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Choice versus no-choice test interpretation and the role of biology and behavior in parasitoid host specificity tests

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ABSTRACT

The need to improve methods and interpretation of host specificity tests for arthropod natural enemies has been clearly identified, yet there remains a paucity of empirical evidence upon which to base recommendations. Factors influencing test outcomes and the mechanisms underlying them must be understood so they can be controlled, and test results can be interpreted correctly. In this study, an established exotic host/parasitoid system was used to assess the outcomes and predictive accuracy of no-choice compared to paired choice tests within small laboratory arenas. Host acceptance by two egg parasitoids, *Enoggera nassaui* and *Neopolycystus insectifurax* (Pteromalidae), was interpreted in light of percent parasitism, offspring sex ratios and observed parasitoid behavior. No-choice tests showed that the four host species, *Paropsis charybdis*, *Dicranosterna semipunctata*, *Trachymela catenata* and *Trachymela sloanei* (Coleoptera: Chrysomelidae) were within the physiological host ranges of both parasitoids. The results of paired choice tests with the first three species supported this interpretation, with two exceptions. *Trachymela catenata* eggs were not accepted by *E. nassaui* and were accepted significantly less often by *N. insectifurax* when compared to no-choice tests. Both test designs predicted that *D. semipunctata* is within the ecological host range of the two parasitoid species, whereas field evidence suggests this is a false positive result. Percent parasitism of all hosts was higher in no-choice compared to choice tests and was predictive of rank order of host preference in choice tests. Presence of the most preferred host did not increase attack on lower ranked hosts. Offspring sex ratios of *E. nassaui* were independent of host preference. In contrast, *N. insectifurax* allocated more females to *P. charybdis* and mostly males to *D. semipunctata* and *T. catenata*. The results support our assertion that both no-choice and choice tests along with detailed behavioral studies should be conducted for correct interpretation of pre-release host specificity tests. This will enable more accurate predictions of parasitoid host ranges and risks parasitoids may pose to non-target organisms in the field.

1. Introduction

Classical biological control is one of the few cost-effective means of sustainable management of exotic pests in productive systems. In the past two decades, awareness of the potential risks posed by introduced biological control agents (BCAs) to non-target organisms has increased and evidence for post release non-target attack by some agents has been presented (Barratt et al., 1997; Duan and Messing, 1999; Follett et al., 1999). Because BCAs are expected to become permanently established in the receiving country it is vital that any risks they may pose are identified and fully assessed before their release. In New Zealand, the introduction of organisms for biological control is governed by legislation that requires extensive pre-release risk assessment (Harrison et al., 2005). Host specificity testing within the confines of quarantine is the most common way to obtain data for this assessment. It is essential to design the most predictive tests possible that can be conducted within the constraints of the laboratory and correctly interpret them for the field. The ultimate aim is to accurately predict field host range from the results of laboratory host specificity tests.

Procedures are fairly well established for predicting herbivore host ranges (Sheppard et al., 2005), but the development of host range testing methods for entomophagous BCAs, especially parasitoids, lags behind (Sands and Van Driesche, 2000). Predicting a parasitoid's host range is intrinsically more difficult because relatedness to the target hosts is less likely to determine whether a non-target organism lies within that range (e.g. Haye et al., 2005). A variety of methods have been used to estimate parasitoid host ranges and assess the possibility of non-target effects (Van Driesche and Murray, 2004), however their relative predictive accuracy have rarely been directly assessed (e.g. Mansfield and

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Mills, 2004; Haye et al., 2005). As a first step towards recommending improved methods Babendreier et al. (2005) reviewed current methods, while van Lenteren et al. (2005) proposed a set of procedures for use in the assessment of agents for inundative biological control. In this study, two common test types, no-choice and choice tests, are assessed to determine if one or the other provides a more accurate estimate of parasitoid host range. Both test types have limitations. No-choice tests are generally believed to overestimate host ranges (Van Driesche and Murray, 2004), which could lead to the rejection of suitable agents. On the other hand, unknown responses to mixed host cues has lead to the concern that choice tests may produce either false negative or false positive results (Vinson, 1976; Gilbert and Morrison, 1997; Van Driesche and Murray, 2004).

A useful way to assess the predictive accuracy of laboratory-based host specificity tests is to study disparities between host ranges predicted in the laboratory and realized ranges of established agents. Since its introduction from Australia in 1987, the egg parasitoid *Enoggera nassaui* Girault (Pteromalidae) has provided effective control of the *Eucalyptus* defoliating beetle *Paropsis charybdis* Stål (Chrysomelidae: Chrysomelinae: Paropsini) in many regions of New Zealand (Bain and Kay, 1989; Kay, 1990). A second Australian egg parasitoid, *Neopolycystus insectifurax* (Girault) (Pteromalidae) was discovered parasitizing *P. charybdis* in 2002, but appears to be less effective as a BCA (Berry, 2003; Jones and Withers, 2003; Murray et al., 2008). Three additional paropsine species, *Trachymela catenata* (Chapuis), *Trachymela sloanei* (Blackburn) and *Dicranosterna semipunctata* (Chapuis) are also established in New Zealand (Anon., 1977; Kay, 1993; Hutcheson, 1998). This assemblage of Australian insects allows us to conduct retrospective host range assessments in order to study the merits of laboratory-based no-choice and choice tests and the value of collecting biological and behavioral data to complement such tests.

