar cases in the future. We are variously involved in developing molecular techniques to assess IGP by *H. axyridis* in the field. Coccinellids, chrysopids, syrphids and anthocorids commonly co-occur spatially and temporally with *H. axyridis* in natural, semi-natural and anthropogenic (e.g. agricultural and urban) habitats, and are potential intraguild prey. In writing this paper we drew upon our own experiences in developing IGP detection techniques to target species from these four families to review the current state of procedures to measure arthropod IGP in the field.

Over recent decades a range of methods have been used to identify or infer predation in natural conditions. Earlier methods include direct observation of predation and visual identification of gut contents involving the use of a microscope. More recently, immunological, chemical and isotopic techniques (Kiritani and Dempster 1973; Sunderland 1988; Harwood and Greenstone 2008), have been used to deduce the diet of coccinellids and other aphids predators (reviewed in Harwood and Obrycki 2005; Weber and Lundgren 2009a). In the context of identifying IGP by arthropods, three methods are of particular interest: the use of monoclonal antibodies, gas chromatography mass spectrometry (GC–MS) and a DNA based approach. Monoclonal antibodies react with an antigen of the target prey, this being typically measured using an enzyme linked immunosorbent assay (ELISA) (Greenstone 1996). Monoclonal antibodies have been developed for and used to identify non-coccinellid preys of *H. axyridis* (e.g. Fournier et al. 2006; Moser et al. 2008), although thus far no antibodies have been developed for coccinellid intraguild prey. In GC–MS, distinctive prey chemicals or suites of chemicals are isolated from the predator in order to identify diet components (Knutsen and Vogt 1985; Becerro et al. 2006). Because ladybirds possess taxonomically-specific defensive alkaloids (Daloze et al. 1995), GC–MS of prey alkaloids in predator bodies is particularly appropriate for studying IGP of other ladybird species by *H. axyridis* (Hautier et al. 2008; Sloggett et al. 2009; Sloggett et al. 2011) and to date are used in the only two published field studies on the ecological impact of *H. axyridis* (Hautier et al. 2008; Hautier et al. 2011). In addition to the above technique, the last decade has seen the development of a PCR DNA based approach to identifying the gut contents of predatory invertebrates (King et al. 2008).

DNA approaches have superseded the use of monoclonal antibodies to detect hidden trophic interactions. After a decade of use, we are now in a position to present a critical assessment of the DNA approach to identifying predation events, in relation to the ubiquitous use of monoclonal antibody in the 1990s and the more recent exploration of GC–MS. In this review, we draw upon both the wealth of literature now available on this topic, and our own

recent experiences in designing molecular detection systems for *H. axyridis*, in order to compare the different techniques available for studying IGP in the field. In comparing the use of these methods, the criteria taken into account are: its prey specificity, detection time after prey consumption, capacity for quantification, possibility of looking at multiple targets simultaneously, likelihood of errors of interpretation and the time and costs involved in developing and using the method.

Principles of a DNA based IGP study

Choice of target gene

A key early decision in developing a PCR based IGP detection system is which prey DNA sequence to target. A clear consensus has emerged from the many studies conducted over the last decade which have used PCR to analyse the gut contents of invertebrate predators. Highest prey detection success has consistently been achieved when the prey target DNA sequence is short (100–200 nucleotides in length), and present in multiple copies within each cell. These two criteria clearly relate to the dynamics of digestion occurring in the predator gut. A shorter target sequence is likely to survive random digestion for the longest time. A multiple copy target site again maximizes the time copies of the target sequence survive digestion, and thus increases PCR sensitivity.

