Hybridisation between populations of *Enoggera nassau* in New Zealand

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Abstract The eucalyptus tortoise beetle (*Paropsis charybdis*) egg parasitoid, *Enoggera nassau*, is distributed throughout New Zealand. The original population released in New Zealand was from Western Australia and was later augmented with two strains from Tasmania (Florentine Valley and Evandale). Analysis of *E. nassau* in 2001 showed a predominance of the Western Australian strain, with only one Florentine Valley individual being detected. In the present study, collections of *E. nassau* specimens from *P. charybdis* eggs made during spring 2010 were subjected to cytochrome oxidase I (COI) sequence analysis. The majority of individuals from Poronui Station (Taupo) were either the Tasmanian Florentine Valley strain or hybrids between the original Western Australian and Tasmanian strains. It is uncertain whether this outcome will improve the biological control of *Paropsis charybdis* in New Zealand.

Keywords eucalyptus tortoise beetle, *Paropsis charybdis*, *Enoggera nassau*, cold tolerance, biological control, strains, COI.

INTRODUCTION

Enoggera nassau (Girault) (Hymenoptera: Pteromalidae) is a primary egg parasitoid of *Paropsis charybdis* Stål (Coleoptera: Chrysomelidae), the eucalyptus tortoise beetle, that is a serious pest of forest plantings of *Eucalyptus nitens* (Deane & Maiden) Maiden in New Zealand. *Enoggera nassau* was first released into New Zealand in 1987 from Busselton, Western Australia (Bain & Kay 1989; Tribe & Cillié 2000). This strain of *Enoggera nassau* is known to be present throughout New Zealand (Murray et al. 2008b). *Enoggera nassau* is the only parasitoid wasp that attacks the first generation of *P. charybdis* eggs that are laid in spring, while the second generation of *P. charybdis* eggs that are laid in summer are very well controlled between *E. nassau* and the second primary egg

parasitoid, *Neopolycystus insectifurax* Girault (Jones & Withers 2003). Intensive post-release monitoring revealed that biological control of *P. charybdis* would be greatly improved if *E. nassau* could appear earlier in spring to parasitise the first egg batches laid (Murphy & Kay 2000). These authors hypothesised that the *E. nassau* population present in New Zealand, having originated from the hot Western Australia region, was insufficiently adapted to cold winters and was subject to high overwintering mortality, causing its late appearance in spring. So two more strains were introduced from Tasmania (Florentine Valley and Evandale) and released into the North Island in May 2000. It was hypothesised that these strains would be more cold-hardy and improved over-wintering

survival could lead to better control of the first spring generation of *P. charybdis* (Murphy et al. 2004). In 2001 collections were made from Poronui Station, Taupo, to determine whether the Tasmanian *E. nassau* strain had established. The cytochrome oxidase I (COI) mitochondrial gene was used to distinguish between the different *E. nassau* strains (Western Australia; Florentine Valley, Tasmania; and Evandale, Tasmania). Results revealed a dominance of the Western Australian strain; the genotype of the Western Australian strain was based on a sample collected from Lyttelton in 1999 (Murphy et al. 2004). One specimen of the Florentine Valley strain was collected but the Evandale strain was not detected (Murphy et al. 2004). This provided evidence for establishment of the Florentine Valley strain released in May 2000. As the sample size in this study was very small it is unclear whether the Evandale strain did not establish or was just not detected.

The objective in the present study was to determine whether the Florentine Valley strain had died out since 2001, or alternatively, had begun to dominate over the Western Australia strain.

