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INVITED REVIEW

Molecular analysis of predation: a review of best practice for DNA-based approaches

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Abstract

Molecular analysis of predation, through polymerase chain reaction amplification of prey remains within the faeces or digestive systems of predators, is a rapidly growing field, impeded by a lack of readily accessible advice on best practice. Here, we review the techniques used to date and provide guidelines accessible to those new to this field or from a different molecular biology background. Optimization begins with field collection, sample preservation, predator dissection and DNA extraction techniques, all designed to ensure good quality, uncontaminated DNA from semidigested samples. The advantages of nuclear vs. mitochondrial DNA as primer targets are reviewed, along with choice of genes and advice on primer design to maximize specificity and detection periods following ingestion of the prey by the predators. Primer and assay optimization are discussed, including cross-amplification tests and calibratory feeding experiments. Once primers have been made, the screening of field samples must guard against (through appropriate controls) cross contamination. Multiplex polymerase chain reactions provide a means of screening for many different species simultaneously. We discuss visualization of amplicons on gels, with and without incorporation of fluorescent primers. In more specialized areas, we examine the utility of temperature and denaturing gradient gel electrophoresis to examine responses of predators to prey diversity, and review the potential of quantitative polymerase chain reaction systems to quantify predation. Alternative routes by which prey DNA might get into the guts of a predator (scavenging, secondary predation) are highlighted. We look ahead to new technologies, including microarrays and pyrosequencing, which might one day be applied to this field.

Keywords: assay optimization, faecal analysis, gut content analysis, molecular diagnostics, multiplexing, predator–prey interaction

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Introduction

Polymerase chain reaction (PCR)-based techniques for detecting prey remains in the guts, faeces and regurgitates of predators are being developed to study complex trophic interactions in the field (reviewed in Symondson 2002; Sheppard & Harwood 2005; Sunderland *et al*. 2005; Gariépy *et al*. 2007). Research in this area has, however, been hampered by a lack of clear guidance through the many techniques and approaches available. Detecting degraded, semidigested DNA is not always easy. Major areas of difficulty seem to be lack of sensitivity, short post-ingestion detection periods and cross-amplification problems. However, attention to some simple guidance, for example on primer design and assay optimization, can in many instances prevent these problems arising in the first place. At an early stage in the application of a new technology, it is good to see people trying a spectrum of different approaches and we do not wish to inhibit that process; this review is certainly not intended to provide a single blueprint that everyone should follow. We cannot hold up our own papers as examples of how to do it either, because at one time or another we have, between us, made just about

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every possible mistake. However, we realize that there is a need for guidelines that will assist both molecular ecology novices and those with experience to advance effectively and rapidly in this challenging field.

In addition to the primary description of techniques, and advice on best practice, we have included simple flow diagrams describing a set of basic steps of wide utility that we recommend (Fig. 1) with further details, and alternative strategies, described in the main text.

Sampling

Collection and trapping of predators

Sampling for population monitoring has been reviewed extensively elsewhere (Southwood 1978; Sunderland *et al*. 1995; McEwen 1997; Sunderland *et al*. 2005) and many of the associated problems (e.g. separating activity from density) may be equally relevant here. However, if the aim is to relate predator, target prey and possibly also alternative prey densities (Harper *et al*. 2005; Harwood *et al*. 2007; Juen & Traugott 2007) to predation frequency, tracked by PCR, then there are a number of further challenges to consider.

Vacuum sampling methods (Sunderland *et al*. 2005) have been used for collecting predators before molecular analysis of their gut contents (e.g. using antibodies, Hagler & Naranjo 1994), but are best avoided wherever possible. Such harsh collecting systems may lead to external contamination of predators by the remains of prey that have broken apart (especially fragile animals such as aphids and whiteflies) or by regurgitated material. Even if the gut is dissected out, rather than the whole predator homogenized, cross-contamination is possible. PCR is such a sensitive technique that the risk of false-positives could be unacceptably high. An even greater danger, possibly, is false-positives from predation occurring within the collection receptacle, immediately after the vacuum is turned off, when high densities of predators and prey are in close proximity. It is our experience that many carabids, spiders and predatory bugs will almost immediately grab the nearest prey. These problems can be mitigated to some extent by using low vacuum pressures and placing samples immediately on ice in the field. If vacuum sampling is used, experiments to check for contamination should be performed.

Pitfall trapping measures activity density (Luff 1975; Sunderland *et al*. 2005), which can be relevant to predator–prey encounter rates. However, predation events occurring within the trap are again a problem. This may be avoided to some degree by using a mesh insert, allowing smaller animals to fall through out of reach of larger predators (Harper *et al*. 2005). If the predators are trapped into a preservative (certainly not formaldehyde which is known to inhibit PCR, Gurdebeke & Maelfait 2002) there is a real danger of regurgitates from one predator contaminating others as they drown. A pragmatic compromise is to use raised covers on dry traps (to keep out prey falling from the vegetation above and encourage any that do fall in to crawl out through positive phototaxis), to provide refugia (stones, leaves, etc.) within the traps to cut down on mutual predation (Sunderland *et al*. 2005) and to collect predators from the traps frequently. The latter is necessary anyway to ensure that they have digested their prey to the minimum extent. A further problem with pitfall traps is that satiated predators are less active than hungry ones (Fournier & Loreau 2001, 2002), potentially causing a bias towards predators with empty guts. Despite these problems, pitfall traps will continue to be used, especially for large, nocturnal, low-density predators such as carabids that bury themselves in the soil during the day and for which no other effective sampling method has been devised.

Ideally, predators required for gut content analysis would be best collected individually, by ground searching within quadrats, using a pooter for small quick-moving predators (e.g. Collembola, Read *et al*. 2006) and flying insects, or hand searching through soil samples (Juen & Traugott 2007). This is particularly appropriate where whole predators, which might be externally contaminated by vacuum sampling, are homogenized (rather than dissected) before DNA extraction (Harwood *et al*. 2007). If Malaise traps are used, catching flying predators within the nets by pooter is acceptable, while analysing those in the collection bottle (where cross-contamination is highly likely) is almost certainly not. In practice, combining such collection of high-quality samples for molecular analysis, with more rapid and efficient collection systems (such as vacuum sampling or extraction of soil samples) to obtain population data, would be recommended.

Aquatic invertebrates present a special problem, particularly marine species that are floating in a planktonic soup of biota. Analysis of the diets of krill, for example, when the water surrounding the predators is teaming with dietary components, is problematic (Martin *et al*. 2006; Passmore *et al*. 2006). Special care is needed to avoid contamination of the gut contents during dissection (Passmore *et al*. 2006) and flaming of dissection tools between samples is always essential. External contamination can also be a problem with soil-dwelling predators. Prey adhering to the outside of very small fragile predators is especially problematic, as such predators may (unlike krill) be impossible to wash. Read *et al*. (2006) had to microscopically examine a subsample of Collembola to ensure that their nematode prey were not attached to the predator.

Invertebrate predators needed for sequencing must be starved after capture, or body parts such as legs that cannot contain prey DNA, are selected for extraction. The latter can be a problem with spiders, where gut diverticula extend even into the legs (illustrated in Ruppert & Barnes 1994).

Vertebrates can also be sampled by killing them and analysing their gut contents, and this has been used for

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birds (Scribner & Bowman 1998) and fish (DeWoody *et al*. 2001; Rosel & Kocher 2002). Although fish are not so problematic (they may be killed for food in any case), we would recommend that most work on vertebrates should now be conducted using noninvasive analysis of faeces, given the proven success of this approach. However, where culling programmes are going ahead in any case, exploitation of the gut contents in order to study the trophic ecology of a predator should be encouraged.

Collection of faecal samples

Most of the literature on faecal analysis is directed towards extraction and analysis of DNA from epithelial cells from the predator (e.g. Garnier *et al*. 2001; Goossens *et al*. 2006). This is now a standard method for noninvasive sampling of vertebrate populations and good quality, relatively undegraded DNA can usually be found. However, where very limited quantities of highly degraded prey DNA are sought, the age and condition of faecal samples can be critical. Vertebrates cannot always be induced to defecate on demand, as found by Deagle *et al*. (2007) with macaroni penguins, *Eudyptes chrysolophus*, even when subjected to stomach flushing. Reptiles can be more obliging, with many, such as slow worms (*Anguis fragilis*) and smooth snakes (*Coronella austriaca*), defecating when gently palpated or as part of a defensive reaction when handled (D.S. Brown, personal communication). Many songbird chicks will produce faecal sacks at the slightest disturbance (Sutherland 2000). Wherever possible, faeces should be collected fresh to minimize further enzymatic action, although freshness is not always easy to determine in the field. Older faecal samples may therefore generate false-negatives for prey that were indeed consumed. Faecal matter in contact with a substrate should be avoided, to minimize contamination, while faeces collected in the sea (Jarman *et al*. 2004), or potentially in fresh water, may again be contaminated with planktonic organisms (see above).

Sample preservation and storage

Once predators or faecal samples have been collected, they need to be preserved as rapidly as possible. In the few predation studies to report field data, preservation of the predator plus prey remains has mainly been by freezing (Agustí *et al*. 2003a, b; Harper *et al*. 2005; Ma *et al*. 2005; Martin *et al*. 2006; Read *et al*. 2006; Harwood *et al*. 2007; Juen & Traugott 2007; Zhang *et al*. 2007a) although Kasper *et al*. (2004), Sheppard *et al*. (2004) and Greenstone *et al*. (2007) found ethanol preservation to be successful. Sutherland (2000) found both freezing and storage in 70% ethanol to be successful for aliquots of bird faeces. Passmore *et al*. (2006) found that preservation in 80% ethanol was superior to freezing, speculating that frozen specimens degrade more during dissection. Eighty per cent ethanol leaves the prey more pliable than higher ethanol concentration, facilitating gut dissection. Sheppard *et al*. (2004), sampling in Hawaii, compared crushing and air drying of predators in the field, followed by storage over silica gel, with ethanol preservation, and found both to be equally successful.

The temperature at which the predators have been frozen has ranged from –20 °C to –80 °C, and we would recommend the latter where possible to accelerate freezing and halt DNA-destroying enzymatic processes. It is sometimes possible to extract DNA from the predators immediately, without preservation (Cuthbertson *et al*. 2003), or to store in ethanol and then freeze at –20 °C (Hoogendoorn & Heimpel 2001). Additional techniques that could be applied to the storage of predators come from the literature on the handling and storage of faecal samples for phylogenetic analysis. Faecal samples share many of the same difficulties as gut samples; the DNA is often degraded, the risk of contamination is high and it is likely that PCR inhibitors may be co-extracted. Methods for the storage of faecal samples have included commercial kits such as RNAlater (Ambion) (Nsubuga *et al*. 2004), two-step storage using ethanol and silica (Roeder *et al*. 2004), storage in dimethyl sulphoxide (DMSO) salt solution (Seutin *et al*. 1991; Frantzen *et al*. 1998) and storage in extraction buffers from commercial kits (Hajkova *et al*. 2006).