The best prey detection success has been achieved in studies that have targeted mitochondrial sequences: there can be one thousand or more copies of mitochondrial genes in an individual cell (Hoy 1994) compared to the two copies of most nuclear genes. The favoured mitochondrial target sequences have been a section of the cytochrome oxidase subunit I (COI) gene. COI has most often been used as the target gene as there is much sequence information already available on internet databases such as GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and in published papers. As a result, useful universal PCR primers exist (e.g. Folmer et al. 1994) and can be used to amplify large sections of the COI gene in both the target prey and predator species under investigation. The resulting sequences can be used to design PCR primer pairs specific to the prey species. It is best to generate one's own COI sequences for the designing of prey specific primers. This guarantees COI sequence from accurately identified specimens. An appraisal of the intraspecific variation of the CO1 or other gene chosen also needs to be undertaken, as occasionally a high level of sequence variation occurs between individuals of a prey species. As a consequence, PCR primers might not bind in all sampled individuals, and so prey detection might fail. High levels of intra-specific variability among lumbricid earthworms (*Aporrectodea caliginosa* (Savigny), *A. longa* (Ude), *A. rosea* (Savigny), *Allolobophora chlorotica* (Savigny), *Lumbricus castaneus* (Savigny), *L. festivus* (Savigny), *L. rubellus* Hoffmeister, *L. terrestris* L. and *Octolasion cyaneum* (Savigny)) foiled attempts by Harper et al. (2005) to design COI specific primers in a study investigating spider prey.

Alternative multicopy genes that have been successfully used in predation studies are mitochondrial cytochrome oxidase subunit II (COII) (e.g. Chen et al. 2000; McMillan et al. 2007), cytochrome b (Pons 2006) and the mitochondrial (12S or 16S) ribosomal genes (e.g. Harper et al. 2005). There have also been a few studies using target sequences within multi-copy nuclear ribosomal genes. For example Hoogendoorn and Heimpel (2001) investigated predation of the European corn borer *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) by *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae) and *H. axyridis* through targeting a section of the ITS 1 region. Other researchers have rejected the ITS 1 region as it can show high levels of intraspecific variation. Zaidi et al. (1999) successfully targeted the multi-copy nuclear esterase gene in their study of predation of the mosquito *Culex quinquefasciatus* (Say) (Diptera: Culicidae) by the carabid beetle *Pterostichus cupreus* (Linnaeus) (Coleoptera: Carabidae).

Many early studies focused on assessing prey detection sensitivity in laboratory based feeding trials. For example, Hoogendoorn and Heimpel (2001) investigated detectability of four different sized fragments (492, 369, 256 and 150 bp) of the nuclear ribosomal ITS 1 sequence of *O. nubilalis* within the gut of *C. maculata*. The shortest (150 bp) fragment was present for the full 12 h of the feeding assay, whereas the two longest fragments disappeared after 4 h and 5 h respectively. Chen et al. (2000) assessed detection within the gut of the lacewing *Chrysoperla plorabunda* (Fitch) (Neuroptera: Chrysopidae) and of

the ladybird *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae) of three fragments (339, 246 and 198 bp) of the COII gene of the corn leaf aphid *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae). They showed that the largest fragment had a statistically shorter half life than the two smaller fragments. Studies from the mid to late 2000s have consistently targeted prey amplicons less than 250 bps (see King et al. 2008). The main steps of a general procedure to design and test primers for use in a DNA detection system for IGP are summarized in Fig. 1. The best practice for most parts of this procedure is reviewed by King et al. (2008). Here, we highlight key aspects.

Calibratory feeding trials

Feeding experiments are an integral part of PCR based prey detection analyses. Not only have they been useful in early generic studies in confirming that shorter target sequences will be detected for the longest time periods, but they form an essential part of the development of each new detection system. Once a target sequence has been chosen, and cross reactivity tests have shown detection to be specific to the desired prey, then feeding trials need to be conducted to gain an insight into the dynamics of digestion of the system under consideration i.e. for how long it is possible to detect prey DNA within the predator gut after feeding. The importance of project specific feeding trials is emphasised by the great variability that has been shown to exist in post-feeding detection periods. Different predator species digest prey at different rates. For example, compared to insects, spider are slow digesters: in a study assessing predation by linyphiid spiders, Agustí et al. (2003) found 100% detection of 211–276 bp fragments of the COII gene after a 24 h digestion. In contrast, Chen et al. (2000) found a half life of only 3.95 h for a 198 bp fragment of the COII gene from the aphid *R. maidis* within the gut of the lacewing *C. plorabunda*.

