

Infiltration by alien predators into invertebrate food webs in Hawaii: a molecular approach

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Abstract

Alien invertebrate predators have been introduced to Hawaii to control pests, particularly in lowland areas where most crops are grown. We developed techniques for assessing the impact of these predators on native food webs in relatively pristine upland areas where, it was hypothesized, few lowland predators might be found. Predator densities were assessed along transects within the Alakaii Swamp on Kaua'i. The most numerous alien biocontrol agents found were *Halmus chalybeus* (Coccinellidae), a species known to feed on Lepidoptera eggs. Laboratory experiments were conducted using two genera of endemic Lepidoptera, *Scotorythra* and *Eupithecia* (Geometridae), that are of considerable conservation value, the former because of its recent speciation across Hawaii, the latter for its unique predatory larvae. Techniques were developed for detecting Lepidoptera DNA within the guts of alien predators using prey-specific PCR primers. General primers amplified fragments of the mitochondrial cytochrome oxidase I gene from beetles and Lepidoptera. The sequences were aligned and used successfully to design target-specific primers for general detection of the remains of Geometridae and for particular species, including *Scotorythra rara* and *Eupithecia monticolans*. DNA fragments amplified were short [140–170 base pairs (bp)], optimizing detection periods following prey ingestion. Trials using the introduced biocontrol agent *Curinus coeruleus* (Coccinellidae) demonstrated detection of Lepidoptera DNA fragments = 151 bp in 85–100% of beetles after 24 h digestion of an early instar larva. This study provides a framework for future use of molecular gut analysis in arthropod conservation ecology and food web research with considerable potential for quantifying threats to endemic species in Hawaii and elsewhere.

Keywords: Biocontrol agents, Coccinellidae, *Eupithecia*, generalist predators, gut analyses, *Scotorythra*

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Introduction

Classical biological control, where exotic predators are introduced to control exotic agricultural weeds and pests, has resulted in the deliberate translocation of hundreds of species between countries and continents, and better regulation is necessary to reduce the potential for deleterious effects upon the indigenous flora and fauna (Howarth 1991; Strong & Pemberton 2000). There is growing evidence of harmful effects, caused by introduced invertebrate

biological control agents, on nontarget native organisms (e.g. Elliott *et al.* 1996), many of which are of considerable scientific and conservation value (Hadfield *et al.* 1993). Many insects released in biocontrol programmes were generalists, feeding on a range of prey species. Indeed, generalist biological control agents were once considered superior, as they have the potential to control several pests (Williams 1931). Moreover, native insects could serve as hosts during times of the year when crop pests were rare. Indeed, the ability of generalist predators to subsist on alternative prey can be a major asset, allowing such predators to provide background control of herbivore populations in their native countries (Symondson *et al.* 2002). Current biocontrol regulations will not allow the release of these types of agents in alien ecosystems, but if we are to understand the present-day impact of past releases

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invasion ecologists need to know more about the diets of these alien predators. However, attempts to study the range of prey utilized by generalist predators in the field, and the ecological processes underpinning demographic changes (inter- and intraspecific competition, switching behaviour, apparent competition, etc.), have until now been hampered by the lack of effective techniques for acquiring quantitative data on prey choice. Predators are particularly difficult to study in the field in comparison to parasitoids which can be reared easily from their hosts (Memmott *et al.* 2000). Given that predators constitute 25% of all biocontrol releases (Greathead & Greathead 1992) ecologists urgently need better tools to study their prey ranges and dietary choices.

There have been numerous releases of biological control agents in Hawaii within the last 100 years (Lai & Funasaki 1986; Funasaki *et al.* 1988), particularly against Lepidoptera. In recent work on the Hawaiian island of Kaua'i quantitative food webs were constructed to examine the community-wide effects of alien parasitoids, particularly deliberately introduced biocontrol agents (Henneman & Memmott 2001). These webs linked host plants, Lepidoptera and parasitoids of those Lepidoptera in the Alaka'i Swamp. Biocontrol agents dominated the parasitoid trophic level of the food web with 83% of parasitoids reared from native moths being alien introductions (Henneman & Memmott 2001). This work clearly demonstrated major infiltration of natural food webs by alien parasitoids in the relatively pristine upland forests of the Alaka'i Swamp, even though most of these biocontrol agents were introduced to control crop pests in the hotter, dryer conditions found at lower elevations where most crops are grown.

Parasitoids, however, constitute only a portion of the whole food web. We wished to develop and calibrate techniques that would form the basis of future work to quantify the effects of invasive alien predators in the Alakai swamp. The ultimate aim will be to link predator and parasitoid food webs, possibly incorporating other mortality factors such as disease. In this study we focused mainly on alien coccinellid beetles attacking endemic Lepidoptera. One of these beetles, *Halmus chalybeus*, was introduced to Kaua'i for biological control in 1894 (Greathead & Greathead 1992). These beetles were absent from the Alaka'i Swamp between 1998 and 1999 but have recently invaded this habitat (Henneman personal observation), this invasion probably being facilitated by several very dry El Niño years. It is not known whether this was a reinvasion by *H. chalybeus*, or whether this was the first appearance of these beetles in the swamp. Despite being introduced to Hawaii to control homopteran pests (Greathead & Greathead 1992), many coccinellids are generalist predators and are known to feed on moth eggs (Roger *et al.* 2000). *H. chalybeus* was observed feeding on the eggs and early

instar larvae of the native tortricid moth *Spheterista pleonectes* in the Alakai Swamp.

The web of trophic interactions affecting the changing population densities of predators and their prey are difficult to model accurately in natural ecosystems, simply because it is difficult to quantify who is eating whom. Traditional methods include direct observation, population monitoring and laboratory-based feeding experiments. All these techniques can provide useful information, especially when combined with post mortem microscopic examination of gut contents (Ingerson-Mahar 2003). Direct observation, however, can rarely provide quantitative information and is labour-intensive, and laboratory feeding trials cannot hope to emulate conditions in the field (especially prey choice and encounter rates). Moreover, microscopic examination of gut contents is not possible with fluid feeding predators (which constitute the majority) and misses many trophic links (Symondson 2002). To overcome these and other difficulties, predation can now be studied more effectively using a combination of population monitoring and predator gut content analyses using a range of biochemical and molecular techniques (Sunderland 1988; Symondson & Hemingway 1997; Symondson 2002). While species-specific monoclonal antibodies combined with enzyme-linked immunosorbent assays are currently considered to be the most accurate method of detecting and quantifying such prey remains (Greenstone 1996; Symondson 2002) considerable progress has been made recently in detecting DNA in predator guts.