Sample preparation

Two factors affect the successful amplification of prey DNA from the guts of invertebrate predators and from faecal material: the amount of target DNA present in the sample and the quantity of that DNA remaining after storage of the predator, dissection (if applicable), then extraction and purification of the DNA. Optimization of each of these steps can enhance the success and consistency of any molecular analysis of predation.

To dissect or not to dissect

Large invertebrate predators such as carabid beetles contain too much tissue for most DNA extraction methods, so the gut may need to be dissected (e.g. Foltan *et al*. 2005; Harper *et al*. 2005, 2006; Sheppard *et al*. 2005), or the amount of predator tissue can be reduced by the removal of legs, elytra and wings (Zaidi *et al*. 1999), potentially improving sensitivity. It is worth repeating that a major danger at this stage is cross-contamination between predators and this can only be avoided by the use of sterile, DNA-free instruments and clean laboratory practice. Where dissection is impractical (very small predators such as Collembola and mites) or difficult (for example centipedes and some beetle larvae), DNA can be effectively extracted from whole

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predators (Cuthbertson *et al*. 2003; de Leon *et al*. 2004; Read *et al*. 2006; Juen & Traugott 2007). Predators can be homogenized in extraction buffer using DNA-free pestles or, where large numbers need to be processed, in a ball mill (e.g. Mixer mill MM 301, Retsch).

Processing faeces

Several studies have now demonstrated that, where time and resources allow, a combination of visual analysis of prey remains in faeces, and PCR, is optimal for establishing trophic links (Casper *et al*. 2007a, b). Larger quantities of faeces should therefore be retained for visual analysis than would be needed for DNA extraction alone.

DNA extraction

A range of methods has been applied to the exacting task of trying to extract small quantities of semidigested prey DNA from predators, including variations of the cetyltrimethyl ammonium bromide (CTAB) extraction protocols (Agustí *et al*. 1999, 2000; Juen & Traugott 2005), phenol–chloroform (Sheppard *et al*. 2004) and Livak methods (Livak 1984; Agustí *et al*. 2003b). However, although these less expensive techniques are still valuable and widely used, they are being superseded increasingly by easy-to-use commercial extraction kits such as those from QIAGEN (Agustí *et al*. 2003a; Foltan *et al*. 2005; Harper *et al*. 2005) and Gentra (Kasper *et al*. 2004).

Thanks to medical and veterinary applications, a number of kits are available for the extraction of DNA from faeces such as the QIAamp DNA Stool Mini Kit, QIAGEN (e.g. Deagle *et al*. 2007) or the Ultra Clean Fecal DNA Isolation Kit, MO BIO Laboratories, Inc. (Casper *et al*. 2007a). It is advisable to extract several subsamples from each scat to increase prey DNA detection rates (A. Juen & M. Traugott, unpublished data). These kits can prove to be effective at overcoming problems of co-extracted PCR inhibitors from invertebrate guts and were successfully used by Foltan *et al*. (2005) to analyse decaying and scavenged insect and mollusc remains.

The importance of the inclusion of negative controls during DNA extraction cannot be overstated. These controls screen for potential contamination by prey DNA between samples and provide a higher degree of confidence in the assay protocol as a whole.

Target genes

Nuclear vs. mitochondrial DNA

Both single and multiple-copy DNA regions from both nuclear and mitochondrial genomes have been used for molecular detection of predation (Table 1). After early attempts using nuclear DNA regions, including randomly amplified polymorphic DNA-derived sequence characterized amplified region (SCAR) markers (Agustí *et al*. 1999), internal transcribed spacer region 1 (ITS-1, Hoogendoorn & Heimpel 2001) and α esterase genes (Zaidi *et al*. 1999), the majority of recent studies have used mitochondrial DNA (mtDNA) genes as the source of their target-specific primers. There are two main reasons for this. First, hundreds or thousands of copies of the mitochondrial genome may be present within each invertebrate cell (Hoy 1994), greatly increasing sensitivity and hence the probability that prey DNA can be amplified from a predator's gut. Second, there are many published sets of 'universal' primers available for the amplification of mtDNA genes (Folmer *et al*. 1994; Simon *et al*. 1994, 2006), facilitating the rapid screening of suitable regions from both predator and prey species. From these sequences, prey-specific primers can be designed. Single- or low-copy number nuclear markers, such as most SCARs, are still being used occasionally (de León *et al*. 2006; Zhang *et al*. 2007a) but generally show lower sensitivity and are best avoided. The ITS region is probably best avoided because it is subject to intra-individual, as well as intraspecific, variation (Parkin & Butlin 2004). However, the multiple-copy nuclear 18S and 28S ribosomal genes have proved to be useful targets for developing group-specific primers in marine systems (Jarman *et al*. 2005; Martin *et al*. 2006; Suzuki *et al*. 2006; Deagle *et al*. 2007).

Choice of mitochondrial gene

The choice of mtDNA gene/region will depend on whether the target is a group of species (e.g. a whole order, family or genus) or a particular species. Protein encoding genes, such as the cytochrome oxidase I and II genes (COI and COII), are less conserved than some other genes and are often appropriate for the design of species-specific primers. The two ribosomal RNA (rRNA) genes within the mitochondria, 12S and 16S, have slower substitution rates and are therefore generally better for the design of group-specific primers (Ballard 2000; Mueller 2006). These ribosomal genes have many indels (insertion/deletion mutations), which can make alignment difficult, but which can provide useful species-specific markers. For example, a 12S primer was developed that was specific to the arionid slugs but, fortuitously, amplified a different sized product for each species tested (Dodd 2004; Harper *et al*. 2005).

Despite this, the choice of gene region for primer design will in practice depend on the levels of variation found within the target prey group. For instance, Dodd (2004) found that the 12S rRNA gene was more useful than COI when designing primers for species-specific detection of predation on the slugs *Deroceras reticulatum* and the *Arion hortensis* agg. Conversely, high levels of intraspecific sequence

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Table 1 Summary of molecular analyses of predation involving terrestrial and marine invertebrate predators and prey, with details of target gene regions and amplicon sizes

Target groups and species	Target region*	Amplicon size (bp)	Reference
Terrestrial ecosystems			
Lepidoptera:			
Helicoverpa armigera	SCAR	254, 600, 1100	Agustí *et al*. 1999
Ostrinia nubilalis	ITS-1	150, 156, 369, 492	Hoogendoorn & Heimpel 2001
Scotorythra rara, Eupithecia monticolans, general *Eupithecia* sp. and general geometrid moths	COI	140–170	Sheppard *et al*. 2004
Plutella xylostella	ITS-1	275	Ma *et al*. 2005
Hemiptera:			
Trialeurodes vaporariorum	SCAR	310, 2100	Agustí *et al*. 2000
Schizaphis graminum, Diuraphis noxia, Rhopalosiphum padi, R. maidis, Sipha flava, Sitobion avenae	COII	77–386	Chen *et al*. 2000
Cacopsylla pyricola	COI	188, 271	Agustí *et al*. 2003b
Rhopalosiphum insertum	ND1 and 16S	283	Cuthbertson *et al*. 2003
Rhopalosiphum maidis	COII	198	Greenstone & Shufran 2003
Homalodisca coagulate, *H. liturata*	SCAR, COI and COII	166–302 166–295	de León *et al*. 2006
Megoura viciae, Sitobion avenae, Metopolophium dirhodum, Rhopalosiphum padi, Myzus persicae, Aphis fabae, general aphids	COI, COII	78–242	Harper *et al*. 2005 (includes two primers developed by Chen *et al*. 2000)
General aphid, *Sitobion avenae*	COI	110, 242	Sheppard *et al*. 2005 (using aphid primers developed by Read 2002 and Harper *et al*. 2005)
General aphid	COI	242	Foltan *et al*. 2005 (using primer developed by Harper *et al*. 2005)
General aphids	COI	242	Harper *et al*. 2006
Bemisia tabaci	SCAR	240	Zhang *et al*. 2007a
Bemisia tabaci	SCAR	93	Zhang *et al*. 2007b
Aphis glycines	COI	255	Harwood *et al*. 2007
Rhopalosiphum padi	COII	331	McMillan *et al*. 2007
Collembola:			
Isotoma anglicana, Lepidocyrtus cyaneus, Entomobrya multifasicata	COI	211, 216, 276	Agustí *et al*. 2003a
Coleoptera:			
Sitona sp.	COI	151	Harper *et al*. 2005
Melolontha melolontha	COI	175, 273, 387, 585	Juen & Traugott 2005
Amphimallon solstitiale	COI	127, 463	Juen & Traugott 2006
Phyllopertha horticola	COI	291	Juen & Traugott 2007
Leptinotarsa decemlineata, *Leptinotarsa juncta*	COI	214 219	Greenstone *et al*. 2007
Diptera:			
Culex quinquefasciatus	α esterase	146, 263	Zaidi *et al*. 1999
Anopheles gambiae	ITS	290	Morales *et al*. 2003
Thysanoptera:			
Neohydatothrips variabilis	COI	160	Harwood *et al*. 2007
Nematoda:			
Phasmarhabditis hermaphrodita, *Heterorhabditis megidis,* *Steinernema feltiae*	COI	154 150 203	Read *et al*. 2006
Mollusca:			
Deroceras reticulatum, Arion hortensis and *Arion* sp.	12S	109–294	Dodd *et al*. 2003, 2005, Dodd 2004

