DETECTION OF A TASMANIAN STRAIN OF THE BIOLOGICAL CONTROL AGENT *ENOGGERA NASSAUI* GIRAULT (HYMENOPTERA: PTEROMALIDAE) USING MITOCHONDRIAL COI

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ABSTRACT

Paropsis charybdis (Coleoptera: Chrysomelidae), an Australian pest of *Eucalyptus* in New Zealand, is subject to biological control by *Enoggera nassaui* (Hymenoptera: Pteromalidae), a solitary egg parasitoid sourced from Western Australia (= Perth Strain) in 1987. Erratic control in inland regions of New Zealand led to the introduction and release in 2000 of two Tasmanian *E. nassaui* strains to attempt expansion of the climatic range of biocontrol. Samples recovered a year later were analysed using partial sequences of the Cytochrome oxidase I (COI) mitochondrial gene to test for establishment. This method detected a haplotype corresponding with a Tasmanian (Florentine Valley) strain. However, as sequences could not be obtained from original Perth strain stocks, some doubt remains as to the genetic source of the other current *E. nassaui* populations in New Zealand. COI proved adept at distinguishing between different parasitoid populations and shows promise for similar studies.
Keywords: *Paropsis charybdis*, *Enoggera nassaui,* Cytochrome oxidase I, biological control.

INTRODUCTION

The *Eucalyptus* tortoise beetle *Paropsis charybdis* Stål is an accidentally established pest of *Eucalyptus* in New Zealand. The solitary egg parasitoid *Enoggera nassaui* Girault was obtained from Western Australia (= Perth strain) and released as a classical biological control agent in 1987 (Kay 1990). Bain & Kay (1989) were concerned that this population may not be climatically tolerant of inland New Zealand conditions, a theory supported by Murphy & Kay (2000), who found that low parasitism rates during spring were associated with high levels of *P. charybdis* defoliation in the central North Island region.

Climatic matching of biocontrol agents is considered important in New Zealand (Cameron et al. 1993) where a range of climatic conditions are encountered. Natural enemies should be sought from the region of origin of a pest to ensure they are phenologically adapted to the host and can survive in a similar climate (Legner & Bellows 1999). It was hypothesised that populations (strains) of Tasmanian *E. nassaui* could potentially provide better control of *P. charybdis* than currently occurs in cooler areas, because of a presumably better climate match and because *P. charybdis* occurs in Tasmania (de Little 1989) but not Western Australia.

A suitable method was required to test for Tasmanian strain establishment. The morphological features of *E. nassaui* were considered insufficient for such a task (Nauman 1989), whereas molecular tools have proven useful in detecting subtle population differences in insects (e.g. Scheffer & Lewis 2001; Mander et al. 2003). Mitochondrial DNA (mtDNA) was considered suitable because it is maternally inherited and does not undergo recombination. The Cytochrome oxidase I (COI) gene was anticipated to provide sufficient genetic variation to discriminate between even closely related *E. nassaui* populations because of its rapid mutation rate (Simon et al.1994).

METHODS

Collection and importation of Tasmanian *Enoggera nassaui* strains

Enoggera nassaui were collected from paropsine eggs in Tasmania during 1999/2000, identified using the key of Naumann (1989), and reared on *P. aegrota* var *Elliottii* Selman eggs. Adults emerging from the same egg batch were assumed siblings (i.e. from the same female wasp). To maintain mtDNA purity, each strain was maintained separately. Live adults and parasitised *P. aegrota* eggs were sent to New Zealand in May 2000, but only two strains, from the Florentine Valley (146°29'E, 42°38'S, ex *P. aegrota*) and Evandale (147°24'E, 41°24'S, ex *P. deboeri*), were established in culture. Rearing occurred at 22°C under 16:8 h light:dark on *P. charybdis* eggs. Identifications were confirmed by the Australian National Insect Collection.

Release protocols

Voucher specimens of both strains were deposited with the New Zealand Arthropod Collection prior to release at four central North Island *Eucalyptus nitens* Maiden (Deane et Maiden) plantations during November 2000 (Table 1). Releases consisted of approximately equal numbers of free adults and parasitised eggs hung in plastic containers from foliage.

TABLE 1: The numbers of Tasmanian *Enoggera nassaui* released for the Florentine Valley and Evandale strains at the four New Zealand release sites. Releases consisted of approximately similar numbers of free adults and parasitised eggs.

Site	Location	Florentine	Evandale	Total
Smythes Rd	Kinleith Forest	1000	100	1100
Poronui	Poronui Station	1000	1000	2000
Cpt. 1060	Kaingaroa Forest	1200	800	2000
Kapenga	Rotorua	300	300	600
Total		3500	2200	5700

Molecular analysis

To detect whether Tasmanian *E. nassaui* had established at the release sites, samples were collected from parasitised *P. charyb*dis egg batches during January 2001. Fifteen samples collected at Poronui and one from Kapenga were compared with one specimen of each Tasmanian strain, a South Island sample (Lyttelton, collected in 1999), and five alcohol-preserved samples of the original Perth strain cultures. No samples could be recovered from Cpt 1060 and the Smythes Rd site had been felled.

Whole individuals were macerated, Proteinase K digested and cleaned using phenol/chloroform extraction (Sambrook et al. 1989). DNA was precipitated in equal volumes of 3M sodium acetate and isopropanol overnight at –20°C. After centrifuging at 12,000 rpm for 25 minutes the pellet was washed in 1 ml 70% ETOH for 5 minutes, air-dried and re-suspended in 100 ml Tris EDTA (pH 8) buffer.