Studies have now used molecular approaches to detect DNA from pest Lepidoptera and/or Homoptera in heteropteran bugs (Heteroptera: Miridae and Anthocoridae), coccinellid beetles (Coleoptera: Coccinellidae), spiders (Araneae) and lacewings (Neuroptera: Chrysopidae) (Agustí *et al.* 1999, 2000, 2003a, 2003b; Chen *et al.* 2000; Hoogendoorn & Heimpel 2001; Greenstone & Shufran 2003; Hoogendoorn & Heimpel 2004). Recently that this technology was used for the first time to measure predation on natural populations of prey in the field, in studies of spiders feeding on Collembola (Agustí *et al.* 2003a) and carabid beetles (Coleoptera: Carabidae) feeding on slugs (Pulmonata) (Dodd *et al.* 2003). The first of these studies demonstrated clear prey choice; Collembola species were consumed by linyphiid spiders in ratios that were very different from those found in the habitat from which the spiders were sampled.

Detection of prey DNA in predator guts is dependent upon the capacity of the DNA to resist digestion and the successful retrieval/amplification of these prey-specific semidigested fragments by polymerase chain reaction (PCR). For this reason it is desirable to target relatively short [< 300 base pairs (bp)] multiple copy sequences, as has been reported previously (Zaidi *et al.* 1999). Mitochondrial

DNA provides multiple targets, with hundreds or thousands of copies per insect cell, and has been useful in other studies of arthropod predation (Chen *et al.* 2000; Agustí *et al.* 2003a, 2003b). All of these studies exploited the cytochrome oxidase genes (COI and COII), mainly because they have proved to be useful for species-level discrimination. These genes are used widely in invertebrate phylogenetic analyses and general primers are available for amplifying the whole or parts of these protein-coding genes (Simon *et al.* 1994; Folmer *et al.* 1994; Caterino *et al.* 2000).

Our approach was to conduct a first survey of the densities of arthropod generalist predators in the Alaka'i swamp and to test the hypothesis that alien predators were penetrating the swamp in significant numbers. We wished to identify alien species and to select an appropriate model group of predators for use in the development of a molecular diagnostic technique. We intended to test the hypothesis that species-specific DNA fragment from relatively recently radiated endemic Lepidoptera could be amplified and identified from the gut contents of alien predators, despite expected low levels of genetic divergence. Any primers that could do this would provide practical and powerful tools for quantifying the infiltration of alien predators into native food webs. Experiments were conducted using two genera of endemic Lepidoptera, *Scotorythra* and *Eupithecia* (Geometridae), both of which are of considerable conservation value. The *Scotorythra* provide a striking example of recent speciation across the islands of Hawaii, while many species of *Eupithecia* have

predatory larvae with unique adaptations for ambushing their insect prey (Montgomery 1982).

Materials and methods

Field site and predator sampling

The field study was conducted in the Alaka'i Swamp on the Hawaiian island of Kaua'i (159°37'-W, 22°8'-9'-N; elevation, 1200 m). This site is designated by the state of Hawaii as a wilderness preserve and was selected due to its geographical, climatic and ecological isolation from agricultural areas as well as its significance as a conservation area.

The abundance of potential lepidopteran predators was quantified at the field site by collecting arthropod predators along 10 transect lines in two study plots. The plots (200 × 25 m) and transects (25 m long, 1 m wide and 2 m high) were the same as those used in Henneman & Memmott (2001). Predator samples were collected by beating the vegetation onto a tray between 12 and 20 June 2002.

Feeding trial protocol

The transect data (Table 1) identified introduced Coccinellidae as the most important group of alien predators in the Alaka'i Swamp. Neither of the two coccinellids found at the site (*H. chalybeus* or *Olla v-nigrum*) were abundant enough to use as model predators at the time when large numbers of specimens were needed for feeding trials. Consequently

Table 1 Numbers of predaceous arthropods collected in the Alaka'i Swamp, June 2002. Species introduced deliberately have been marked as 'Introduced', while those introduced accidentally are marked as 'Adventive'

Species	Order	Family	Status ^a	No. recorded ^b
<i>Nabis keraosphoros purpureus</i>	Hemiptera	Nabidae	Native	3
<i>N. sharpianus</i>	Hemiptera	Nabidae	Native	12
<i>N. capsiformis</i>	Hemiptera	Nabidae	Adventive	3
<i>N. lolupe</i>	Hemiptera	Nabidae	Native	15
<i>N. sylvestris</i>	Hemiptera	Nabidae	Native	4
<i>Halmus chalybus</i>	Coleoptera	Coccinellidae	Introduced	36 ^c
<i>Olla v-nigrum</i>	Coleoptera	Coccinellidae	Introduced	2
<i>Rhyzobius forestieri</i>	Coleoptera	Coccinellidae	Introduced	2
<i>Anamalochrysa maclachlani</i>	Neuroptera	Chrysopidae	Native	1
<i>Micromus timidus</i>	Neuroptera	Hemerobiidae	Introduced	2
Larvae (also <i>A. malachlani</i> ?)	Neuroptera	Chrysopidae	Native	3
4 unidentified species	Araneae	Tetragnathidae	All native	68
5 morphs (< 5 spp.?)	Araneae	Salticidae	Probably all native	57
5 morphs (< 5 spp.?)	Araneae	Thomisidae	All native	74
2–3 spp.?	Araneae	Linyphiidae	?	17
Several spp.?	Araneae	Araneid	?	26
Unidentified spiders	Araneae			14
Unidentified mites	Acari		?	162

^aStatus according to Nishida (2002).

^bThe sum of all individuals over the total volume of vegetation sampled (1000 m³).

^c21 of these were larvae.

we chose to use the more abundant lowland coccinellid *Curinus coeruleus* as our model alien predator because it is morphologically very similar in size, shape and colour. *C. coeruleus* was introduced to Kaua'i in 1922 to control pseudococcids on Avocado (Lai & Funasaki 1983). As prey for the feeding trials we used larvae of *S. rara*, one of the most abundant moth species in the Alakai Swamp, and another geometrid, *Eupithecia monticolans*.