Feeding experiments represent an artificial situation. The predator is starved beforehand, generally for 24 h (King et al. 2008). The prey species of interest is the sole source of food for the predator, yet in the field it may represent only a small percentage of the predator's daily diet. In feeding experiments, meal size is controlled. Generally, newly moulted or starved individuals are offered large amounts of prey material e.g. large numbers of eggs or whole, late instar larvae. This may bias towards longer post ingestion detection. In the field, predators might be partially satiated, and therefore ingest smaller amounts. Rates of digestion might be affected by many factors such as the amount of food present in the gut, the temperature and the predator's activity level.

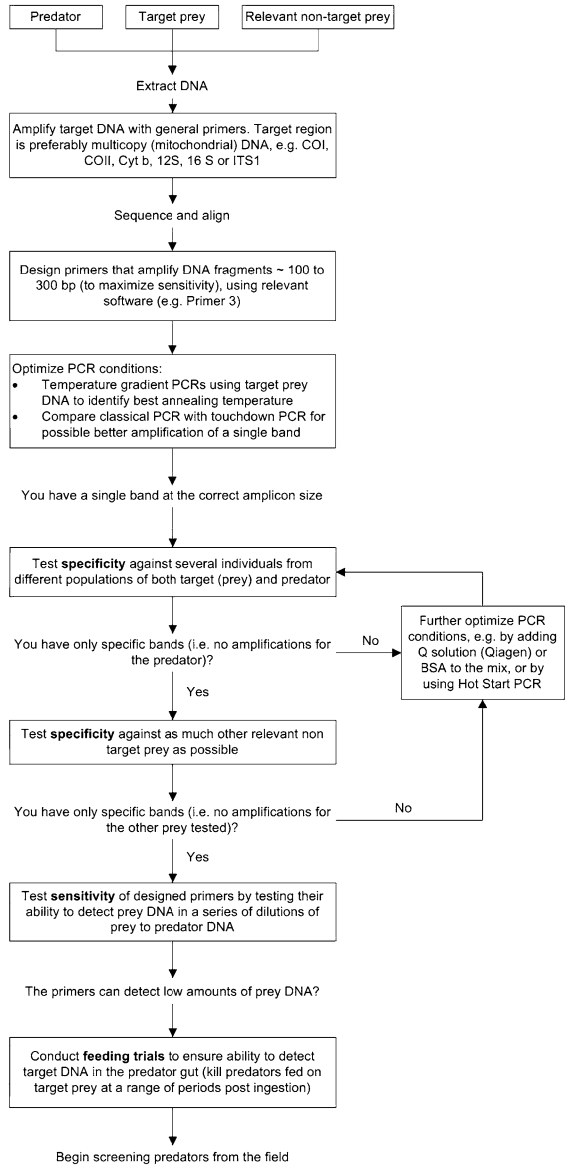

Fig. 1 Basic procedure to design and test primers for use as molecular markers for intraguild predation studies under field conditions. Adapted from King et al. (2008). For further details, see main text or King et al. (2008)

Field studies

The ultimate goal of DNA based prey detection is to use it to detect interactions occurring in the field. In recent years a series of papers have been published which confirm the effectiveness of this molecular technique to aid investigations of key ecological issues. How, for example, PCR based DNA prey detection can give insights into previously impenetrable trophic interactions? As part of a study characterising below ground trophic interactions in Austrian alpine meadows, Juen and Traugott (2005) investigated the invertebrate predator guild of the garden chafer *Phyllopertha horticola* L. (Coleoptera: Scarabaeidae). Targeting a 291 bp section of the chafer COI gene was highly informative in identifying centipedes and predatory beetle larvae as the main consumers of *P. horticola* during summer months. Other studies have shown the utility of DNA based prey detection to investigate the effectiveness of a BCA. The knowledge gained from the studies described in the next paragraph hint at ways of enhancing the biological control of pests.