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Table 1 *Continued*

Target groups and species	Target region*	Amplicon size (bp)	Reference
Deroceras reticulatum	12S	109	Foltan *et al*. 2005 (using primer developed by Dodd 2004)
Deroceras reticulatum, Arion hortensis, A. intermedius, A. distinctus, general *Arion* sp., *Vallonia pulchella, Candidula intersecta*	12S	109–221	Harper *et al*. 2005 (*Deroceras* and *Arion* primers developed by Dodd 2004)
Annelida:			
General earthworms	12S	225–236	Harper *et al*. 2005, 2006
General earthworm	COI	523	Admassu *et al*. 2006
General and group-specific†:			
General invertebrates	12S	165–400	Sutherland 2000
General invertebrates and vertebrates	16S	500–650	Kasper *et al*. 2004
General arthropods	Cyt *b*	358	Pons 2006
General invertebrates	COI	332	Harper *et al*. 2006
Anserinae, Anatidae	microsatellites	variable	Scribner & Bowman 1998
Aquatic ecosystems			
Actinopterygii			
Kareius bicoloratus	D-loop	1.46 k	Asahida *et al*. 1997
Clupea pallasii, *Hypomesus pretiosus*, *Oncorhynchus nerka*	16S	65–69	Deagle & Tollit 2007
Clupea pallasii	16S	69–304	Deagle *et al*. 2006
Krefftichthys anderssoni	16S	169	Deagle *et al*. 2007
Paralichthys olivaceus	mtDNA control region	153	Saitoh *et al*. 2003
Salmo trutta, *Salmo salar*	16S, Cyt *b*	162, 327	Parsons *et al*. 2005
Gadus morhua	16S	132	Rosel & Kocher 2002
Etheostoma olmstedi	microsatellites	variable	DeWoody *et al*. 2001
Crustacea:			
Ceropagis pengoi	16S	154	Gorokhova 2006
Calanus helgolandicus	COI	172	Vestheim *et al*. 2005
General and group-specific†:			
General fish and general squid	16S, 28S	~180, ~250	Deagle *et al*. 2005a
General marine vertebrates and invertebrates	16S	183–280	Deagle *et al*. 2005b
Euphausiids, Nototheniodei, Amphipoda, Cephalopoda	16S, 18S, 28S	169–375	Deagle *et al*. 2007
General Krill	28S	224–327	Jarman *et al*. 2002
General Krill	28S	326–336	Jarman & Wilson 2004
General Eukaryota, Bilateria, Chordata and Nototheniodei	18S, 12S, 16S	220–370	Jarman *et al*. 2004
Amphipoda, Chordata, Copepoda, Dinophyceae, Foraminifera, Gastropoda, Ostracoda, Tintinnida, Eukaryote	18S, 28S, 12S, ITS-1	188–600	Passmore *et al*. 2006
Amphipoda, Cephalopoda, Echinodermata, Gastropoda, Isopoda, Ostracoda, Thoracica	18S, 28S, 12S, 16S	134–375	Jarman *et al*. 2005
General marine invertebrates	COI	~700	Blankenship & Yayanos 2005
General invertebrates, vertebrates, plants	18S	~500	Suzuki *et al*. 2006
General invertebrates and plants	18S	~240	Martin *et al*. 2006
Squid, Arripidae, Sillaginidae	16S, 28S	100–237	Casper *et al*. 2007a
Squid, Nototheniodei, Myctphidae	16S, 28S	90–275	Casper *et al*. 2007b

*Abbreviations are for: COI and COII (cytochrome oxidase I and II genes, mtDNA), 12S and 16S (ribosomal RNA genes, mtDNA), Cyt *b* (cytochrome *b*, mtDNA), ND1 (NADH dehydrogenase 1, mtDNA), 18S (ribosomal RNA gene, nuclear DNA), ITS-1 (internal transcribed spacer 1, nuclear DNA), SCAR (sequence characterized amplified region markers, mainly nuclear DNA).
†Several of the other studies used general primers as controls for the presence of amplifiable DNA. These have not been included in the table.

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diversity in the COI gene of lumbricid earthworms foiled attempts to design species-specific primers in this important prey group (Harper *et al*. 2005, 2006; Admassu *et al*. 2006), while a lack of suitable primer sites in the 12S gene allowed only for the design of group-specific earthworm primers (Harper *et al*. 2005). Therefore, before attempting to design primers for any species or group of species, it is advisable to sequence several mtDNA gene regions in order to identify the one most appropriate for the target prey species.

An alternative multicopy region that has been used successfully in predation studies is the nuclear 18S rRNA gene (Jarman *et al*. 2004). The ribosomal gene cluster, comprising the 5.8S, 18S and 28S rRNA gene, two internal transcribed spacers (ITS) and an external transcribed spacer, is a tandemly repeated region that is found in several hundred copies within nuclear genomes (Beebee & Rowe 2004). The 18S gene has been used extensively in phylogenetic studies and many sequences are available on databases such as GenBank. Using only 18S rRNA gene sequences from public databases, Jarman *et al*. (2004) were able to design group-specific primers for the amplification of a wide range of potential prey groups found in marine ecosystems. (To avoid any possible confusion, when we design primers for the rRNA genes, we are actually targeting the DNA that codes for these genes, not the RNA produced by the ribosomes in the mitochondria or nucleus).

How many individuals should be sequenced before primer design?

Many recent studies have shown the presence of cryptic species complexes in invertebrates (Hebert *et al*. 2004; Bickford *et al*. 2007). This raises questions as to how many individuals per species need to be sequenced and from how many different populations. If primers will be used on numerous field sites, then it would be advisable to sequence several individuals from each of the proposed sites in order to measure the extent of variation within target prey species. Few studies state the number of individuals initially sequenced or the levels of intraspecific variation found. Even within sites, extensive variation can be found, as shown in lumbricid earthworms (Harper *et al*. 2005, 2006; Admassu *et al*. 2006; R. A. King, A. L. Tibble, W. O. C. Symondson, unpublished data). This variation can make it difficult to design primers that will amplify all lineages or even haplotypes within a prey species (R. A. King, A. L. Tibble, W. O. C. Symondson, unpublished data). If inadequate preparatory sequencing is performed, there is a danger that substitutions at the primer sites may prevent some haplotypes being detected, leading to false-negatives during predator screening. For this reason and others, it is advisable to do your own sequencing, rather than basing your primer design solely on sequences taken from GenBank or other published sources.

Nuclear copies

Another potential danger, using mtDNA, is the presence of nuclear copies of mitochondrial genes (NUMTS) in many species (Bensasson *et al*. 2001). For instance, there are numerous nuclear copies of both COI and COII in *Sitobion* aphids, many of which are important crop pests (Sunnucks & Hales 1996). Techniques for the detection of NUMTS and methods for avoiding PCR amplification of NUMTS exist and have been reviewed elsewhere (Zhang & Hewitt 1996; Bensasson *et al*. 2001), and these techniques should be used if their presence is suspected. Designing primers to such NUMTS is best avoided, as they are likely to be single- or low-copy number markers, and this will adversely affect post-ingestion detection times.

Designing primers

Although the library of available primers targeting DNA of specific prey taxa is steadily growing, new primers usually have to be designed. A simple flow diagram showing the steps involved is shown in Fig. 1a. Primer design is based on alignment of sequences from target prey, nontarget prey and predators. The process can be assisted by inclusion of sequences from GenBank when available, particularly when looking for group-specific primer sites. Alignment and display can be carried out using specific software packages such as BIOEDIT (Hall 1999) or LASERGENE (DNAStar). In multiple-species systems involving highly generalist predators in biodiverse ecosystems, it is not possible (or necessary) to include sequence information for all potential prey taxa in the alignment. Major alternative prey might be included, especially any species closely related to the target. By aligning target prey and nontarget DNA sequences, primer sites can be identified. For general rules on primer design, see Hawkins (1997) or Apte & Daniel (2003). Primer design software such as PRIMER 3 (Rozen & Skaletsky 2000), PRIMERPREMIERE (PREMIER Biosoft International) or LASERGENE (DNAStar) can be used to identify suitable primer sites from multiple-sequence alignments.

Specificity depends critically on the 3′ end of the primer, because extension of the DNA strand only occurs when this end of a primer is fully matched to the template sequence (Hawkins 1997). Therefore, primers that contain several mismatches at the 3′ end are desirable. Wherever possible, primers used for prey DNA detection should have high melting temperatures (T_m > 55 °C) as this allows PCRs to be run with high annealing temperatures (T_a > 60 °C). This reduces the risk of nonspecific amplification and primer annealing to false priming sites, as well as increasing PCR efficiency.

Group-specific primers are sometimes more useful than species-specific ones where, for example, the ecological

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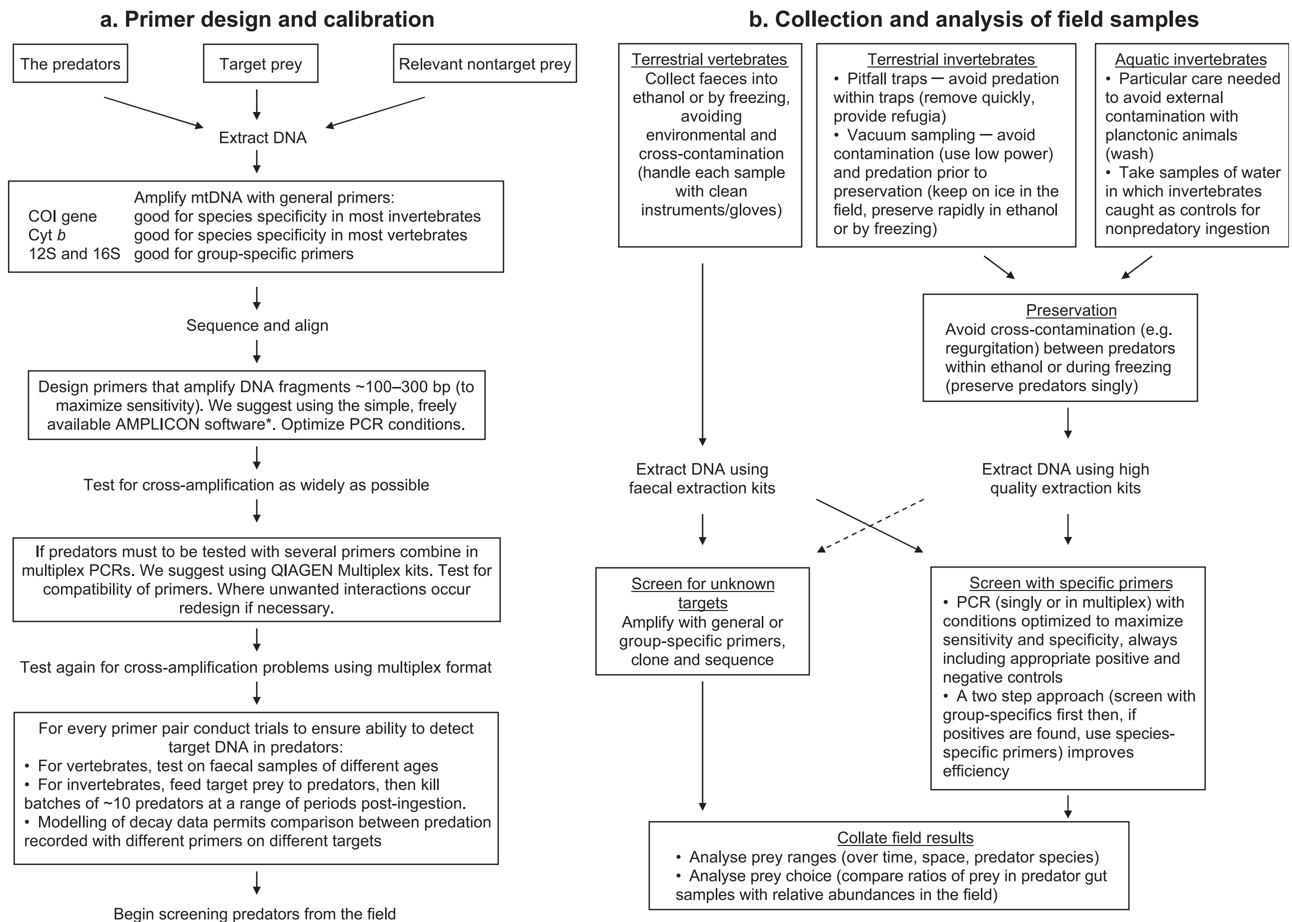