C. coeruleus larvae were starved for at least 3 days before the trials. A relatively short starvation period was acceptable, as the *C. coeruleus* larvae had not come into contact with any of the targeted prey items. Each predator was isolated in a plastic 33 mL vial and presented with one prey caterpillar (*S. rara* or *E. monticolans*) at instar 1–3 and watched until it began to feed. Predators were allowed to feed for 30–60 min, at which time any remains of the prey were removed from the vial. Beetle larvae were then either killed immediately or kept isolated and alive for 6, 12, 24 or 36 h for *S. rara*; the 36-h sample was taken for *E. monticolans*. Beetles were killed and preserved either by submersion in 100% ethyl alcohol or were crushed between filter paper and air-dried. As specimens had to be shipped back to the United Kingdom for DNA analysis, both techniques were used to determine which gave the best results. All samples were frozen in clean glass vials until shipment. In the case of *S. rara* six specimens of *C. coeruleus* (three in alcohol and three on filter paper) were preserved for each time period, except for those killed immediately after feeding, when 10 were preserved (five in alcohol, five on paper). For *E. monticolans*, three specimens of *C. coeruleus* were preserved at each time interval (two in alcohol, one of filter paper). In practice three replicates proved adequate.

DNA extraction

The same procedure was used to extract DNA from Lepidoptera larvae, beetle larvae, beetle larvae fed on Lepidoptera larvae and dried specimens of adult Lepidoptera collected by light trapping in 2000. Acceptable DNA yields were obtained following homogenization of moth legs (three per extraction) or whole beetle larvae. DNA extraction was carried out using two methods. In the first, total DNA was extracted in 5.0 mL volumes using proteinase K digestion, phenol–chloroform purification, NaCl extraction and ethanol precipitation (Ausubel *et al.* 1989). The protocol was modified by reducing extraction volumes to 1.0 mL. DNA was suspended in 75–150 µL 1 mM EDTA (TE) buffer (pH 8.0) and stored at –20 °C. The second, more sensitive DNA extraction method, employed the commercially available QIAamp® DNA Stool Mini Kit (Qiagen GMBH, Hilden, Germany). Extraction was carried out according to the manufacturer's instructions and the DNA was resuspended in 75–150 µL 1 mM TE buffer and stored at –20 °C.

PCR amplification and sequencing of COI gene

Large fragments (710 bp) of the *COI* gene were amplified using the general invertebrate primers of Folmer *et al.* (1994): LCO 1490f (5'-GTCAACAAATCATAAAGATATTGG-3') and HCO 2198r (5'-TAAACTTCAGGGTGACCAAAAA-ATCA-3'). PCR was performed on moths (*Scotorythra rara*, *S. euryphaea* and *Eupithecia monticolans*) and unfed beetle larvae in order to sequence sections of the *COI* gene for subsequent design of family, genus and species-specific primers. Amplification was carried out in 25 µL reaction volumes containing 25 ng/mL of DNA (in TE buffer), 100 µM dNTPs (Boehringer Mannheim, Indianapolis, IN, USA), 0.75 units *Taq* polymerase (Promega, Madison, WI, USA), 2.5 µL 10× PCR buffer (provided by the manufacturer), 2.5 mM MgCl₂ and 50 µM of each primer. PCR conditions were optimized and reactions were carried out in a Hybaid thermal cycler (Hybaid, UK). Cycling parameters were as follows: 95 °C for 1.5 min, then 40 cycles of 95 °C for 1 min, 47 °C for 1.3 min and 73 °C for 1.1 min, with a final extension period at 74 °C for 5 min. Twelve µL of PCR products were visualized with ultraviolet transillumination following electrophoresis at 100 V (for 1 h) on a 1.5% (w/v) agarose gel in 1×TAE buffer (1 mM EDTA, 40 mM Tris-acetate). Twenty µL or less of amplification products were then concentrated and purified using Geneclean™ Turbo Kit (Bio 101) following the manufacturer's instructions. Aliquots (25–50 pg) of purified DNA template were then used for Big Dye™ (Promega, Madison, WI, USA) terminator cycle sequencing (ABI Prism) following the manufacturer's protocol. DNA template was sequenced using forward (LCO 1490f) and reverse (HCO 2198r) *COI* primers for five specimens of all three moth species and unfed beetle larvae. Sequencing products were resolved on an automated capillary sequencer (ABI Prism model 3100). Sequence alignment was performed using the SEQUENCHER™ 4.0 software package. Final adjustments were made by eye (Table 2).

Primer design

Visual alignment of the 710 bp *COI* sequences from the different species of Lepidoptera and the beetle predators allowed the identification of regions where the gene sequence differed between species. Aggregations of single and multiple base pair differences were targeted as regions for primer design. Regions of less than 200 bp were targeted to ensure that partially degraded DNA would be amplified easily from predator gut samples (Zaidi *et al.* 1999). A number of oligonucleotide primers were designed (Table 3) and tested for species specificity (Table 4). PCR conditions were optimized as follows: 94 °C for 3 min; 30–35 cycles at 94 °C for 30 s, 45–57 °C (annealing temperature is specific to primer pair, Table 4) for 50 s and 72 °C for 1.3 min; with a final extension period of 10 min at 72 °C.

Table 2 Cytochrome oxidase subunit I (COI) nucleotide sequences for three moths (*Scotorythra euryphaea*, *S. rara* and *Eupithecia monticolans*), and one coccinellid beetle (*Curinus coeruleus*) species from the Alakai Swamp. Sequences are shown in reference to *Drosophila yakuba*. Nucleotide no. 1 corresponds to position no. 1513 in the published *D. yakuba* sequence. Base lettering nomenclature is included for the Hawaiian invertebrates at positions where a difference from the *D. yakuba* sequence was observed. Where no base information was obtained a (-) is entered. Associated amino acids are denoted by standard abbreviations