METHODS

Paropsis charybdis egg batches (n=150) were collected from each of Northland, Bay of Plenty and Taupo regions, and three egg batches from Southland, from the foliage of *E. nitens* or *Eucalyptus globulus* Labill. trees during late spring of 2010. These were taken to the laboratory and separated with one leaf portion bearing one egg cluster per plastic Petri dish. These samples were stored in 20°C and 70% RH temperature cabinets. Monitored three times a week, hatching eggs either produced *P. charybdis* larvae or a parasitoid, namely *E. nassau*, *N. insectifurax* or the hyperparasitoid *Baeoanusia albifunicle* Girault (Encyrtidae). Only individual adults of *E. nassau* were collected and stored in labelled Eppendorf tubes and frozen until ready for COI analysis. Only one individual *E. nassau* was analysed from each egg batch, in order to avoid pseudo-replication of the data with excessive

collections of full-siblings. This was important as behavioural observations reveal that female *E. nassau* not only readily parasitise an entire egg batch of their host, but also vigorously defend those eggs against other parasitoids (Murray et al. 2008a).

DNA was extracted from each *E. nassau* using a DNeasy blood and tissue® kit, according to the manufacturer's instructions. The DNA was PCR amplified using the COI primers C1-J-1751 (alias Ron) (5'-GGATCACCTGATATAGCATTC-3') and C1-N-2191 (alias Nancy) (5'-CCC GGTAATTAATAATATAAACTTC-3') (Simon et al. 1994). The PCR reaction mixture, total volume 25 µl, contained: 8.0 – 10.0 µl H₂O, 6.0 µl dNTPs (1.25 mM), 0.5 µl Taq Polymerase or Expand Hifi Polymerase (5 Units/µl) (Roche Applied Science, Mannheim, Germany), 2.5 µl of the supplied 10× reaction buffer (15 mM MgCl₂), 1.0 µl MgCl₂ (25 mM) to adjust the final magnesium concentration to 2.5 mM, 1.0 µl of each COI primer (10 µM) and 3.0 – 5.0 µl of 1/5 diluted template DNA. The PCR reaction mixtures were incubated at 94°C for 2 min, followed by 35 cycles of 94°C for 15 sec, 50°C for 30 sec, 72°C for 45 sec, with a final extension phase of 5 min at 72°C. The resulting PCR products (4 µl) were run on a 1.5% agarose/ 1× TAE gel and inspected for single band products of the expected size. Single band PCR products were then purified using ExoSAP before being sequenced by Macrogen (Seoul, Korea).

To determine the current proportional establishment of the origins of *E. nassau* collected, sequences generated were compared with the sequences of populations of *E. nassau* from Appendix 4 in Murphy (2006).

RESULTS

In total, only 18 *E. nassau*-infected samples were successfully reared, with the biggest sample from Poronui Station, Taupo (Table 1). The COI gene was sequenced from the samples and 12 single-nucleotide polymorphisms (SNPs) were identified. Eight of the SNPs had been previously

Table 1 The origin of *E. nassaui* collected from five sites within New Zealand during 2010 based on analysis of the COI gene region.

Location	N individuals	Sequenced to origin:
Rust, Taneatua, Bay of Plenty	1	1 Western Australia
Longmile, Rotorua	3	3 Western Australia
Kapenga, Rotorua	2	2 Western Australia
Poronui Station, Taupo	11	1 Western Australia 7 Florentine Valley 3 heterozygous hybrids (Florentine Valley × Western Australia)
Morris, Invercargill	1	1 Western Australia

identified by Murphy (2006) and the remaining four SNPs were new to this study.

All of the *E. nassaui* collected from Rust, Longmile, Kapenga and Morris had the same gene sequence as the Western Australia *E. nassaui* identified by Murphy (2006) (Table 1). Conversely, only one of the eleven *E. nassaui* identified from Poronui Station had the same gene sequence as Western Australia *E. nassaui* (Table 1). Seven of the *E. nassaui* from Poronui Station had the same gene sequence as parasitoids from Florentine Valley, and the remaining three *E. nassaui* samples had SNPs belonging to both Western Australia and Florentine Valley, suggesting a hybridisation between the two strains. The four new SNPs identified in this study were all unique to these three putative hybrid samples.