Harwood et al. (2007) used PCR based detection to assess predation by the anthocorid bug *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) of the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), in North America. They had previously tested, in a series of feeding experiments, the effectiveness of primers designed to target the COI gene. When applied in the field, 32% of *O. insidiosus* tested positive for *A. glycines* DNA over their early June to late August sampling periods. Critically, disproportionately high levels of the aphid were consumed early in the sample period, when aphid abundance was low, identifying *O. insidiosus* as a potential biocontrol agent. In another study Zhang et al. (2007) showed the effectiveness of DNA detection to access the potential of native natural enemies to control the cotton whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), a recently introduced horticultural pest in Northern China. A nuclear marker was developed and tested in laboratory feeding experiments and then used to look for the presence of *B. tabaci* in the guts of 185 arthropod predators representing seven species. In some species, close to 100% of individuals showed the presence of *B. tabaci*, and generally more larval than adult predators tested positive. Agustí et al. (2003) investigated predation of three springtail species by a range of linyphiid spider species in UK wheat fields where these spiders are important aphid control agents. Forty-eight percent of spiders tested positive for *Isotoma anglicana* Lubbock (Collembola: Isotomidae) and a further 16% for the other two species. This preferential predation was unexpected as *I. anglicana* was the least abundant of the three springtail species. This discovery rose questions regarding the spiders' predation habits, and their potential role as natural enemies of pest aphids and how higher beneficial spider population levels may be maintained between crop growing seasons.

A comparison of DNA detection of IGP with the use of monoclonal antibodies or GC–MS

Detection period of prey after a predation event

For all three approaches, the period for which prey can be detected after being eaten varies in relation to a wide range of factors including what the predator has eaten, temperature and the type of prey involved as well the exact experimental conditions (e.g. Hoogendoorn and Heimpel 2001; Juen and Traugott 2005; Weber and Lundgren 2009b). It is, however, possible to make some generalisations about detection periods. In general, DNA detection times are a few to many hours (McMillan et al., 2007; Greenstone et al., 2007), while detection using ELISA, can extend to days (Sopp and Sunderland, 1989; Symondson and Liddell, 1995). Results from GC–MS are highly variable, because target chemicals, especially defensive ones, can be broken down (Sloggett and Davis 2010; Sloggett et al. 2011), sequestered or may remain in the predator as unmetabolised compounds. While Sloggett et al. (2009) found that hippodamine from single *H. convergens* eggs persisted on average for 13.5 h in *H. axyridis* intraguild predators, Hautier et al. (2008) found that adaline from a single *A. bipunctata* first instar larva could persist for many days.

Prey specificity

The highest specificity of all techniques is provided by monoclonal antibodies as they can be species- and even life stage-specific (Boreham and Ohiagu 1978).

DNA techniques enable prey identification to the species level according to primer specificity, but cannot differentiate between life stages (Symondson 2002; Sheppard and Harwood 2005). GC–MS exhibits the least specificity, because chemical compounds are rarely unique to a single species. Ladybird alkaloids are often shared by a number of species, for example adaline in *A. bipunctata* and *A. decempunctata* (Daloze et al. 1995) thus posing potential prey identification limitations if these species share a similar ecological distribution (Sloggett et al. 2009).

Detection of multiple prey species

All of the studies mentioned in our review of a DNA based approach used separate PCR reactions to detect each prey species. Thus, Agustí et al. (2003) scanned the DNA from 50 spiders three times for the three Collembola species, while Zhang et al. (2007) in their screen for nine different prey species in 185 predator individuals conducted nine separate PCR reactions per individual. One solution to increase the scope, and efficiency, of a PCR based study is to perform 'multiplex PCR reactions' i.e. to include in a single PCR reaction multiple primer pairs designed to detect different prey species. A multiplex approach was developed by Harper et al. (2005), who recognised that certain kinds of food web studies needed a PCR based system that could simultaneously screen a generalist predator for a range of prey species. As standard agarose gel electrophoresis cannot resolve multiple short fragments, the analysis of multiplex PCR products requires the use of detection systems discriminating fragments amplified with primer pairs labeled with fluorescent markers which can substantially add to the experimental costs. An alternative way to detect multiple PCR amplifications is to perform quantitative or Real Time PCR (RT-PCR). Again, this technique uses fluorescent markers as probes to bind to and identify PCR amplicons. The advantage of RT-PCR is that it achieves detection in 'real-time', i.e. as amplification happens. RT-PCR is cost effective when the presence of many target sequences can be assessed in a single PCR reaction.