D. yakuba	Gly	Thr	Leu	Tyr	Phe	Ile	Phe	Gly	Ala	Trp	Ala	Gly	Met	Val	Gly	Thr	Ser	Leu	Ser	Ile	60		
S. eury	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---			
S. rara	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---			
E. monti	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---			
Beetle	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---			
D. yakuba	Leu	Ile	Arg	Ala	Glu	Leu	Gly	His	Pro	Gly	Ala	Leu	Ile	Gly	Asp	Asp	Gln	Ile	Tyr	Asn	120		
S. eury	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---			
S. rara	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---			
E. monti	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---			
Beetle	---	---	---	---	---	---	- A	ACA	T T	AGT					A								
D. yakuba	Val	Ile	Val	Thr	Ala	His	Ala	Phe	Ile	Met	Ile	Phe	Phe	Met	Val	Met	Pro	Ile	Met	Ile	180		
S. eury	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---			
S. rara	---	-		A	T				C				C	G	T		A	C					
E. monti	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---			
Beetle	T		A	A	T		C					C											
D. yakuba	Gly	Gly	Phe	Gly	Asn	Trp	Leu	Val	Pro	Leu	Met	Leu	Gly	Ala	Pro	Asp	Met	Ala	Phe	Pro	240		
S. eury	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---			
S. rara	---	A	C						C	C	T			G	C		T		C				
E. monti	---	A	A		C					C	T		C	C			T		T				
Beetle	---	A	A			A								C		T		T		C			
D. yakuba	Arg	Met	Asn	Asn	Met	Ser	Phe	Trp	Leu	Leu	Pro	Pro	Ala	Leu	Ser	Leu	Leu	Leu	Val	Ser	300		
S. eury	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---			
S. rara	---	---	---	C					C	T		C	A	T	A	A	A	C	T	A	T	T	T
E. monti	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
Beetle		T						G		T	C	A		T	A	A	C	C	A	T	T	T	T
D. yakuba	Ser	Met	Val	Glu	Asn	Gly	Ala	Gly	Thr	Gly	Trp	Thr	Val	Tyr	Pro	Pro	Leu	Ser	Ser	Gly	360		
S. eury	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---			
S. rara	---	T	A		T	G		A	T	G		A	A				C	T	C	T	AA		
E. monti	---	T	A		T			A	T		A				G		C	T		T	AA		
Beetle	---	C	A		T		C	A	T	A				C			C		T	AA			
D. yakuba	Ile	Ala	His	Gly	Gly	Ala	Ser	Val	Asp	Leu	Ala	Ile	Phe	Ser	Leu	His	Leu	Ala	Gly	Ile	420		
S. eury	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---			
S. rara	---	T		A		AG		T			A			A	A					T			
E. monti	---	T	A		A		AG		T	C	C	T	A		C	T	A			T			
Beetle	---	T	GCT		A	T	AGA	A	T					T	A					T			
D. yakuba	Ser	Ser	Ile	Leu	Gly	Ala	Val	Asn	Phe	Ile	Thr	Thr	Val	Ile	Asn	Met	Arg	Ser	Thr	Gly	480		
S. eury	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---			
S. rara	---	A				A	T				A	A	A	T				T	A	AAT			
E. monti	---	A		C		A	T				A	A	A	T		C			T	A	AAT		
Beetle	---	A	T			A	T				A		A	T					T	A	AAT		
D. yakuba	Ile	Thr	Leu	Asp	Arg	Met	Pro	Leu	Phe	Val	Trp	Ser	Val	Val	Ile	Thr	Ala	Leu	Leu	Leu	540		
S. eury	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---			
S. rara	---	T	A	T		T	A				G		G	C		G		A	C	T	---	---	
E. monti	---	T	A	T		T	A						G	T		T	G		A		C	---	---
Beetle	---	A	TTT		T	T	A	T			T		G	T		GA		---	---	---	---	---	---
	---	A	T		A	AA	CT								T		A	G	T		-		

Table 2 *Continued*

D. yakuba	Leu	Leu	Ser	Leu	Pro	Val	Leu	Ala	Gly	Ala	Ile	Thr	Met	Leu	Leu	Thr	Asp	Arg	Asn	Leu	600
S. eury	TTA	CTA	TCT	TTA	CCA	GTT	CTT	GCC	GGA	GCT	ATT	ACT	ATA	TTA	TTA	ACA	GAC	CGA	AAT	TTA	
S. rara	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
E. monti	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
Beetle	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
D. yakuba	Asn	Thr	Ser	Phe	Phe	Asp	Pro	Ala	Gly	Gly	Gly	Asp	Pro	Ile	Leu	Tyr	Gln	His	Leu	657	
S. eury	AAT	ACT	TCT	TTT	TTT	GAT	CCA	GCT	GGA	GGA	GGA	GAT	CCT	ATT	TTG	TAC	CAA	CAT	TTA		
S. rara	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
E. monti	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		
Beetle	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		

Table 3 Family-, genus- and species-specific cytochrome oxidase subunit I (*COI*) primers. Sequences are presented from 5' to 3' and the position of each primer on the *COI* gene is provided

Primer name ^a	Sequence 5'–3'	Position ^b
Geom f	TGCWCATGGAGGAAGTTCTGTT	1876
Scot f	GCCTCTTATACTCGGAGCYCCT	1717
Eup f	ACCTTTAATATTAGGAGCC	1717
Rara f	CTCGGAGCTCCTGATATAGCT	1727
Geom r1	GATCAAATGATAAATTTAATCG	2007
Geom r2	AAGCTGTAATACCWACAGC	2046
Scot r1	AGCTCCATTTCTTACA	1846
Scot r2	AACAGAACTTCTCCATGTGCA	1898
Eup r1	GAAGAGTGATAGAGGGAGGA	1814
Eup r2	GGATAAAGGGGGTAAACAGTTCAT	1868
Rara r1	ATGTAATAGATGGAGGTAG	1792
Rara r2	AGAAAGAGGCGGGTAAACTGTTCAA	1868
Rara r3	AGGAAAAAATTGCAAGC	1917

^ar and f denotes forward and reverse.

^bPosition on the *COI* gene.

Following optimization, standard reaction mixes were as follows: 2–5 µL of DNA extract; 10 mM dNTPs (Boehringer Mannheim, Indianapolis, IN, USA); 5 units *Taq* polymerase (Promega, Madison, WI); 2.5 µL 10× PCR buffer (provided by the manufacturer); 2.5–3 mM MgCl₂ and 50 µM of each primer in a 25 µL reaction volume. Thermal cycling and DNA visualization were carried out as described above.

Following optimization of PCR conditions primer specificity experiments were conducted using other moth species (dried specimens) from the Hawaiian food web and the two predatory beetle species to select the most appropriate primers for feeding experiments. These species included *Eupithecia monticolans*, *E. orichloris*, *Scotorythra rara*, *S. euryphaea*, *S. pachyspila*, *S. hecatae*, *S. trachyopsis*, *S. apicalis* and *S. ortharcha*. Three primer pairs were selected: Geom f/Geom r2, specific to Lepidoptera of the family Geometridae, Eup f/Eup r2, specific to the genus *Eupithecia* and rara f/rara r2, specific to *S. rara* (Tables 3 and 4). The gene sequence data for regions of the *COI* gene amplified

Table 4 Primer pair specificity and optimum annealing temperatures for polymerase chain reactions (PCR)

Primer pair ^a	PCR annealing temperature (°C)	Positive PCR products (primer specificity) ^b
Geom f/	Geom r1	<i>E. monticolans</i> , <i>S. rara</i> and <i>S. euryphaea</i>
	Geom r2	<i>E. monticolans</i> , <i>E. orichloris</i> , <i>Scotorythra</i> species complex ^c
Scot f/	Scot r1	<i>S. rara</i>
	Scot r2	<i>E. monticolans</i> , <i>E. orichloris</i> , <i>Scotorythra</i> species complex ^c
Eup f/	Eup r1	—
	Eup r2	<i>E. monticolans</i> and <i>E. orichloris</i>
Rara f/	Rara r1	—
	Rara r2	<i>S. rara</i>
	Rara r3	<i>E. monticolans</i> , <i>S. rara</i>

^ar and f denotes forward and reverse.