Fig. 1 Recommended basic steps, with wide applicability, needed to design and test primers, then use them as molecular markers for field experiments. For alternative genes and protocols, and further details, see main text.
*amplicon.sourceforge.net

interaction in question only requires information on predation at this level (e.g. predation on any species of annelid worm, pulmonate mollusc or 'aphid'). These primers can be used efficiently to screen predators for predation on a 'group' followed by re-screening with a set of species-specific primers to species within that group (Fig. 1b). Group-specific primers are also used extensively before a more detailed analysis of gut contents using cloning and sequencing. Such intensive effort is particularly appropriate in vertebrates (Sutherland 2000; Rollo *et al*. 2002; Jarman *et al*. 2004; Deagle *et al*. 2007) and also invertebrates (Blankenship & Yayanos 2005; Deagle *et al*. 2005b), where the aim is to obtain maximum information on the dietary range of a relatively small number of individuals. Group-specific primers are designed using multiple sequence alignments to identify sites that are conserved within group but unique between groups (e.g. Jarman *et al*. 2004, 2005). Degeneracy can be tolerated at the 5′ end of the primer, but mismatches at the 3′ end should always be avoided. Apart from commercially available software (see above), AMPLICON (Jarman 2004) is a software package for designing group-specific PCR primers sets that is freely available on the Web.

Prey DNA detection success in gut and faecal samples has (in most instances) been enhanced by targeting short DNA fragments (< 300 bp) (Agustí *et al*. 1999; Zaidi *et al*. 1999; Chen *et al*. 2000; Hoogendoorn & Heimpel 2001). This is because DNA molecules are broken into smaller fragments during digestion. For that reason, group-specific primers should be designed to be within this size range (e.g. Sutherland 2000; Harper *et al*. 2005; Jarman *et al*. 2005). It has been shown, for example, in sea lion faeces that there is a rapid decrease in copy number of prey DNA as fragment size increases (Deagle *et al*. 2006). However, PCR efficiency is also determined by factors such as the quality of the template DNA extract, PCR reagents, cycle conditions and the efficiency of the primers used. Thus, optimized PCR assays will sometimes allow detection of larger prey DNA fragments, even up to 500 bp, for extended time periods post feeding (Juen & Traugott 2005, 2006, 2007). However, to optimize detectability, shorter fragments < 300 bp should be targeted wherever possible (Table 1).

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Assay optimization and evaluation

Optimization to minimize cross-amplification of nontargets

Primers play a key role in determining the sensitivity and specificity of the PCR (He *et al*. 1994), both critical for accurate analysis of the heterogeneous mixtures of degraded DNA in the guts of predators. Those designed to amplify DNA from a specific prey taxon should have high melting temperatures and be run at the highest annealing temperature possible without compromising amplicon yield. The optimum annealing temperature has to be determined empirically, ideally using a temperature gradient PCR machine. It is advisable to determine this temperature using a low-target DNA concentration, emulating the low quantities of prey DNA that have to be detected in gut samples. Sensitivity and specificity of PCR also depends on other parameters including primer and Mg^{2+} concentration, polymerase enzymes, cycle number and PCR enhancing agents such as DMSO and bovine serum albumin (BSA, Roux 2003).

Sensitivity tests

The sensitivity of a diagnostic PCR assay to identify prey consumption is best determined by feeding experiments to establish how long after prey ingestion prey DNA can be detected within predator guts (see below). The sensitivity of an assay can be evaluated using serial dilutions of prey DNA in a constant concentration of predator DNA (Chen *et al*. 2000; Admassu *et al*. 2006; Traugott *et al*. 2006). Such tests help to identify, for example, differences in primer sensitivity that can be useful when interpreting detection rates among different prey taxa.

Cross-amplification tests on nontarget organisms

It is important to empirically test whether a PCR assay, designed to detect a specific prey, also amplifies DNA from the predators themselves or nontarget organisms that might be consumed by the predators in the system under study. Some compromise is inevitable here, given that field sites will normally contain thousands of species (including microorganisms). Species chosen for cross-reactivity tests should include those known from the literature to be consumed by the predator plus representative species from other major taxonomic groups. The number of species chosen depends to some extent upon the complexity of the system under study, but should be as large as is practicable. For example, Juen & Traugott (2007) and Harwood *et al*. (2007) tested their primers against 93 and 84 nontarget invertebrate taxa, respectively. To avoid the danger of false-negatives, the DNA from these nontarget organisms should also be tested with general invertebrate primers, to ensure that they contain amplifiable DNA. If prey-specific primers do cross-amplify, but the amplicons from nontarget species are clearly of a different size to that of the target prey, this may not matter, as long as they are not dominating the PCR and potentially reducing detectability of any target DNA in the same gut sample. Within multiplex PCRs, where amplicons of several sizes are generated, these nonspecific fragments may, however, interfere with prey fragment assignment and thus extra bands should be avoided whenever possible.

Screening of predators

The importance of controls

As in all PCR work, but especially in diagnostic applications, it is essential to include positive and negative controls, which indicate whether the reaction was successful and whether DNA contamination occurred. As positive controls, DNA from the organisms targeted by the primers should be used. It is advisable to minimize the amount of target DNA in the positive controls to ensure robust amplification and to reduce the chances that this DNA might itself serve as a source of contamination (Neumaier *et al*. 1998). The best positive control might be DNA from a predator fed with the target prey or a mixture of predator and prey DNA.

Negative PCR controls are critical and serve to check for sample-to-sample contamination and contamination of PCR reagents. The negative controls should include all reagents except the DNA, which is substituted by PCR water. It is strongly recommended that several negative controls be used for each PCR ($n > 5$) as low levels of DNA contamination can result in random amplification patterns (i.e. some controls may show a band while others do not). In addition, DNA from the predator may be used as a negative control, ensuring that nothing endogenous to the predators is being amplified.

False-negatives can arise when prey DNA cannot be amplified because of failures during DNA extraction, the presence of PCR inhibitors or simply errors during PCR. To avoid this type of error, all samples should ideally be tested in parallel with an internal control in the form of a primer that will amplify DNA from any invertebrate, including the predator. This has been carried out, for example, by Zaidi *et al*. (1999) with primers targeting the actin gene; only samples that were actin-positive were included in the results. De León *et al*. (2006) used a 28S primer in a similar way. Multiplexing allows primers targeting the predator and prey to be amplified simultaneously (Juen & Traugott 2006, 2007).

Contamination

The high sensitivity of PCR is a double-edged sword as it also facilitates the amplification of minute quantities of

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contaminating DNA. PCR to detect prey remains has to follow the same stringent contamination control strategies as used by those working with ancient DNA. Contamination-free conditions can be best ensured by physically separating pre- (DNA extraction, PCR preparation) and post-PCR (PCR execution, visualization of PCR products) activities and the workflow should always be from pre-PCR to post-PCR areas. Separate sets of pipettes should be used with filter tips. For further information on setting up a contamination-free environment in a PCR laboratory, see Neumaier *et al*. (1998).

Overcoming PCR inhibition

PCR inhibition can lead to false-negative results. Inhibitors may originate from the invertebrate or faecal sample itself or from the environment from which it is taken (Rossen *et al*. 1992). Modern DNA extraction and purification kits have greatly reduced this problem which can be further reduced using PCR facilitators such as BSA, Betaine, or TritonX-100. Juen & Traugott (2006) found that addition of BSA greatly improved amplification of DNA from soil-dwelling predators. Inhibitor-tolerant thermostable polymerases can also be used to overcome PCR inhibition (Rådström *et al*. 2004). Simply diluting the DNA extract is an easy, and in many cases successful, approach to overcoming PCR inhibition (Muelhardt 2000), but cannot be applied when using PCR to detect prey remains, simply because concentrations of undigested prey DNA will usually be low and any further dilution will jeopardise detection.

Singleplex and multiplex PCR

If predators or faecal material need to be screened for many prey taxa, a number of separate singleplex PCRs may be conducted, one PCR for each prey type (Agustí *et al*. 2003a). If the predator needs to be tested for many targets, this becomes a lengthy, costly and tedious process, and effectively precludes the analysis of the hundreds of predators that might be required for a meaningful ecological study.

Multiplex PCR offers a more rapid approach. The use of fluorescent primers, to improve sensitivity, to allow separation of fragments only 1 bp apart and to allow multiloading of gel channels, was first exploited in predation studies by Dodd (2004) and reported later in Dodd *et al*. (2005). This was further developed by Harper *et al*. (2005), who showed that it is possible to simultaneously amplify mitochondrial DNA fragments of up to 12 different prey from the gut content of invertebrate predators within a single PCR. Fluorescently labelled primers were used to separate PCR products by size on a sequencer and record the results on electropherograms. Multiplexing (without fluorescent labelling) has also been used to simultaneously test for PCR inhibition and check for false-negative results (Juen & Traugott 2006, 2007). By including predator-specific primer pairs, it is possible to identify the producer of faecal material or the identity of the predator (Juen & Traugott 2006). By comparing prey DNA detection rates in laboratory-fed beetle larvae assayed with either singleplex (targeting prey DNA only) or multiplex PCR (targeting both prey and predator DNA), Juen & Traugott (2006) found that prey DNA detection success was not significantly different between the two PCR methods.