Each 25 µl PCR reaction consisted of 3 µl Roche (10x) Taq polymerase buffer (+ $MgCl_2$), 2.5 µl dNTPs at 4 mM, 1 unit Taq (1 U/µl), 12.5 µl double distilled H_2O, 2.5 µl of the primers C1-J-1751 'Ron' and C1-N-2191 'Nancy' from Simon et al. (1994) at 5 µM, and

1 µl of template. PCR and sequence reactions were run on an Applied Biosystems GeneAmp, PCR System 9700. After denaturation at 94°C for four minutes, samples were subjected to 30 cycles of 94°C/30 seconds, 55°C/30 seconds, 72°C /45 seconds, and a final extension phase of 4 minutes at 72ºC. Two µl of PCR product were visualised on 2% agarose gel against a 1KB Plus ladder. Sequencing reactions (Version 3.0 Big Dye® Terminator mix Applied Biosystems) followed the manufacturer's recommendations and were run on an ABI Prism, 3100 16 capillary-array gene analyser. The approximately 370 bp sequences were aligned and a Neighbour Joining (NJ) tree generated under the Kimura 2-parameter model with 1000 bootstraps in MEGA version 2.1 (Kumar et al. 2001).

RESULTS

PCR products and sequences were obtained for all specimens except the original Perth stocks. The resulting NJ tree (Fig. 1) shows three haplotypes were present. The Lyttelton, Kapenga and 11 Poronui samples had the same haplotype, with three Poronui samples sharing a unique substitution.

The Tasmanian strains differed from each other at three positions and from the majority of other samples by six nucleotides. One Poronui sample had the same haplotype as the Florentine Valley strain.

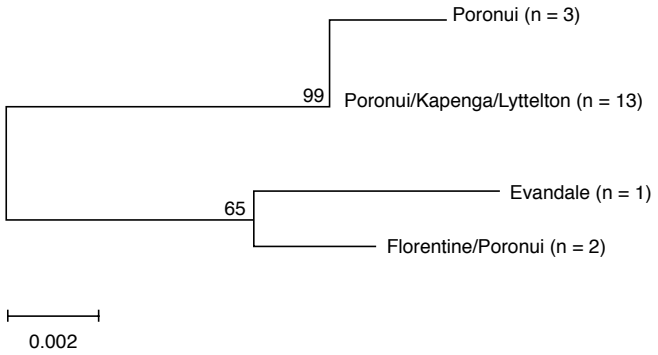

FIGURE 1: Neighbour-joining dendogram of *Enoggera nassaui* sequences using Kimura 2 Parameter. Numbers at branches represent 1000 bootstrap values. Scale = genetic distance. n = sample size.

DISCUSSION

This study was undertaken to determine whether Tasmanian *E. nassaui* strains could improve biocontrol of *P. charybdis* and to evaluate the ability of COI to discriminate between different parasitoid populations. However, two aspects of this study undermined our ability to draw clear conclusions from the results.

Firstly, recovery of *E. nassaui* was reduced by *Baeoanusia albifunicle* Girault (Hymenoptera: Encyrtidae), an obligate hyperparasitoid of *E. nassaui* (Tribe 2000). High hyperparasitism rates and competition from another self introduced egg parasitoid were recorded by Jones & Withers (2003). Because of this situation, there appears to be little future value in monitoring the effect of Tasmanian *E. nassaui* on *P. charybdis* since the parasitoid has been rendered relatively ineffective. This also means that the climate-matching hypothesis cannot be tested.

The second issue was the inability to extract DNA from the original Perth strain samples. Because two parasitoid species have now accidentally established in New Zealand from Australia, we cannot exclude the possibility that *E. nassaui* populations may also have self introduced since 1987, although the material recovered in this study

is likely to be the Perth strain. The similarity of the Lyttelton haplotype (collected before the Tasmanian strains were released) to the North Island samples confirms that the extant material in New Zealand is different from either Tasmanian strain. Although only one sample of each Tasmanian strain was analysed, we believe that the maternal inheritance, lack of recombination and separate rearing of strains maintained any mtDNA differences within a strain, and therefore one sample was a sufficient sample size. The recovery of a sample from Poronui sharing the same haplotype as the Florentine Valley strain suggests that a Tasmanian strain did establish in at least one location in New Zealand. In this aspect, the programme appears to have been successful.

COI proved useful for the task of discriminating between *E. nassaui* populations. In addition to separating the two Tasmanian strains, it also indicated a slight genetic diversity in the population previously established in New Zealand. It therefore seems a useful tool for monitoring the establishment or dispersal of biocontrol agents where different population sources may be used. Further COI analysis of Tasmanian strains collected but not released and the original Perth strain samples would clarify the remaining issues raised in this study.

CONCLUSIONS

Two Tasmanian strains of the egg parasitoid *Enoggera nassaui* were introduced to New Zealand in an attempt to improve biological control of *Paropsis charybdis*. Cytochrome oxidase I sequences were evaluated as a method to distinguish between different populations and to test for establishment. Sufficient variation was detected to discriminate both between Tasmanian strains and between geographic races (Tasmanian and presumably Western Australian). Three haplotypes were detected in New Zealand, one of which appears to be the Tasmanian Florentine Valley strain. However, the detection of an obligate hyperparasitoid of *E. nassaui* reduced the recovery of samples and mitigates any advantage that may have been obtained from the release of Tasmanian strains.

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