^bIn all cases PCR products resulted from a single amplification. (—) symbols indicate that no products were recorded.

^cIn the context of these experiments the *Scotorythra* species complex includes the following species: *Eupithecia monticolans*, *E. orichloris*, *Scotorythra rara*, *S. euryphaea*, *S. pachyspila*, *S. hecatae*, *S. trachyopsis*, *S. apicalis*, *S. ortharcha*.

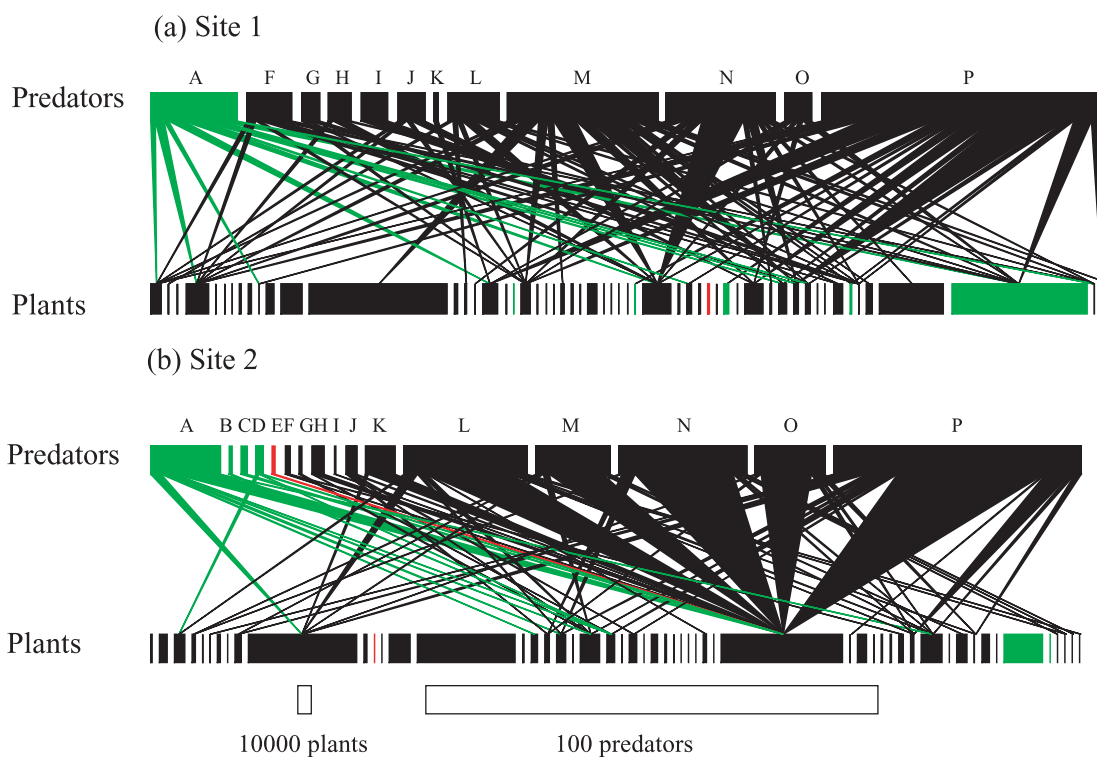


Fig. 1 A food web showing the distribution of predators on the plants in each plot. The predators are shown on the top line of the figure, each box representing a different species or species group. The size of the box is proportional to its abundance. The plants are shown on the lower line of the figure. The triangles linking predators and plants show the frequency of occasions they were found on that plant species. Native species are shown in black, deliberately introduced alien species are shown in green and adventive species in red. Predators are listed A–P (A: *Halmus chalybeus*, B: *Olla v-nigrum*, C: *Rhyzobius forestieri*, D: *Micromus timidus*, E: *Nabis capsiformis*, F: *N. sharpianus*, G: *N. lolupe*, H: *Araniedae*, I: *Araniedae* [orb weavers], J: *Linyphiidae*, K: *Oonopidae*, L: *Salticidae*, M: *Tetragnathidae*, N: *Thomicidae*, O: unidentified spiders, P: mites).

using general invertebrate primers (LCO 1490f and HCO 2198r) appear in the GenBank nucleotide sequence database under Accession nos AY283619–AY283622 relating to *S. rara*, *S. euryphaeae*, *E. monticolans* and *C. coeruleus*, respectively.

Results

Transect data on predator abundance

Numbers of predaceous arthropods collected in the Alakai Swamp are shown in Table 1. Data on species observed/collected represents numbers of individuals per 1000 m³ of vegetation (i.e. 20 transects each 25 m long, 1 m wide and 2 m high) in the swamp. The majority of species captured were native, but there were a number of aliens including an adventive nabid, a hemerobiid lacewing (*Micromus timidus*) (introduced for the control of aphids) and three species of coccinellids (also introduced to control Homoptera). Of these *H. chalybeus* was the most abundant. There were many mites and spiders, but none were known to be alien species.

The predator transect data are presented as food webs showing the distribution of predators on plants (Fig. 1), highlighting the parts of the food web that comprised

alien species. The abundance of predators was correlated with the abundance of different species of plant, both for the total predator counts (Site 1, Spearman's correlation = 0.521, $P < 0.001$, Site 2, Spearman's correlation = 0.474, $P < 0.001$) and for *H. chalybeus* distribution (Site 1, Spearman's correlation = 0.362, $P < 0.006$; Site 2, Spearman's correlation = 0.495, $P < 0.001$). Such correlations would be expected if the predators were distributed randomly.