Harper *et al*. (2005) were greatly assisted in their work by the availability of multiplex PCR kits (QIAGEN). These kits are highly tolerant to differences between primers in, for example, annealing temperatures. Indeed, the multiplex PCR mastermix provided in these kits can often be used to get 'difficult' primers to work in singleplex, overcoming problems of inhibition.

Visualizing of PCR products

Gel systems and fluorescent primers

When the aim is to measure predation on a single target pest species, agarose gel electrophoresis of prey-specific PCR amplicons will usually be adequate. However, as the number of target prey species increases, so will the time and effort needed to screen each predator for multiple prey items. Given adequate size differences between the amplicons for different prey targets, agarose gels can still be used to detect multiple prey within a single predator following multiplex PCR (Juen & Traugott 2007). However, when differences between amplicon sizes are smaller, polyacrylamide gels can give a higher resolution. Other high-resolution gel systems, including *e*Gene (http://www.egeneinc.com/) and Elchrom (http://www.elchrom.com), are also available. For analysis of many targets within highly generalist predators, multiplex PCR utilizing fluorescently labelled primers and separation on highly sensitive DNA sequencer-based detection systems allows the simultaneous detection of multiple prey species from a single predator (Harper *et al*. 2005).

DGGE and TGGE

Recent studies have utilized 'universal' and group-specific primers for detection of prey DNA on temperature or denaturing gradient gels (TGGE and DGGE) (Deagle *et al*. 2005a, b; Harper *et al*. 2006; Martin *et al*. 2006). These gels allow amplicons of the same length to be separated on the basis of differences in their sequence and may be useful when trying to distinguish closely related species for which specific primers could not be developed. This approach is potentially excellent for examining predator responses to prey diversity, overall or within prey groups (Harper *et al*. 2006). Control samples must be run on the same gel, in parallel, in order to identify the prey species consumed (Harper *et al*. 2006).

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PCR artefacts, as encountered by Deagle *et al*. (2005b), may complicate the interpretation of gels. TGGE and DGGE can be difficult techniques to master and can give confusing results where, for example, haplotype diversity within target species may produce very different band mobilities on the gels (Harper *et al*. 2006). Although others may have had more success, in our own experience it is difficult to get consistently good gels using these techniques for gut analyses and, in view of the other problems outlined, this is not an approach we could recommend.

Quantitative systems

It is not usually possible to quantify the amount of prey DNA amplified from the gut or faeces of a predator using conventional PCR. Deagle *et al*. (2005a, 2007) used both clone libraries and quantitative PCR (qPCR) to estimate proportions of the different fish species in captive sea lion faeces. Both studies showed that the prey proportions estimated from faecal DNA samples corresponded reasonably well with the known dietary proportions. Zhang *et al*. (2007b) went further, quantifying the number of copies of their DNA target that equated to a *Bemisia tabaci* egg, nymph or adult. They then screened a range of predators from the field and estimated the numbers of nymph equivalents present in the guts of each predator from the DNA copy number. They also showed that qPCR, using *Taq*Man, improved sensitivity compared with conventional PCR. Two recent studies, both using qPCR, have attempted to quantify ingestion rates of algal-feeding zooplankton species. Troedsson *et al*. (2007) demonstrated that, in the appendicularian *Oikopleura dioica*, algae of varying sizes were trapped and ingested at different rates, while Nejstgaard *et al*. (in press) quantified feeding rates in three calanid copepod species in both laboratory and seminatural conditions.

Before quantitative methods can routinely be used for molecular detection of predation, several barriers have to be overcome. Given that DNA copy number will often be very different between species and inevitable differences in primer efficiency, calibration of such quantitative methods will require considerable effort, especially when the aim is to detect the remains of many prey targets simultaneously in highly polyphagous predators. For instance, Nejstgaard *et al*. (in press) found differences in 18S rDNA copy number between different aged cultures of *Emiliania huxleyi* which has major implications for the choice of standards for qPCR. There are also questions as to the applicability of quantitative approaches outside of the controlled settings under which the above studies have been conducted. It is not clear how quantitative approaches should be calibrated when applied in field situations and further experiments are also needed to investigate the effects of meals of different ages within the same predator on qPCR signal. At best, it can provide a semiquantitative measure of the biomass of undigested prey at the time of analysis (a valuable quantitative measure in its own right), but not the biomass consumed and certainly not the number of prey killed and/or eaten. High-specification qPCR machines can detect many different fluorescent labels simultaneously, offering the prospect of multiplex qPCRs in future predation work. At its simplest, qPCR can be recommended for quantifying predation on a single target prey species using a single primer, but only in well-calibrated systems, when levels of predation are likely to be low. Such a system could be used in a similar way to enzyme-linked immunosorbent assays using monoclonal antibodies, to obtain data on the relative quantities of prey consumed over time and between locations or field treatments (e.g. Symondson *et al*. 1999).

Calibratory feeding experiments to determine DNA survival during digestion

Before a new PCR primer can be used to assess predation in the field, its ability to detect prey DNA in the gut of a predator has to be assessed (Fig. 1a). For invertebrates, this is achieved by carrying out a feeding trial, in which predators are fed the target prey and then killed at various time points post-ingestion of the prey. After DNA extraction, the predators are screened for prey DNA, and the number testing positive at each time point can be expressed as a percentage or proportion. As the ability to detect DNA in the guts of invertebrate predators can be affected by temperature (Hoogendoorn & Heimpel 2001), feeding trials should be conducted in a controlled environment emulating conditions (day/night cycles, temperature) found at the field site (e.g. Greenstone *et al*. 2007). Predator activity levels may also influence digestion rates; high activity has been shown to increase the production of haematin (a by-product of digestion) in tsetse flies (Loder *et al*. 1998). Therefore, if feeding trials are conducted indoors, predators should be maintained in a light : dark cycle that is similar to field conditions. Many invertebrate predators are nocturnal, so it is advisable to begin the feeding trials (by feeding the predators) during the period when predation is likely to be occurring in the field.

Feeding trials are usually conducted using field-caught predators, which reflect the ranges of ages, sizes and physiological states that will later be encountered when screening predators for predation. Previous feeding trial studies have starved the predators from 48 h to 14 days before the start of the feeding trial (de León *et al*. 2006; Harper *et al*. 2006). This is advisable to ensure predators have empty guts at the start of the feeding trial and equal hunger levels. It also increases the chances that most of the predators will consume the prey when presented with it, facilitating the experiment greatly. However, this may not be analogous to conditions in the field, as many predators will have the

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remains of previous meals in their guts before feeding. The consumption of alternative prey after feeding on target prey has been shown to increase prey DNA detection times in laboratory feeding trials (Dodd 2004). So far, no studies have investigated the influence of the consumption of alternative prey before feeding on target prey, but it is likely this will have a similar effect. In some predators, such as spiders, the quantity of food eaten (or the degree of hunger) can affect metabolic rates (Anderson 1974), probably explaining the relatively long post-ingestion detection periods found for spiders not fed alternative prey in feeding trials (Sheppard *et al*. 2005). Similarly, in a study using C-14 labelled food, starvation after feeding by the collembolan *Tomocerus flavescens* caused an increase in gut retention times (Wolters 1985).

Most previous studies have involved feeding predators in Petri dishes or vials, with damp filter paper or cotton wool as a substrate (Agustí *et al*. 1999; Chen *et al*. 2000; Greenstone *et al*. 2007). This approach avoids contamination of the predator with extraneous material, and ensures that the predator can find the prey quickly and that the experimenter can observe and confirm predation taking place. Once feeding has occurred, the predator should be moved to a more natural environment and substrate with suitable refugia, otherwise stress might affect digestion rates. Food intake during the feeding period is either limited to a set number or weight of prey, or feeding is *ad libitum*. Limiting food intake is preferable, reducing a potential source of error in the results and probably reflecting better the limited prey availability predators may often experience in the field (Lovei *et al*. 1985). For example, the mean foregut biomass of the carabid beetle *Pterostichus melanarius* caught in the field over 5 years was ~7.5 mg (Symondson *et al*. 2002a), whereas when allowed to feed *ad libitum* on slugs in the laboratory for 2.5 h, the mean foregut biomass was much greater (females 22.4 mg, males 16.7 mg) (Symondson *et al*. 1999). Meal size has been shown to influence the detection of prey DNA in the gut of predators, where larger meals caused an increase in prey detection times (de León *et al*. 2006), although other studies have observed no correlation (Zaidi *et al*. 1999; Hoogendoorn & Heimpel 2001; Juen & Traugott 2005).

The number and range of the postfeeding sampling times depends upon the type of predator being studied and must be planned from the start. Maximum detection times have ranged from a few hours to 5 days postfeeding (Chen *et al*. 2000; Sheppard *et al*. 2005). Greenstone *et al*. (2007) found that the detection times for prey DNA in the guts of predators that process their prey in very different ways can vary considerably. Colorado beetles, *Leptinotarsa decemlineata*, were fed to a soldier bug, *Podisus maculiventris*, a fluid feeder that pre-orally partially digests its prey, and a ladybird, *Coleomegilla maculata*, a predator that consumes macerated prey. The mean detection period was seven times longer in the former than in the latter. Prey DNA in the spider *Tenuiphantes tenuis* fed on the aphids *Sitobion avenae* was still being detected 120 h after ingestion (Sheppard *et al*. 2005). The more time points that can be included in a feeding trial, the more accurately the decay of prey DNA can be modelled. The number of replicates at each time point in previous feeding trials has ranged from 1 (Cuthbertson *et al*. 2003) to over 30 (Juen & Traugott 2006), but we would recommend no less than 8–10 to allow for the many sources of error associated with predator physiological state and quantities of prey consumed.

Calibratory feeding experiments have also been applied to vertebrates, although time periods cannot be very precise as one has little if any control over when (or where) a vertebrate will defecate. With vertebrates, the aim is usually to determine whether the DNA survives complete transit through the gut. Deagle *et al*. (2005a) examined the effects of feeding different quantities and ratios of prey to sea lions and used clone numbers (and later real-time PCR, Deagle & Tollit 2007) to quantify prey detectability. The main parallel with invertebrate work is to determine how long, postfeeding, prey DNA can still be found in fresh faeces (Deagle *et al*. 2005a; Casper *et al*. 2007b), and this is recommended where practicable, before application in the field.