Thus, it is possible to detect multiple prey types in a single analysis using a multiplex PCR (Harper et al. 2005). This is also the case with GC–MS techniques, if retention times and/or mass spectra of each compound are sufficiently different (Sloggett et al. 2009; Hautier et al. 2011). A separate analysis is required for each prey type when ELISA is used.

Quantification

Quantification of prey biomass in the predator is possible using ELISA, GC–MS and RT-PCR. However, King et al. (2008) pointed out that biological interpretation of the results is difficult. RT-PCR is, at best, a semi-quantitative measure, as it only indicates the biomass of prey material at the time of sampling. It cannot indicate the biomass consumed or the number of prey items eaten. Sopp et al. (1992) estimated biomass consumed with an equation based on known quantity of biomass detected in the predator from ELISA and the prey digestion rate. Although Sloggett et al. (2009) considered that it might be possible to similarly estimate intraguild prey biomass in *H. axyridis* from recorded alkaloid amounts, the finding that even within life stages there is considerable intraspecific variation in alkaloid quantity (Kajita et al. 2010) suggests that this could be difficult to achieve with any great accuracy.

In addition to how much prey material has been consumed a related issue is an assessment of how recently the predation occurred. ELISA, GC–MS and, on occasions, DNA detection methods all enable detection for days following ingestion. An extended post-feeding detection period increases the chances of obtaining a positive result, yet it confounds data interpretation: it is not possible to distinguish between an old and a recent predation event. There is a solution to this dilemma. As early as 2001, Hoogendoorn and Heimpel used a set of four target sequences of different sizes (with different digestion times) to study predation on the eggs of the corn borer *O. nubilalis* by *C. maculata*. If prey DNA was detected using the PCR primers for the shortest fragment, PCR was repeated with the primer sets amplifying the longer sequences to estimate the time that had elapsed since predation.

Errors of interpretations

For all methodologies, errors of interpretation are possible due to false positives or negatives (detection failure due to a poor extracted DNA quality from the tested specimen). Confounding variables giving false positives are the possibility of secondary predation or

scavenging. Positive detection of a prey species in a predator does not necessarily indicate that the predator has directly eaten or killed and eaten the prey species. The detected prey species could have been eaten by another predator which in turn was consumed by the predator under investigation or the detected prey could be dead when consumed by the predator (Harwood et al. 2001; Foltan et al. 2005; Sheppard et al. 2005). Using an aphid-spider-beetle model system, Sheppard et al. (2005) showed secondary predation by the beetle *Pterostichus melanarius* (Illiger) (Coleoptera: Carabidae) of the grain aphid *Sitobion avenae* (F.) (Hemiptera: Aphididae) which had been eaten by the spider *Tenuiphantes tenuis* (Blackwall) (Araneae: Linyphiidae). They could detect aphid DNA in beetles up to 4 h after having consumed spiders that themselves had ingested their aphid prey within the previous 4 h, and up to 8 h if the spiders were fed to the beetles immediately after they had ingested an aphid. While this aphid detection does indicate that secondary predation could be a source of error in some predation studies, this sensitivity could also be used to advantage in certain food web studies.

Time and costs

The long duration and associated high cost of the development phase is the principal limitation of the use of monoclonal antibodies in comparison to DNA and GC–MS techniques. Many months are required to produce and validate specific antibodies from hybridoma cell lines (Symondson 2002; Harwood and Obrycki 2005). DNA and GC–MS require the identification of, respectively, prey-specific sequences or chemical compounds, and the speed of methodological development will thus depend on the availability of sequence data or knowledge of suitable target chemicals. However, for both techniques the developmental phase is likely to be measured in weeks or months.