Primer design

The 'universal' DNA primers, LCO 1490 and HCO 2198, amplified 650–710 bp regions of the *COI* gene from fresh beetles and moths and those dried or preserved in alcohol. In practice, acceptable yields of DNA were obtained from all samples, regardless of preservation technique. *COI* sequences for *S. rara*, *S. euryphaeae*, *E. monticolans* and *C. coeruleus* were compared to sequence information for *Drosophila yakuba*, GenBank Accession no. X03240 (Clary & Wolstenholme 1985). To identify differences in the *COI* gene sequences between the four species and to enable species-, genus- and family-specific primer design, sequence alignments were performed (Table 2). Comparison of

Moth fed to beetle	Time from end (h) of feeding period	<i>n</i>	170 bp (%)	140 bp (%)
<i>S. rara</i>	1	10	100	100
	6	6	100	100
	12	6	100	100
	24	6	67	83
	36	6	33	33
<i>E. monticolans</i>	1	3	100	100
	6	3	100	100
	12	3	67	100
	24	3	67	100

Table 5 Polymerase chain reaction (PCR) products of DNA extracted from *Curinus coeruleus* fed with one of two geometrid moth species (*Scotorythra rara* or *Eupithecia monticolans*). Primer pairs Geom f/Geom r2, rara f/rara r2 and Eup f/Eup r2 gave PCR products of 170, 151 and 140 bp, respectively. Predators were deemed positive for moth predation if two of five replicate PCRs per specimen yielded amplifiable moth DNA

these sequences with *COI* from *D. yakuba* revealed the positions where nucleotide variations occurred. Most of the variations occurred at the third-position nucleotides. Intermediate levels of resolution were retained in first- and second-position nucleotides, sufficient for species/genus level discrimination. Analysis of these fragments suggested that phylogenetic resolution at the class, order, family, genus and species level could be obtained from inferred amino sequences, even therefore within the recently radiated *Scotorythra*. From this information primers that discriminated between these sequences were designed.

Altogether, three coding-strand and six anticoding strand primers were tested for amplification efficiency and species specificity. The details of these primers are given in Tables 3 and 4. In all cases where positive PCR products were recorded they resulted from five replicate amplifications per specimen to eliminate potential PCR errors incurred as a result of targeting such small amounts of DNA. Primer pairs Geom f/Geom r2, Eup f/Eup r2 and rara f/rara r2 gave PCR products of 170, 151 and 140 bp, respectively. No product was amplified from unfed *C. coeruleus* using any of these primers and *C. coeruleus* DNA was included as a second negative control in all subsequent PCR experiments.

Feeding experiments

These three primer pairs were tested in PCR amplifications of prey DNA from homogenized predators that had been fed the target Lepidoptera. Geometrid-specific primers (Geom f/Geom r2) were applied to coccinellids fed with either *S. rara* or *E. monticolans*. Two other primers pairs, rara f/rara r2 and Eup f/Eup r2, were used in PCR amplifications of homogenized coccinellid samples fed with *S. rara* and *E. monticolans*, respectively. DNA was amplified successfully using all three primer pairs and percentages of prey-positive PCRs are shown in Table 5. The per-

centage of positives varied with prey species and primers, with best results obtained from primers that amplified shorter fragments, = 151 bp. With these primers prey could be detected in 83–100% of predators after 24 h. Prey DNA was still detectable in many predators after 36 h. Although there were just three replicate beetles fed on *E. monticolans* at each time period, giving very crude percentages, the results suggest that both prey species could be detected reliably within a workable time-frame (up to 24 h).

Discussion

The transect data showed that alien predators were indeed penetrating the Alaka'i Swamp on Kaua'i in significant numbers. The webs provide a visual picture of the 'living sheet of enemies' (Janzen 1993) to which the Lepidoptera larvae found in this habitat are exposed. They show that the different groups/species of predators are distributed over a range of plant species and that many of them are found on more than one plant species. Predators overall, and *H. chalybus* in particular, do not seem to show any obvious preference for particular plant species; rather, they are distributed over the plant species according to plant abundance. Thus common plants have a higher abundance of predators on them than the rare plants. The level of taxonomic resolution is not sufficient to test whether this pattern extends to species-richness patterns.

The level of infiltration by alien predators into the web is small in comparison to that seen by parasitoids. Henneman & Memmott (2001) showed that 83% of parasitoids in these plots were alien biological control agents and 14% were accidentally introduced adventive wasps. Native parasitoids were relatively rare at 3% of parasitized caterpillars. In contrast, the alien and adventive predators constitute only 11% of predators overall and the remaining 89% of predators appear to be native.

However, while the proportion of alien predators was smaller, the picture is complicated, as our data indicate that predators were more abundant than parasitoids in the Alakai swamp. It is not possible to compare directly predator and parasitoid abundance because of differences in the sampling procedure. However, the abundance of the parasitoids collected 2 years earlier from the same transects shows that parasitoids were only 12% of total natural enemies (i.e. actual parasitoids in 2001 plus potential predators in 2003). Alien predators may predate many caterpillars, while parasitoids will kill only a single caterpillar. The impact of alien predators may therefore be commensurate with the alien parasitoids. Obviously further work is needed to verify such speculations.

Although designed originally for use on marine invertebrates, primers LCO 1490f and HCO 2198r were used initially to amplify 710 bp DNA fragments from all moth and beetle specimens. These primers were found to work better on Hawaiian Lepidoptera than the *COI* primers (C1-J-1718, C1-J-1751, C1-J-1859, C1-J-2183, C1-N-2191, C1-N-2329) described in Simon *et al.* (1994). From the sequences amplified using these general primers, new primers were created that would amplify different species and genera of Geometridae. This was by no means certain, given the supposed recent radiation of species such as the *Scotorythra* within Hawaii, limiting genetic diversity (Gillespie & Roderick 2002). There is no reason to suppose that similar primers could not be developed for other species and genera of Lepidoptera (or other invertebrates) in the Alakai'i, providing a library of probes for the analysis of the prey range of native and exotic predators.

It was clear that lepidopteran DNA (and that of *S. rara* and *E. monticolans* in particular) could be amplified from the guts of coccinellid beetle larvae for extended periods following ingestion. Although similar approaches have been developed for the study of predation on pests, this is the first time DNA-based techniques have been applied to the field of arthropod conservation ecology. The techniques used could probably be applied to detect DNA from almost any arthropod prey in almost any arthropod predator in natural or anthropogenically modified ecosystems; the only limitation would be the availability of suitable primer sites within the target genes of the prey. Should such sites be unavailable within the *COI* gene, other mitochondrial genes could be used (Simon *et al.* 1994) or even multiple-copy nuclear genes, such as *ITS1* and *ITS2*, within ribosomal gene clusters (Hoogendoorn & Heimpel 2002). From a conservation perspective, we now have the means to identify potential threats to endangered invertebrate prey species from identifiable predators, or to identify the primary prey that may help to sustain rare invertebrate predators. Given that these predators are usually nocturnal, cryptic and small, such molecular techniques provide the most practical means for analysing such trophic links in the field

and have considerable potential for future research into the impact of alien predators.