Data analysis

One method for comparison of feeding trial data, for each predator–prey and primer combination, is to calculate a median detection time or molecular half-life (Greenstone & Hunt 1993). This is defined as the time after feeding when 50% of the predators test positive for the target prey DNA. For this to be calculated, the feeding trial data need to be fitted with a regression model that describes the decline in positives over time. The most frequent method of analysis has been to fit a linear regression to the number of positives against time since feeding (Agustí *et al*. 2003a; Harper *et al*. 2005, 2006; Sheppard *et al*. 2005; Read *et al*. 2006), although Probit models (Chen *et al*. 2000; Ma *et al*. 2005; Greenstone *et al*. 2007) and logisitic regressions (Foltan *et al*. 2005) have also been used. Additionally, 95% confidence limits can be calculated for the observed amplification success at specific time points after feeding (Juen & Traugott 2007) and differences in prey DNA detection compared by *G*-test (Dytham 2003). A comparative weighting can then be placed on prey positives obtained from field-collected predators, so that predation by predators with long detection times is not overestimated in comparison with predators with shorter detection times.

Scavenging and secondary predation

The fact that a predator tests positive for a target prey species does not necessarily mean that it killed that prey

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animal nor even that it intended to eat it. Scavenging has been shown to be a potential cause of false-positives for predation by carabids feeding on slugs (*Deroceras reticulatum*) and aphids (*Sitobion avenae*) (Foltan *et al*. 2005), and chafer larvae (*Melolontha melolontha*) (Juen & Traugott 2005). Foltan *et al*. (2005) found that DNA could still be amplified from 50% of dead slugs and aphids, placed on field soil, after 175 h and 134 h, respectively. They went on to show that these dead and decaying slugs were readily consumed by carabids after more than a week and that the DNA from scavenged slugs could be amplified from the guts of the beetles. Juen & Traugott (2005) obtained similar results with chafer grubs. Both studies showed that acceptance of dead prey was negatively correlated with carrion age. DNA-based detection of prey cannot currently distinguish between scavenging and predation, and to date, nobody has managed to effectively monitor the availability of dead prey in the field. In some studies, it may not matter whether the prey are dead or alive when eaten, if the aim is simply to identify sources of predator nutrition. However, in most cases, the main interest is in the population dynamics of the predator–prey interaction and for that we really do need to know who is killing whom and in what numbers.

Another source of error can be secondary predation (Harwood *et al*. 2001). Secondary predation errors are a consequence of a predator consuming a second predator, shortly after the latter has consumed the target prey. In a DNA-based study, secondary predation was found to be a significant potential source of error (Sheppard *et al*. 2005). In the worst case a secondary predator (the carabid *Pterostichus melanarius*) tested positive for aphid 4 h after eating the primary predator (the spider *Tenuiphantes tenuis*), that itself had been digesting its aphid prey (*S. avenae*) for 4 h. It is likely that such sensitivity to secondary predation was helped by the long post-ingestion detection times recorded for the spiders, with a median detection period of ~60 h, twice as long as that recorded for the beetles. Thus, the importance of secondary predation as a potential source of error may depend critically upon the digestion rates of the predators involved.

As far as we know, the problems of scavenging and secondary predation have not as yet been addressed in vertebrate systems, but are probably an equally serious source of error.

Future directions

The use of gel-based PCR detection systems to monitor predation on single or small numbers of prey species will continue to expand. However, the real prize will be to exploit invertebrate genomics to develop mass-target detection systems, allowing us to identify the complete prey range even of highly generalist species in biodiverse communities. This is important, because rates of predation on a target species (such as an agricultural pest) are strongly affected by prey choice and the relative densities of alternative prey species (Symondson *et al*. 2002b). How far we go down this route is currently limited mainly by cost rather than technology. To date, the nearest approach has been through the use of multiplexing with fluorescent-labelled primers (Harper *et al*. 2005), which managed to detect and identify up to 12 targets simultaneously. However, microarray technology is now well established, with the potential to identify tens of thousands of targets on a single surface-based chip. There are many formats available and these are used extensively to detect viruses and bacteria in medical diagnostics (reviewed in Striebel *et al*. 2003) as well as the identification of fungi and other plant pathogens (Lievens *et al*. 2005; Szemes *et al*. 2005). Bead-based microarrays are also available, in which DNA is hybridized to fluorescent-labelled reporters on the beads which are read individually by laser (Armstrong *et al*. 2000). At present, up to 100 distinct bead populations can be generated, more than enough for most predation work. Where necessary microarrays can be constructed that are capable of detecting species-specific single nucleotide polymorphisms (SNP, Pastinen *et al*. 2000; Erdogan *et al*. 2001), that will separate closely related taxa where suitable primer sites cannot be found. Such an approach could allow predation to be monitored not just on different species but even on individual haplotypes. Such a tool would permit comparison between genotypes existing in the field and those found in the guts of predators, allowing direct study of genetic selection through predation.

Another limitation on the use of such technology is the need to sequence and find primer sites for all the target species. In time, these will become available, but this process could be accelerated by use of mass pyrosequencing, which is capable of detecting many thousands of sequences simultaneously from mixed samples (Margulies *et al*. 2005). This approach is already being used to mass sequence marine bacteria (Goldberg *et al*. 2006). At present, it is prohibitively expensive, costing thousand of pounds per run, but may one day become cheap enough to use for direct screening of predator gut and faecal samples.

However, the main task ahead is to start applying molecular analysis techniques to study complex trophic interactions in the field. The potential is enormous but at present we have barely scratched the surface. A great deal of valuable background work in going on, developing primers, characterizing their use in the laboratory, developing new quantitative methods, factoring in, for example, the effects of temperature, amplicon size, primer efficiency, meal size, predator activity and sex, but all this now needs to be applied to address fundamental ecological questions. The few PCR-based field studies to date have done little more than record semiquantitatively a few trophic links. There are notable exceptions. For example, the study by Kasper *et al*. (2004)

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showed, through field data, the degree of prey overlap and resource partitioning between a native and alien wasp. Resource partitioning, between closely related birds feeding on Lepidoptera, was also addressed by Sutherland (2000). The study by Agustí *et al*. (2003a) compared the proportions of different prey (Collembola) in the field with the ratios found in predator (spider) guts and was thus able to demonstrate clear prey choice. DeWoody *et al*. (2001) were able, with the aid of microsatellites, to study filial cannibalism in fish. We have some way to go yet but potentially molecular analysis of the diets of generalist predators, vertebrate or invertebrate, should allow us to construct quantitative foodwebs, similar to those currently published for host–parasitoid interactions (e.g. Henneman & Memmott 2001). Major areas with future potential include molecular tracking of the diets of individuals from faeces (individual profiling of DNA from the predator (Reed *et al*. 1997) combined with use of prey-specific markers), analysis of herbivory, and evolutionary processes such as selection among prey genotypes by predators (Taberlet & Fumagalli 1996). Molecular analysis of predation can also make major contributions in the more practical fields of biological control and conservation, and is clearly beginning to do so. Given all this potential, the current almost exponential rise in the numbers of papers published in this field is likely to continue.

References

Admassu B, Juen A, Traugott M (2006) Earthworm primers for DNA-based gut content analysis and their cross-reactivity in a multi-species system. *Soil Biology and Biochemistry*, **38**, 1308–1315.

Agustí N, De Vicente MC, Gabarra R (1999) Development of sequence amplified characterized region (SCAR) markers of *Helicoverpa armigera*: a new polymerase chain reaction-based technique for predator gut analysis. *Molecular Ecology*, **8**, 1467–1474.

Agustí N, de Vicente MC, Gabarra R (2000) Developing scar markers to study predation on *Trialeurodes vaporariorum*. *Insect Molecular Biology*, **9**, 263–268.

Agustí N, Shayler SP, Harwood JD *et al*. (2003a) Collembola as alternative prey sustaining spiders in arable ecosystems: prey detection within predators using molecular markers. *Molecular Ecology*, **12**, 3467–3475.

Agustí N, Unruh TR, Welter SC (2003b) Detecting *Cacopsylla pyricola* (Hemiptera: Psyllidae) in predator guts using COI mitochondrial markers. *Bulletin of Entomological Research*, **93**, 179–185.

Anderson JF (1974) Responses to starvation in the spiders *Lycosa lenta* and *Filistata hibernalis* (Hentz). *Ecology*, **55**, 576–585.

Apte A, Daniel S (2003) PCR primer design. In: *PCR Primer: a Laboratory Manual*, 2nd edn (eds Dieffenbach CW, Dveksler GS), pp. 61–74. Cold Spring Harbor Laboratory Press, New York.

Armstrong B, Stewart M, Mazumder A (2000) Suspension arrays for high throughput, multiplexed single nucleotide polymorphism genotyping. *Cytometry*, **40**, 102–108.

Asahida T, Yamashita Y, Kobayashi T (1997) Identification of consumed stone flounder, *Kareius bicoloratus* (Basilewsky), from the stomach contents of sand shrimp, *Crangon affinis* (De Haan) using mitochondrial DNA analysis. *Journal of Experimental Marine Biology and Ecology*, **217**, 153–163.

Ballard JWO (2000) Comparative genomics of mitochondrial DNA in members of the *Drosophila melanogaster* subgroup. *Journal of Molecular Evolution*, **51**, 48–63.

Beebee TJC, Rowe G (2004) *An Introduction to Molecular Ecology*. Oxford University Press, New York.

Bensasson D, Zhang DX, Hartl DL, Hewitt GM (2001) Mitochondrial pseudogenes: evolution's misplaced witnesses. *Trends in Ecology & Evolution*, **16**, 314–321.

Bickford D, Lohman DJ, Sodhi NS *et al*. (2007) Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution*, **22**, 148–155.

Blankenship LE, Yayanos AA (2005) Universal primers and PCR of gut contents to study marine invertebrate diets. *Molecular Ecology*, **14**, 891–899.

Casper RM, Jarman SN, Deagle BE, Gales NJ, Hindell MA (2007a) Combining DNA and morphological analysis of faecal samples improves insight into trophic interactions: a case study using a generalist predator. *Marine Biology*, **152**, 815–825.

Casper RM, Jarman SN, Deagle BE, Gales NJ, Hindell MA (2007b) Detecting prey from DNA in predator scats: a comparison with morphological analysis, using *Arctocephalus* seals fed a known diet. *Journal of Experimental Marine Biology and Ecology*, **347**, 144–154.

Chen Y, Giles KL, Payton ME, Greenstone MH (2000) Identifying key cereal aphid predators by molecular gut analysis. *Molecular Ecology*, **9**, 1887–1898.

Cuthbertson AGS, Fleming CC, Murchie AK (2003) Detection of *Rhopalosiphum insertum* (apple-grass aphid) predation by the predatory mite *Anystis baccarum* using molecular gut analysis. *Agricultural and Forest Entomology*, **5**, 219–225.