After method development, two kinds of costs can be distinguished: one-off costs of analytical equipment and ongoing running costs from reagents and consumables (costs were based on 2011 prices, see Table 1). For GC–MS, analytical equipment costs about €70,000–€100,000 each, while PCR thermocyclers and equipment for agarose gel electrophoresis will cost about €15,000–€20,000, and a machine for

Table 1 Comparison of techniques for gut analysis of arthropod predators

	PCR	ELISA	GC–MS
Development time	Months; dependent on sequence availability (e.g. in GenBank)	Months or years	Weeks, assuming suitable target chemical compounds already known (e.g. alkaloid)
Equipment costs (at 2011 prices)	€15,000–20,000 €25,000–35,000 (Real Time PCR)	€5,000–10,000	€70,000–100,000
Estimated running costs per sample for 100 samples (at 2011 prices)	<€1	<€0.5	€2.5
Estimated running time to analyse 100 samples	PCR: at the most 1–2 days Real Time PCR: around one day	Around one day	Around 2–3 days
Detection period of prey	Hours	Hours-days	Hours-days
Specificity	To species	To life stage or instar	To genus or species
Detection of multiple preys possible in one analysis	Yes, using multiplex PCR	No, separate analysis with specific antibody must be done for each prey	Yes, if each prey chemical compound has a distinct retention time
Potential for biomass quantification	Yes, using real time PCR	Yes	Yes using a standard and calibration curve
Non destructive monitoring	Unknown, has been carried out for vertebrates	Unknown	Yes

RT-PCR costs about €25,000–€35,000. For ELISA, only an immunological plate reader, costing €5,000–€10,000, is needed.

ELISA and RT-PCR are faster than classical PCR or GC–MS. In ELISA and RT-PCR, results are provided directly after sample extraction, while with classical PCR, electrophoresis of PCR products is required. GC–MS analyses are processed individually and require tens of minutes per sample, while in ELISA and PCR many samples are processed simultaneously. The running times given in Table 1 are estimated for 100 samples. It is worth noting that running times will vary markedly depending on the equipment used. For example, PCR running time will vary depending on the sizes of the thermocycler and electrophoresis tank available. Similarly the use of an autosampler, facilitating continuous sample analysis in GC–MS, will reduce total running time when multiple samples are analysed.

Conclusion

The above mentioned techniques based on the use of monoclonal antibodies, GC–MS or PCR based detection are all able to track intraguild predation in the field. An evaluation of the interactions among the introduced BCA and native species is required in current ERA schemes, before the actual introduction of the BCA. An overview of the current ERA procedure and definitions of risk categories are given in Fig. 1 and Table 1 of Gibbs et al. 2011. The techniques here can be used in the context of an environmental risk assessment before or after the release of a BCA. By studying in detail case studies such as the *H. axyridis* example, we will gain expertise in predicting which prey might be eaten by an introduced BCA (as it is impossible to test all potential species). Biological control has contributed to the control of over 165 pest species in more than 120 years, in most cases without causing environmental damage (Bigler et al. 2006; De Clercq et al. 2011). By improving our understanding of the rare failures of biological control programs, the techniques reviewed here will contribute to its further development.

An insight into the broader prey spectrum of a predator is probably best gained using DNA techniques, using specific primers for each potential prey. However, if the researcher is interested in a narrower range of prey species, with suitably specific chemical markers, such as ladybird alkaloids, GC–MS can be a valuable technique. Immunology is particularly suitable for studies of a single interaction between a predator and a specific prey species. The choice of technique will depend on the number of samples to be analysed and on the laboratory equipment available. For large-scale predation surveys, the use of specific antibodies and ELISA may be most desirable, in spite of the initial costs. By contrast a small-scale non-destructive survey would be best achieved by GC–MS. Thus, different techniques may prove suitable for different situations.

These techniques provide a promising way to investigate desired and non-desired trophic interactions in the field. Nevertheless, they have seldom been used in studies in the context of risk assessment for biological control. We believe that molecular tools have the potential to facilitate and to reduce the time necessary for the study of potential non-target effects such as intraguild predation, apparent competition or enrichment associated with the release of a BCA. The potential of molecular technologies to answer unresolved ecological questions is further simplified by the availability of high throughput sequencing methodologies such as for example Roche/454 pyrosequencing (see Lawson Handley et al. 2011). A better understanding, aided by molecular techniques, of the trophic interactions among members of a community will certainly shed light on potential non-target effects associated with a candidate BCA and facilitate its early recognition as an unsuitable BCA.

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