The primers used in this study, that amplified the shorter species-specific 140 and 151 bp fragments from *S. rara* and *E. monticolans*, respectively, were able to detect the remains of these prey in 83–100% of the predators tested 24 h after consumption by the beetle. A number of studies have shown that shorter sequences are detectable for longer periods in predators than longer sequences (Symondson 2002), and the trend found in our study was in the same direction. The technique was highly sensitive with single early instar larvae providing sufficient prey DNA to allow detection by PCR. This was true despite the presence of far larger amounts of beetle tissue and there was no evidence that this nontarget material masked the detection of very small amounts of prey DNA. This level of sensitivity, and the requirement that only a single prey item needs to be consumed for detection to be successful, has been noted in previous predation experiments involving small prey such as mosquitoes, aphids and Collembola (Zaidi *et al.* 1999; Chen *et al.* 2000; Agustí *et al.* 2003b). There was no need to dissect the predators, which were simply homogenized. This is a great advantage, as it not only makes the sample processing quicker but also reduces the chances of cross-contamination.

The ability to detect prey remains over such extended time periods (24 h) shows clearly that DNA-based approaches are comparable to monoclonal antibody techniques (Symondson 2002), which until now represented the most practical (fast and sensitive) method of detecting prey remains in predator guts. Although monoclonal antibodies can be a powerful tool in predation experiments and have been used to detect predation on agricultural pests, their application is limited largely to analysis of the predators attacking single-target prey species. Multiple antibody probes can be used (Hagler & Naranjo 1994) but the generation and characterization of monoclonal antibodies, that are specific and can detect digestion-resistant epitopes, may easily take a year or more (Symondson 2002) with no guarantee of success. The viability of this method in the study of food webs that include polyphagous predators is therefore questionable.

The decay rate of lepidopteran prey DNA within coccinellid predators in this study was similar to that observed in some previous studies. For example, 146 bp mosquito DNA fragments were detected using PCR in the guts of 86–98% of carabid-beetle predators, 28 h after the prey were consumed (Zaidi *et al.* 1999). However, detection periods can be very different with different predators, even where the same probe is used, as has been shown in studies using antibodies (Symondson & Liddell 1993; Hagler & Naranjo 1997). Antibody work shows that some spiders, for example, digest prey proteins slowly compared with other invertebrates (Harwood *et al.* 2001). If this

were to hold true for detection of DNA, which is by no mean certain (Agustí *et al.* 2003b; Greenstone & Shufan 2003), any screening of spiders in the Alaka'i for predation on Lepidoptera would be expected to show a greater number of positive gut samples, even if actual rates of predation were the same as those for coccinellids. Agustí *et al.* (2003b), using primers that amplified a larger 276 bp fragment of DNA, found that 100% of linyphiid spiders tested positive for consumption of Collembola 24 h after feeding on these insects. Table 1 shows that spiders are found in significant numbers in the Alaka'i and therefore are likely to be important components of the food webs surrounding Lepidoptera, as predators of both adults and larvae. Quantitative comparison of predation rates by different predators in the Alaka'i will require measurement of digestion rates for each species of predator using each set of primers (discussed in Chen *et al.* 2000 and Agustí *et al.* 2003b).

There have been 121 releases of coccinellids into Hawaii of 111 species: 86 of these releases failed to establish, 23 releases established in the field and four releases were deemed to be successful or partially successful in controlling their target pest (Greathead & Greathead 1992). Neither *H. chalybeus* nor *C. coeruleus* were considered to be successful at controlling their target pests (Lai & Funasaki 1983). *Olla v-nigram* is not recorded as being introduced purposely and so could be either adventive or an unrecorded biological control release. Given that alien coccinellids have proven problematic in the past (Elliot *et al.* 1996) and that *H. chalybeus* and *O. v-nigram* are recent additions to the fauna of the Alakai swamp, it would be timely to investigate their impact.

In conclusion we have developed, through a combination of field sampling protocols, food web construction method and primer development, a model for a future programme of field research into the impact of alien predators on native food webs in Hawaii. We have demonstrated that the remains even of closely related island populations of Lepidoptera can be amplified from predators using species-specific primers. Once we have screened predators for the presence of prey Lepidoptera in their guts an intermediate trophic level will be added to the food web in Fig. 1, linking predators with both prey and host plants [in a similar way to those already constructed for Hawaiian parasitoids (Henneman & Memmott 2001)]. PCR-based techniques may be a powerful tool for analysing food webs involving invertebrate generalist predators. DNA-based systems have already been used successfully in vertebrate conservation ecology. For example, Scribner & Bowman (1998) used a similar approach to analyse the gut contents of glaucous gulls, *Larus hyperboreus*. Microsatellite primers were applied to the remains of chicks eaten by the gulls. This revealed that they were not significant predators of the spectacled eider, *Somateria fischeri*, but were preying

the declining emperor goose, *Chen canagica*. As a result of such work control of the gulls might be justified. Less invasive approaches are also (fortunately) available. Reed *et al.* (1997) identified prey remains in seal scats visually, then used a combination of microsatellite and *SRγ* gene primers to determine the species, sex and even the individual identity of the seals. Farrell *et al.* (2000) used the same approach (targeting the mitochondrial cytochrome *b* gene) to match scats to species of large carnivore in Venezuela. Invertebrate DNA has also been identified from the faeces of birds in studies of resource partitioning and the importance of different prey species to the birds on organic vs. conventional farms (Sutherland 2000). These issues in conservation ecology are equally important further down the food chain where disruption of plant–herbivore–predator food webs, caused by alien introductions, can have profound effects upon the vertebrates that share the same habitat.

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References

- 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, Vaughan IP, Sunderland KD, Symondson WOC (2003b) 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 (2003a) Detecting *Cacopsylla pyricola* (Hemiptera: Psyllidae) in predator guts using COI mitochondrial markers. *Bulletin of Entomological Research*, **93**, 179–185.
- Ausubel FM (1989) In: *Current Protocols in Molecular Biology* (eds Ausubel FM, Brent R, Kingston RE), pp. 70–225. John Wiley and Sons, New York.
- Caterino MS, Cho S, Sperling AH (2000) The current state of insect molecular systematics: a thriving tower of Babel. *Annual Review of Entomology*, **45**, 1–54.