Deagle BE, Tollit DJ (2007) Quantitative analysis of prey DNA in pinniped faeces: potential to estimate diet composition? *Conservation Genetics*, **8**, 743–747.

Deagle BE, Tollit DJ, Jarman SN *et al*. (2005a) Molecular scatology as a tool to study diet: analysis of prey DNA in scats from captive Steller sea lions. *Molecular Ecology*, **14**, 1831–1842.

Deagle BE, Jarman SN, Pemberton D, Gales NJ (2005b) Genetic screening for prey in the gut contents from a giant squid (*Architeuthis* sp.). *Journal of Heredity*, **96**, 417–423.

Deagle BE, Eveson JP, Jarman SN (2006) Quantification of damage in DNA recovered from highly degraded samples — a case study on DNA in faeces. *Frontiers in Zoology*, **3**. doi: 10.1186/1742-9994-3-11.

Deagle BE, Gales NJ, Evans K *et al*. (2007) Studying seabird diet through genetic analysis of faeces: a case study on macaroni penguins (*Eudyptes chrysolophus*). *PLoS One*, **2** (9), e831. doi: 10.1371/journal.pone.0000831.

DeWoody JA, Fletcher DE, Wilkins SD, Avise JC (2001) Genetic documentation of filial cannibalism in nature. *Proceedings of the National Academy of Sciences, USA*, **98**, 5090–5092.

Dodd CS (2004) *Development and optimization of PCR based techniques in predator gut analysis*. PhD Thesis, Cardiff University, Cardiff, UK.

Dodd CS, Bruford MW, Symondson WOC, Glen DM (2003) Detection of slug DNA within carabid predators using prey-specific PCR primers. In: *Slug and Snail Pests: Agricultural, Veterinary and Environmental Perspectives* (ed. Dussard GBJ), pp. 13–20. British Crop Protection Council, Alton, UK.

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Dodd C, Bruford MW, Glen DM, Symondson WOC (2005) Molecular detection of slug DNA within carabid predators. In: *Insect Pathogens and Entomoparasitic Nematodes: Slugs and Snails* (ed. Bohan DA). *IOBC Bulletin*, **28** (6), 131–134.

Dytham C (2003) *Choosing and Using Statistics: a Biologist's Guide*. Blackwell, Oxford, UK.

Erdogan F, Kirchner R, Mann W, Ropers HH, Nuber UA (2001) Detection of mitochondrial single nucleotide polymorphisms using a primer elongation reaction on oligonucleotide microarrays. *Nucleic Acids Research*, **29**, E36.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for the amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294–299.

Foltan P, Sheppard S, Konvicka M, Symondson WOC (2005) The significance of facultative scavenging in generalist predator nutrition: detecting decayed prey in the guts of predators using PCR. *Molecular Ecology*, **14**, 4147–4158.

Fournier E, Loreau M (2001) Activity and satiation state in *Pterostichus melanarius*: an experiment in different agricultural habitats. *Ecological Entomology*, **26**, 235–244.

Fournier E, Loreau M (2002) Foraging activity of the carabid beetle *Pterostichus melanarius* Ill. in field margin habitats. *Agriculture Ecosystems and Environment*, **89**, 253–259.

Frantzen MAJ, Silk JB, Ferguson JWH, Wayne RK, Kohn MH (1998) Empirical evaluation of preservation methods for faecal DNA. *Molecular Ecology*, **7**, 1423–1428.

Gariépy TD, Kuhlmann U, Gillott C, Erlandson M (2007) Parasitoids, predators and PCR: the use of diagnostic molecular markers in biological control of arthropods. *Journal of Applied Entomology*, **131**, 225–240.

Garnier JN, Bruford MW, Goossens B (2001) Mating system and reproductive skew in the black rhinoceros. *Molecular Ecology*, **10**, 2031–2041.

Goldberg SMD, Johnson J, Busam D *et al*. (2006) A Sanger/pyro-sequencing hybrid approach to the generation of high-quality draft assemblies of marine microbial genomes. *Proceedings of the National Academy of Sciences, USA*, **103**, 11240–11245.

Goossens B, Setchell JM, James SS *et al*. (2006) Philopatry and reproductive success in Bornean orangutans (*Pongo pygmaeus*). *Molecular Ecology*, **15**, 2577–2588.

Gorokhova E (2006) Molecular identification of the invasive cladoceran *Cercopagis pengoi* (Cladocera: Onychopoda) in stomachs of predators. *Limnology and Oceanography: Methods*, **4**, 1–6.

Greenstone MH, Hunt JH (1993) Determination of prey antigen half-life in *Polistes metricus* using a monoclonal antibody-based immunodot assay. *Entomologia Experimentalis et Applicata*, **68**, 1–7.

Greenstone MH, Shufran KA (2003) Spider predation: species-specific identification of gut contents by polymerase chain reaction. *Journal of Arachnology*, **31**, 131–134.

Greenstone MH, Rowley DL, Weber DC, Payton ME, Hawthorne DJ (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.

Gurdebeke S, Maelfait JP (2002) Pitfall trapping in population genetics studies: finding the right 'solution'. *Journal of Arachnology*, **30**, 255–261.

Hagler JR, Naranjo SE (1994) Qualitative survey of two coleopteran predators of *Bemisia Tabaci* (Homoptera: Aleyrodidae) and *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) using multiple prey gut content ELISA. *Biology Control*, **23**, 193–197.

Hajkova P, Zemanova B, Bryja J *et al*. (2006) Factors affecting success of PCR amplification of microsatellite loci from otter faeces. *Molecular Ecology Notes*, **6**, 559–562.

Hall TA (1999) BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**, 95–98.

Harper GL, King RA, Dodd CS *et al*. (2005) Rapid screening of invertebrate predators for multiple prey DNA targets. *Molecular Ecology*, **14**, 819–827.

Harper GL, Sheppard SK, Harwood JD *et al*. (2006) Evaluation of temperature gradient gel electrophoresis for the analysis of prey DNA within the guts of invertebrate predators. *Bulletin of Entomological Research*, **96**, 295–304.

Harwood JD, Phillips SW, Sunderland KD, Symondson WOC (2001) Secondary predation: quantification of food chain errors in an aphid-spider-carabid system using monoclonal antibodies. *Molecular Ecology*, **10**, 2049–2057.

Harwood JD, Desneux N, Yoo HJS *et al*. (2007) Tracking the role of alternative prey in soybean aphid predation by *Orius insidiosus*: a molecular approach. *Molecular Ecology*, **16**, 4390–4400.

Hawkins JR (1997) *Finding Mutations*. IRL Press, Oxford, UK.

He Q, Marjamäki M, Soini H, Mertsola J, Viljanen MK (1994) Primers are decisive for sensitivity of PCR. *BioTechniques*, **17**, 82–87.

Hebert PDN, Penton EH, Burns JM, Janzen DH, Wallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences, USA*, **101**, 14812–14817.

Henneman ML, Memmott J (2001) Infiltration of a Hawaiian community by introduced biological control agents. *Science*, **293**, 1314–1316.

Hoogendoorn M, Heimpel GE (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.

Hoy MA (1994) *Insect Molecular Genetics: An Introduction to Principals and Applications*. Academic Press, San Diego, California.

Jarman SN (2004) AMPLICON: software for designing PCR primers on aligned DNA sequences. *Bioinformatics*, **20**, 1644–1645.

Jarman SN, Gales NJ, Tierney M *et al*. (2002) A DNA-based method for identification of krill species and its application to analysing the diet of marine vertebrate predators. *Molecular Ecology*, **11**, 2679–2690.

Jarman SN, Deagle BE, Gales NJ (2004) Group-specific polymerase chain reaction for DNA-based analysis of species diversity and identity in dietary samples. *Molecular Ecology*, **13**, 1313–1322.

Jarman SN, Redd KS, Gales NJ (2005) Group-specific primers for amplifying DNA sequences that identify Amphipoda, Cephalopoda, Echinodermata, Gastropoda, Isopoda, Ostracoda and Thoracica. *Molecular Ecology Notes*, **6**, 268–271.

Jarman SN, Wilson SG (2004) DNA-based species identification of krill consumed by whale sharks. *Journal of Fish Biology*, **65**, 586–591.

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 and 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.

Kasper ML, Reeson AF, Cooper SJB, Perry KD, Austin AD (2004)

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Assessment of prey overlap between a native (*Polistes humilis*) and an introduced (*Vespula germanica*) social wasp using morphology and phylogenetic analyses of 16s rDNA. *Molecular Ecology*, **13**, 2037–2048.

de León JH, Fournier V, Hagler JR, Daane KM (2006) Development of molecular diagnostic markers for sharpshooters *Homalodisca coagulata* and *Homalodisca liturata* for use in predator gut content examinations. *Entomologia Experimentalis et Applicata*, **119**, 109–119.

Lievens B, Brouwer M, Vanachter ACRC *et al*. (2005) Quantitative assessment of phytopathogenic fungi in various substrates using a DNA macroarray. *Environmental Microbiology*, **7**, 1698–1710.

Livak KJ (1984) Organization and mapping of a sequence of the *Drosophila melanogaster* X chromosome and Y chromosome that is transcribed during spermatogenesis. *Genetics*, **107**, 611–634.

Loder PMJ, Hargrove JW, Randolph SE (1998) A model for blood meal digestion and fat metabolism in male tsetse flies (Glossinidae). *Physiological Entomology*, **23**, 43–52.

Lovei GL, Monostori E, Ando I (1985) Digestion rate in relation to starvation in the larva of a carabid predator, *Poecilus cupreus. Entomologia Experimentalis et Applicata*, **37**, 123–127.

Luff ML (1975) Some features influencing the efficiency of pitfall traps. *Oecologia*, **19**, 345–357.

Ma J, Li D, Keller M, Schmidt O, Feng X (2005) A DNA marker to identify predation of *Plutella xylostella* (Lep., Plutellidae) by *Nabis kinbergii* (Hem., Nabidae) and *Lycosa* sp (Aranaea, Lycosidae). *Journal of Applied Entomology*, **129**, 330–335.

Margulies M, Egholm M, Altman WE *et al*. (2005) Genome sequencing in microfabricated high-density picolitre reactors. *Nature*, **437**, 376–380.

Martin DL, Ross RM, Quetin LB, Murray AE (2006) Molecular approach (PCR-DGGE) to diet analysis in young Antarctic krill *Euphausia superba*. *Marine Ecology Progress Series*, **319**, 155–165.

