

ASSESSING INSECT ENDOPARASITISM BY PCR: APPLICATIONS TO CLASSICAL BIOLOGICAL CONTROL

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INTRODUCTION

Insect endoparasitism is inherently difficult to study, usually involving minute organisms embedded within other organisms that are themselves small. X-raying may provide useful estimates of parasitism rates when there is a single large parasitoid species in the system (Biever and Boldt, 1970). But for the majority of systems, in which the parasitoid larvae tend to be small, estimates have usually been made by rearing hosts in the laboratory for adult emergence or by dissecting hosts.

Rearing samples requires space and other facilities for maintaining hosts individually on live plants or artificial diet. Because development and emergence take time, the results usually come too late to be used by growers or consultants to make tactical pest management decisions. Furthermore, because a portion of the sample population may succumb to disease or simply die of unknown causes without producing adult parasitoids or hosts, some rearing samples fail to produce a usable estimate of parasitism rate (see Stuart and Greenstone, 1996, for references).

Dissection, on the other hand, may provide a timely and accurate estimate, but is tedious and requires special skills. Also, if several closely related parasitoid species attack a given host, distinguishing them as larvae may be difficult or impossible. This has led to the development of a variety of molecular assays to detect and distinguish endoparasitoid larvae within their hosts.

HISTORY OF MOLECULAR ASSAYS FOR ENDOPARASITISM

Molecular assays that have been developed to measure endoparasitism detect either proteins or DNA (Table 1). Enzyme electrophoresis, the first type of assay developed to detect parasitoid proteins, has proven in some cases to be a sensitive and reproducible method; however, it requires highly trained practitioners and cannot always separate closely related species reliably (Tomiuk *et al.*, 1979; Castañera *et al.*, 1985). A second method, serological assay with monoclonal antibodies, has been used only twice: once to develop a species- and instar-specific assay (Stuart and Greenstone, 1996) and once to develop an assay that is generally parasitoid-specific (Stuart and Greenstone, 1997). Although this approach shows great promise because of the inherent exquisite specificity of monoclonal antibodies and their adaptability to widely used immunoassays (ELISA and immunodot), the expense and expertise required to develop the antibodies may preclude wide adoption of the assay by entomologists.

The first DNA-detecting assays employed randomly-amplified polymorphic DNA– polymerase chain reaction (RAPD-PCR). Although assays using RAPD-PCR are species-specific, they proved unable to detect parasitism until nearly a week following parasitization (Black *et al.*, 1992). Two other DNA methods employed microsatellite variability (Vanlerberghe-Masutti and Chavigny, 1997) and a hybridization assay employing a probe fashioned from a moderately repetitive fragment from a recombinant DNA library (Greenstone and Edwards, 1998). The hybridization assay was specific and easy to perform but not terribly sensitive: it was able to detect first instars but had a threshold of around nine eggs for a fairly large braconid species.

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Table 1. Molecular methods for assessing endoparasitism.

Target type	Assay method	Reference
Protein	Allozyme electrophoresis	Tomiuk et al., 1979 Castañera et al., 1985 Walton et al., 1990
	Immunoassay	Stuart and Greenstone, 1996, 1997
DNA	RAPDs	Black et al., 1992 Roehrdanz et al., 1993 Kazmer et al., 1995
	DNA hybridization	Greenstone and Edwards, 1998
	Microsatellite variability	Vanlerbrghe-Masutti and Chavigny, 1997
	PCR	Campbell et al., 1993 Orrego and Aguidelo-Silva, 1993 Zhu and Greenstone, 1999

The most effective assay using DNA detection is specific PCR, which is able to detect, with great sensitivity, even closely related species in which target sequences may differ by a single basepair (see Zhu and Greenstone, 1999, and references therein).