- Chen Y, Giles KL, Payton ME, Greenstone MH (2000) Identifying key cereal aphid predators by molecular gut analysis. *Molecular Ecology*, **9**, 1887–1898.
- Clary DO, Wolstenholme DR (1985) The mitochondrial DNA molecule of *Drosophila yakuba*: nucleotide sequence, gene organization and genetic code. *Journal of Molecular Evolution*, **22**, 252–271.
- Dodd CS, Bruford MW, Symondson WOC, Glen (2003) Detection of slug DNA within carabid predators using prey-specific PCR primers. In: *Slugs and Snails: Agricultural, Veterinary and Environmental Perspectives. Symposium Proceedings 80* (ed. Dussart GBJ), pp. 13–20. British Crop Protection Council, Alton UK.
- Elliott N, Kieckhefer R, Kauffman W (1996) Effects of an invading coccinellid on native coccinellids in an agricultural landscape. *Oecologia*, **105**, 537–544.
- Farrell LE, Roman J, Sunquist ME (2000) Dietary separation of sympatric carnivores identified by molecular analysis of scats. *Molecular Ecology*, **9**, 1583–1590.
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294–299.
- Funasaki GY, Lai PY, Nakahara LM, Beardsley JW, Ota AK (1988) A review of biological control introductions in Hawaii: 1890–1985. *Proceedings of the Hawaiian Entomological Society*, **28**, 105–160.
- Gillespie RG, Roderick GK (2002) Arthropods on islands: colonization, speciation, and conservation. *Annual Review of Entomology*, **47**, 595–632.
- Greathead DJ, Greathead AH (1992) Biological control of insect pests by insect parasitoids and predators: the BIOCAT database. *Biocontrol News and Information*, **13**, 61–68.
- Greenstone MH (1996) Serological analysis of arthropod predation: past, present and future. In: *The Ecology of Agricultural Pests: Biochemical Approaches* (eds Symondson WOC, Liddell JE), pp. 265–300. Chapman & Hall, London.
- Greenstone MH, Shufran KA (2003) Spider predation: species-specific identification of gut contents by polymerase chain reaction. *Journal of Arachnology*, **31**, 131–134.
- Hadfield MG, Miller SE, Carwile AH (1993) The decimation of endemic Hawaiian tree snails by alien predators. *American Zoologist*, **33**, 610–622.
- 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. *Environmental Entomology*, **23**, 193–197.
- Hagler JR, Naranjo SE (1997) Measuring the sensitivity of an indirect predator gut content ELISA: detectability of prey remains in relation to predator species, temperature, time and meal size. *Biological Control*, **9**, 112–119.
- 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.
- 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-I fragments from prey to estimate predation frequency. *Molecular Ecology*, **10**, 2059–2068.
- Hoogendoorn M, Heimpel GE (2002) PCR-based gut content analysis of insect predators: using ribosomal ITS-1 fragments from prey to estimate predation frequency. *Proceedings of the 1st International Symposium on Biological Control of Arthropods*, Honolulu, Hawaii, January 2002.
- Hoogendoorn M, Heimpel GE (2004) Competitive interactions between an exotic and a native lady beetle: a field case study. *Entomologia Experimentalis et Applicata*, **111**, 19–28.
- Howarth FG (1991) Environmental impacts of classical biological control. *Annual Reviews of Entomology*, **36**, 485–509.
- Ingerson-Mahar J (2003) Relating diet and morphology in adult carabid beetles. In: *The Agroecology of Carabid Beetles* (ed. Holland J), pp. 111–136. Intercept, Andover, UK.
- Janzen DH (1993) Caterpillar seasonality in a Costa Rican dry forest. In: *Caterpillars: Ecological and Evolutionary Constraints on Foraging* (ed. Stamp NE), pp. 448–477. Chapman & Hall, New York.
- Lai PY, Funasaki GY (1983) *List of Biological Control Introductions in Hawaii*. Hawaii Department of Agriculture, Hawaii.
- Lai PY, Funasaki GY (1986) Introductions for biological control in Hawaii. *Proceedings of the Hawaiian Entomology Society*, **26**, 89–90.
- Memmott J, Martinez ND, Cohen JE (2000) Predators, parasitoids and pathogens: species richness, trophic generality and body size in a natural food web. *Journal of Animal Ecology*, **69**, 1–15.
- Montgomery SL (1982) Biogeography of the moth genus *Eupithecia* in Oceania and the evolution of ambush predation in Hawaiian caterpillars (Lepidoptera: Geometridae). *Entomologia Generalis*, **8**, 27–34.
- Nishida GM (2002) Hawaiian terrestrial arthropod checklist, 2nd edn. In: *Hawaii Biological Survey* (ed. Nishida GM), p. 313. Bishop Museum, Honolulu.
- 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.
- Roger C, Coderre D, Boivin G (2000) Differential prey utilization by the generalist predator *Coleomegilla maculata lengi* according to prey size and species. *Entomologia Experimentalis et Applicata*, **94**, 3–13.
- Scribner KT, Bowman TD (1998) Microsatellites identify depredated waterfowl remains from glaucous gull stomachs. *Molecular Ecology*, **7**, 1401–1405.
- Simon C, Frati F, Beckenbach A *et al.* (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America*, **87**, 651–701.
- Strong DR, Pemberton RW (2000) Biological control of invading species: risk and reform. *Science*, **288**, 1969–1970.
- Sunderland KD (1988) Quantitative methods for detecting invertebrate predation occurring in the field. *Annals of Applied Biology*, **112**, 201–224.
- Sutherland RM (2000) *Molecular analysis of avian diets*. PhD Thesis, University of Oxford, UK.
- Symondson WOC (2002) Molecular identification of prey in predator diets. *Molecular Ecology*, **11**, 627–641.
- Symondson WOC, Hemingway J (1997) Biochemical and molecular techniques. In: *Methods in Ecological and Agricultural Entomology* (eds Dent DR, Walton MP), pp. 293–350. CAB International, Oxford, UK.

- Symondson WOC, Liddell JE (1993) Differential antigen decay rates during digestion of molluscan prey by carabid predators. *Entomologia Experimentalis et Applicata*, **69**, 277–287.
- Symondson WOC, Sunderland KD, Greenstone MH (2002) Can generalist predators be effective biocontrol agents? *Annual Review of Entomology*, **47**, 561–594.
- Williams FX (1931) *Handbook of the Insects and Other Invertebrates of Hawaiian Sugar Cane Fields*. Advertiser Publishing Co., Honolulu, HI.
- 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.

This study was part of a long-term research programme investigating invertebrate food webs in the Alakai'i Swamp on the Hawaiian island of Kaua'i. It arose from a collaboration between Bill Symondson at Cardiff, who leads a research group developing a range of molecular ecology techniques to study predator–prey interactions, and Jane Memmott, who is interested in using food webs to address key environmental problems. Experimental work depended upon the ideas and expertise of postdocs Sam Sheppard, who designed and performed the molecular experiments, and Laurie Henneman, who conducted the fieldwork and feeding trials.