2. Materials and methods

2.1. Hosts

Colonies of *P. charybdis*, *T. catenata* and *T. sloanei* were maintained separately in large, ventilated perspex cages (1.0 m tall × 0.7 × 0.7 m) in an environmentally-controlled room (22 ± 2 °C, 65% RH, 14:10 h L:D). Adult beetles were provided fresh new-growth *Eucalyptus nitens* (H. Deane and Maiden) Maiden foliage twice weekly for food. Egg batches laid by *P. charybdis* and *T. catenata* were harvested every 2–3 days by plucking off the leaf-tips to which they were adhered. As *T. sloanei* naturally deposit their eggs under bark or in crevices, artificial oviposition sites were created following the methods of Millar et al. (2000). Stacks of six pieces of 1 mm thick × 20 mm^2 cork were pinned to foam blocks and placed among the *E. nitens* stems. Cork pieces bearing egg batches were harvested every 2–3 days. Eggs were held at 4 ± 1 °C for up to two weeks before being used to maintain parasitoid colonies (*P. charybdis*), or for up to 48 h before use in experiments (all species).

Dicranosterna semipunctata adults were held in a large perspex cage placed beside a laboratory window under ambient conditions because natural light is essential for egg production. Every two days, beetles were supplied fresh new-growth *Acacia melanoxylon* R. Br. foliage, and eggs, which are laid individually rather than in batches like the other three host species, were harvested by plucking off phyllodes to which they were adhered. Eggs were stored at 4 °C until required. For paired choice tests, groups of four individual eggs were glued to *A. melanoxylon* phyllodes with chicken-egg albumen to create artificial 'batches' equal in size to the natural *P. charybdis* and *T. catenata* batches with which they were paired.

2.2. Parasitoids

Enoggera nassaui and *N. insectifurax* were maintained in a temperature-controlled room separate from beetle colonies (22 ± 2 °C, 65% RH, 14:10 h L:D). Solitary female parasitoids were presented with egg batches of colony-reared *P. charybdis* for 24 h. Parasitized batches were transferred to plastic Petri dishes (90 mm diameter) in groups of five, and parasitoids emerged after nine (*E. nassaui*) or 11 (*N. insectifurax*) days. These parasitoids were supplied with undiluted honey on 20 mm^2 squares of paper towel and allowed to feed and mate in the presence of the empty natal host eggs from which they had emerged.

2.3. No-choice tests

Naïve (defined as having had no access to any live host eggs, only the remains of the natal egg, as described above) 3-day-old *E. nassaui* and *N. insectifurax* adults were placed into individual 55 mm Petri dishes with honey and either one single *D. semipunctata* egg or a batch of *P. charybdis*, *T. catenata* or *T. sloanei* eggs. Sample sizes varied due to the differential availability of each host species, and inability to sex live parasitoids (Table 1). All eggs remained attached to the leaf or cork on which they had been laid. Eggs were exposed to *E. nassaui* for 24 h. *Dicranosterna semipunctata* eggs were exposed to *N. insectifurax* for 24 h and *P. charybdis*, *T. catenata* and *T. sloanei* for 48 h. The 48 h duration was used because *N. insectifurax* were slow to initiate parasitism and, in contrast to *E. nassaui*, there was minimal risk that eggs would be probed excessively, which can lead to desiccation and collapse during long periods of exposure. As neither *E. nassaui* nor *N. insectifurax* are sexually dimorphic (Bouček, 1988; Naumann, 1991), parasitoids were frozen after experiments and dissected to verify their sex. Egg batches that had been exposed inadvertently to male parasitoids were discarded. Egg batches exposed to females were separately labeled and incubated (22 °C, 65% RH, 14L:10D). Beetle larvae that hatched were removed to prevent cannibalism of adjacent eggs. The proportion of individual eggs and egg batches parasitized was recorded and parasitoid offspring were dissected to verify their sex.

2.4. Choice tests

Two hours before experiments, 3-day-old *E. nassaui* and *N. insectifurax* females were identified by presenting individual parasitoids with a *P. charybdis* egg batch under a dissecting microscope and observing the ovipositor as the abdomen was raised to commence oviposition. The egg batches were then removed before parasitoids could successfully drill into the host egg and begin oviposition. In this way, female parasitoids were consistently selected with a high degree of motivation to oviposit (see Barton Browne and Withers, 2002).

Paired choice tests were undertaken separately for *E. nassaui* and *N. insectifurax* with each pairing of the three host species *P. charybdis*, *D. semipunctata,* and *T. catenata*. *Trachymela sloanei* was not included in these tests because its eggs are laid in jumbled masses, preventing batches of a known, fixed number of eggs being obtained so that equal numbers of eggs of each host species could be presented in the paired tests. Solitary parasitoids were presented individually with egg batch pairs (n = 29–33 per species, see Table 2) in 55 mm Petri dishes for 1–6 h (22 °C, 65% RH). Eggs were presented on a leaf-tip/phyllode from the beetle's host plant (*E. nitens* or *A. melanoxylon*). Individual eggs were removed from the outside edge of an egg batch with a scalpel to obtain equal-sized batches. Batches of eight eggs were used in choice tests between *P. charybdis* and *T. catenata*. Batches of four eggs were used in all choice tests including *D. semipunctata* as eggs of this species