A CHALLENGING CASE FOR CLASSICAL BIOCONTROL

Aphelinus spp. (Hymenoptera: Aphelinidae) are important biological control agents of aphids worldwide. Four species have been introduced into the United States and some other countries to control several cereal aphids: *Aphelinus albipodus* (Hayat and Fatima), *Aphelinus asychis* (Walker), *Aphelinus varipes* (Foerster), and *Aphelinus hordei* (Kurdjumov). These tiny wasps are extremely difficult to distinguish morphologically. Recently we have shown that, based on their 16s rDNA genes, these species are only about as divergent from one another (0 to 0.38%) as different populations within a typical aphelinid or aphidiine species (Chen *et al.*, 2002). Separating such closely related organisms, especially when several species occur together, is a high challenge (Chen *et al.*, 2002; Pinsloo *et al.*, 2002).

Aphelinus hordei, originally collected from *Diuraphis noxia* (Mordvilko) in Ukraine, was released in the Free State Province of South Africa in 1998 and 1999, and in the United States southern Great Plains in 1999 (Zhu *et al.*, 2000). At the time, *A. asychis* and *A. varipes* were already established in South Africa, and those two species and *A. albipodus* were already established in the United States. The object of the work reported here was to develop a reliable, species-specific assay to distinguish *A. hordei* from all of these congeners and use the assay to verify its establishment in South Africa.

MATERIALS AND METHODS

Rearing of the aphids and parasitoids studied has been described elsewhere for *A. hordei* (Prinsloo *et al.*, 2002) and the other *Aphelinus* spp. (Zhu and Greenstone, 1999). General protocols for DNA extraction, PCR, and gel electrophoresis were presented in Zhu *et al.* (1999). Because of the extremely close sequence similarities of the aphelinids under study, two sets of primers—one for the ITS-2 ribosomal DNA sequence (Zhu and Greenstone, 1999) and the other for the mitochondrial 16s rDNA sequence (Prinsloo *et al.*, 2002)—were required to separate *A. hordei* and *A. varipes* from the other

species. A final restriction digest of the 16s rDNA products, using the endonuclease Hinf1 (Prinsloo *et al.*, 2002), was used to distinguish *A. hordei* and *A. varipes*. Accuracy of the resulting three-step assay was verified by testing *A. hordei* colony material and used to determine whether parasitoids recovered from *D. noxia*-infested fields in 1999 and 2000 in the Free State Province of South Africa and in Lesotho were descendents of the *A. hordei* released in the Free State in 1998 and 1999.

RESULTS AND DISCUSSION

The three-step assay successfully identified the colony material as *A. hordei* and determined that 94 to 100% of the parasitoids recovered from the Free State and Lesotho field sites belonged to that species (Prinsloo *et al.*, 2002). This is the first time that a molecular assay has been used to confirm the establishment of an exotic parasitoid released for biological control.

Because of the importance of aphelinids in aphid biological control, the PCR assays described here should be useful to cereal aphid workers worldwide. Primer pairs are relatively inexpensive and simple to manufacture, and the assays—when properly performed—are reproducible and—when performed in microplate format—are cost-competitive with ELISA (Chen *et al.*, 2000). Nevertheless the need to perform PCR limits it to laboratories that can afford the thermocycler and electrophoresis equipment, and have personnel trained to perform and trouble-shoot the assay. The development of new DNA hybridization formats that are easy to perform and evaluate without the use of thermocyclers, yet have the specificity and sensitivity of PCR (e.g., Taton *et al.*, 2000; Parks *et al.*, 2002), heralds the day when high-powered yet quick-and-dirty assays will be more widely available to entomologists. This will make determination of parasitism rates routine, supporting both research and pest management needs.

ACKNOWLEDGMENTS

I am deeply grateful to Roy Van Driesche and his colleagues for organizing this conference, to Marjorie Hoy for inviting me to participate in her session, to Marshall Johnson and his colleagues for making the local arrangements, and to David Porter and Richard O'Grady for making it possible for me to attend. I also thank the postdoctoral fellows and university and USDA colleagues who assisted me in my research on endoparasitism.

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