McEwen P (1997) Sampling, handling and rearing insects. In: *Methods in Ecological and Agricultural Entomology* (eds Dent DR, Walton MP), pp. 5–20. Cab International, Oxford, UK.

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.

Morales ME, Wesson DM, Sutherland IW *et al*. (2003) Determination of *Anopheles gambiae* larval DNA in the gut of insectivorous dragonfly (Libellulidae) nymphs by polymerase chain reaction. *Journal of the American Mosquito Contrological Association*, **19**, 163–165.

Muelhardt C (2000) *Der Experimentator: Molekularbiologie/Genomics*. Elsevier, Munich, Germany.

Mueller RL (2006) Evolutionary rates, divergence dates and the performance of mitochondrial genes in Bayesian phylogenetic analysis. *Systematic Biology*, **55**, 289–300.

Nejstgaard JC, Frischer ME, Simonelli P *et al*. (in press) Quantitaive PCR to estimate copepod feeding. *Marine Biology*, doi: 10.1007/s00227-007-0830-x.

Neumaier M, Braun A, Wagener C (1998) Fundamentals of quality assessment of molecular amplification methods in clinical diagnostics. *Clinical Chemistry*, **44**, 12–26.

Nsubuga AM, Robbins MM, Roeder AD *et al*. (2004) Factors affecting the amount of genomic DNA extracted from ape faeces and the identification of an improved sample storage method. *Molecular Ecology*, **13**, 2089–2094.

Parkin EJ, Butlin RK (2004) Within- and between-individual sequence variation among ITS1 copies in the meadow grasshopper *Chorthippus parallelus* indicates frequent intrachromosomal gene conversion. *Molecular Biology and Evolution*, **21**, 1595–1601.

Parsons KM, Piertney SB, Middlemas SJ *et al*. (2005) DNA-based identification of salmonid prey species in seal faeces. *Journal of Zoology*, **266**, 275–281.

Passmore AJ, Jarman SN, Swadling KM *et al*. (2006) DNA as a dietary biomarker in Antarctic krill, *Euphausia superba*. *Marine Biotechnology*, **8**, 686–696.

Pastinen T, Raitio M, Lindroos K *et al*. (2000) A system for specific, high-throughput genotyping by allele-specific primer extension on microarrays. *Genome Research*, **10**, 1031–1042.

Pons J (2006) DNA-based identification of preys from non-destructive, total DNA extractions of predators using arthropod universal primers. *Molecular Ecology Notes*, **6**, 623–626.

Rådström P, Knutsson R, Wolffs P, Lövenklev M, Löfström C (2004) Pre-PCR processing: strategies to generate PCR-compatible samples. *Applied Biochemistry and Biotechnology — Part B Molecular Biotechnology*, **26**, 133–146.

Read DS (2002) *Sequencing of aphid DNA and primer design for the detection of aphid remains in predators*. Undergraduate Dissertation, Cardiff University, Cardiff, UK.

Read DS, Sheppard SK, Bruford MW, Glen DM, Symondson WOC (2006) Molecular detection of predation by soil micro-arthropods on nematodes. *Molecular Ecology*, **15**, 1963–1972.

Reed JZ, Tollit DJ, Thompson PM, Amos W (1997) Molecular scatology: the use of molecular genetic analysis to assign species, sex and individual identity to seal faeces. *Molecular Ecology*, **6**, 225–234.

Roeder AD, Archer FI, Poiner HN, Morin PA (2004) A novel method for collection and preservation of faeces for genetic studies. *Molecular Ecology Notes*, **4**, 761–764.

Rollo F, Ubaldi M, Ermini L, Marota I (2002) Ötzi's last meals: DNA analysis of the intestinal content of the Neolithic glacier mummy from the Alps. *Proceedings of the National Academy of Sciences, USA*, **99**, 12594–12599.

Rosel PE, Kocher TD (2002) DNA-based identification of larval cod in the stomach contents of predatory fishes. *Journal of Experimental Marine Biology and Ecology*, **267**, 75–88.

Rossen L, Nørskov P, Holmstrøm K, Rasmussen OF (1992) Inhibition of PCR by components of food samples, microbial diagnostic assays and DNA-extraction solutions. *International Journal of Food Microbiology*, **17**, 37–45.

Roux KH (2003) Optimization and troubleshooting in PCR. In: *PCR Primer: A Laboratory Manual*, 2nd edn (eds Dieffenbach CW, Dveksler GS), pp. 35–41. Cold Spring Harbor Laboratory Press, New York.

Rozen S, Skaletsky H (2000) PRIMER 3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (eds Krawetz S, Misener S), pp. 365–386. Humana Press, Totowa, New Jersey.

Ruppert EE, Barnes RD (1994) *Invertebrate Zoology*. Saunders College Publishing, New York.

Saitoh K, Takagaki M, Yamashita Y (2003) Detection of Japanese flounder-specific DNA from gut contents of potential predators in the field. *Fisheries Science*, **69**, 473–477.

Scribner KT, Bowman TD (1998) Microsatellites identify depredated waterfowl remains from glaucous gull stomachs. *Molecular Ecology*, **7**, 1401–1405.

Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, **69**, 82–90.

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Sheppard SK, Harwood JD (2005) Advances in molecular ecology: tracking trophic links through predator-prey foodwebs. *Functional Ecology*, **19**, 751–762.

Sheppard SK, Henneman ML, Memmott J, Symondson WOC (2004) Infiltration by alien predators into invertebrate food webs in Hawaii: a molecular approach. *Molecular Ecology*, **13**, 2077–2088.

Sheppard SK, Bell J, Sunderland KD *et al*. (2005) Detection of secondary predation by PCR analyses of the gut contents of invertebrate generalist predators. *Molecular Ecology*, **14**, 4461–4468.

Simon C, Buckley TR, Frati F, Stewart JB, Beckenbach AT (2006) Incorporating molecular evolution into phylogenetic analysis, and a new compilation of conserved polymerase chain reaction primers for animal mitochondrial DNA. *Annual Review of Ecology, Evolution and Systematics*, **37**, 545–579.

Simon C, Frati F, Beckenbach A *et al*. (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene-sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America*, **87**, 651–701.

Southwood TRE (1978) *Ecological Methods*, 2nd edn. Chapman & Hall, London, UK.

Striebel H-M, Birch-Hirschfeld E, Egerer R, Foldes-Papp Z (2003) Virus diagnostics on microarrays. *Current Pharmaceutical Biotechnology*, **4**, 401–415.

Sunderland KD, De Snoo GR, Dinter A *et al*. (1995) Density estimation for invertebrate predators in agroecosystems. *Acta Jutlandica*, **70**, 133–162.

Sunderland KD, Powell W, Symondson WOC (2005) Populations and communities. In: *Insects as Natural Enemies: a Practical Perspective* (ed. Jervis MA), pp. 299–434. Springer, Berlin, Germany.

Sunnucks P, Hales DF (1996) Numerous transposed sequences of mitochondrial cytochrome oxidase I–II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Molecular Biology and Evolution*, **13**, 510–524.

Sutherland RM (2000) *Molecular analysis of avian diet*. PhD Thesis, University of Oxford, Oxford, UK.

Suzuki N, Murakami K, Takeyama H, Chow S (2006) Molecular attempt to identify prey organisms of lobster phyllosoma larvae. *Fisheries Science*, **72**, 342–349.

Symondson WOC (2002) Molecular identification of prey in predator diets. *Molecular Ecology*, **11**, 627–641.

Symondson WOC, Erickson ML, Liddell (1999) Development of a monoclonal antibody for the detection and quantification of predation on slugs within the *Arion hortensis* agg. (Mollusca: Pulmonata). *Biological Control*, **16**, 274–282.

Symondson WOC, Glen DM, Ives AR, Langdon CJ, Wiltshire CW (2002a) Dynamics of the relationship between a generalist predator and slugs over five years. *Ecology*, **83**, 137–147.

Symondson WOC, Sunderland KD, Greenstone MH (2002b) Can generalist predators be effective biocontrol agents? *Annual Review of Entomology*, **47**, 561–594.

Szemes M, Bonants P, de Weerdt M *et al*. (2005) Diagnostic application of padlock probes — multiplex detection of plant pathogens using universal microarrays. *Nucleic Acids Research*, **33**, Art No. e70.

Taberlet P, Fumagalli L (1996) Owl pellets as a source for genetic studies of small mammals. *Molecular Ecology*, **5**, 301–305.

Traugott M, Zangerl P, Juen A, Schallhart N, Pfiffner L (2006) Detecting key parasitoids of lepidopteran pests by multiplex PCR. *Biological Control*, **39**, 39–46.

Troedsson C, Frischer ME, Nejstgaard JC, Thompson EM (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.

Vestheim H, Edvardsen B, Kaartvedt S (2005) Assessing feeding of a carnivorous copepod using species-specific PCR. *Marine Biology*, **147**, 381–385.

Wolters V (1985) Resource-allocation in *Tomocerus flavescens* (Insecta, Collembola) — a study with C-14-labeled food. *Oecologia*, **65**, 229–235.

Zaidi RH, Jaal Z, Hawkes NJ, Hemingway J, Symondson WOC (1999) Can multiple-copy sequences of prey DNA be detected amongst the gut contents of invertebrate predators? *Molecular Ecology*, **8**, 2081–2087.

Zhang DX, Hewitt GM (1996) Nuclear integrations: challenges for mitochondrial DNA markers. *Trends in Ecology & Evolution*, **11**, 247–251.

Zhang GF, Lü ZC, Wan FH (2007a) Detection of *Bemisia tabaci* remains in predator guts using a sequence-characterised amplified region marker. *Entomologia Experimentalis et Applicata*, **123**, 81–90.

Zhang GF, Lü ZC, Wan FH, Lövei GL (2007b) Real-time PCR quantification of *Bemisia tabaci* (Homoptera: Aleyrodidae) B-biotype remains in predator guts. *Molecular Ecology Notes*, doi: 10.1111/j.1471-8286.2007.01819.x.

This review has emerged from the research group of Dr Bill Symondson at Cardiff University, a group that specialises in the development and application of new techniques for studying predator–prey interactions in the field. The authors include Dr Michael Traugott (now developing a similar research group at Innsbruck), Dr Andrew King (senior postdoc developing multiplex approaches to analysis of the spatial dynamics of predation by carabid beetles), Dr Daniel Read (working on micro-arthropod predators and soil foodwebs) and Dr Bill Symondson (Reader in Invertebrate Molecular Ecology). All authors contributed equally.

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