DISCUSSION

These results confirm that the Florentine Valley strain of *E. nassaui* established at the Poronui Station site from a release of approximately 1000 individuals made there in November 2000 (Murphy et al. 2004). The small samples sizes from the other locations prevented a conclusion being drawn about whether this Tasmanian strain has spread to other areas or established at any of the other release sites, as it was not detected elsewhere. The Evandale *E. nassaui* strain was not detected in this study; it is still unclear whether this strain established in New Zealand after it was released.

Since the arrival of the hyperparasitoid of *E. nassaui*, *Baeoanusia albifunicle* in New Zealand in 2002 (Murphy et al. 2004) it has been increasingly difficult to collect *P. charybdis* egg batches that contain viable primary parasites, as there is only a small period of time between the first parasitism of *E. nassaui* and the rapid domination by *B. albifunicle* (on average 29 days later) (Mansfield et al. 2011). Although the presence of the hyperparasitoid reduces the populations of *E. nassaui*, this parasitoid remains an important biological control agent for New Zealand, as eggs attacked by both parasitoid and hyperparasitoid still fail to hatch into *P. charybdis* larvae. The presence of the hyperparasitoid, along with the increasingly common aerial spraying of insecticide onto *E. nitens* plantations and continued forest harvesting, has increased the difficulty in locating *P. charybdis* biological control agents. However, until now *P. charybdis* has held some value as food for pheasants at Poronui Station, so defoliation of the high altitude *E. nitens* plantations on that station had been tolerated. The cutting rights to any *E. nitens* trees that have survived defoliation by *P. charybdis* at Poronui Station were recently sold to a forestry company. This company has started aerial spraying of synthetic pyrethroids to control *P. charybdis* until the trees are harvested in 2012. This will undoubtedly reduce even further the population density of *E. nassaui* as its host becomes locally scarce.

Analysis of SNPs from Poronui Station individuals indicate interpopulation hybridisation of *E. nassau* at this site. The heterozygosity observed in the three individuals suggests mating has occurred between the two strains. It is also possible there may have been mating between all three *E. nassau* strains as one of the heterozygous SNPs (SNP = A/G) could have arisen from an Evandale (SNP = G) mating with either a Western Australian or Florentine Valley (both SNP = A). The influence of a hybrid *E. nassau* on the biological control of the pest *P. charybdis* is unclear. It is unknown whether these hybrids would have an advantage, such as increased cold temperature tolerance. It is also possible these hybrids may confer a disadvantage to biological control. They could inherit the lack of cold tolerance and potentially be more susceptible to the hyperparasitoid, as it is known that *B. albifunicle* heavily attacks *E. nassau* in Tasmania (Murphy 2006). For instance, in laboratory crosses between strains of *M. aethiopoides* that had arisen from different hosts, the hybrids showed reduced efficacy at parasitising one of the hosts, although not the other (Goldson et al. 2003).

The apparent dominance of the Florentine Valley strain at Poronui Station after merely 10 years presence there, as well as hybrids between the Florentine Valley and Western Australia *E. nassau*, could suggest the Florentine Valley strain has some ecological advantage over the Western Australian strain. If so, this might benefit biological control of *P. charybdis* elsewhere in New Zealand. Certainly the Florentine Valley population should be better matched climatically to the central North Island and non-alpine areas of the South Island of New Zealand. This was demonstrated by a CLIMEX Match Sites comparison between Florentine Valley and New Zealand. Ludlow Tuart Forest, Busselton, Western Australia, was climatically similar to Auckland and further north where there are no *E. nitens* plantations (Murphy 2006). The Western Australia *E. nassau* strain is known to have a low temperature development threshold of 6.5°C and reaches

highest parasitism levels of *P. charybdis* eggs in December, which is a mean of 30 days after the first appearance of *P. charybdis* eggs (Mansfield et al. 2011). Conversely, Murphy & Kay (2000) hypothesised that the Tasmanian strain may be better synchronised with the appearance of the first generation of eggs in November in cooler parts of New Zealand. However, the collections made in this study were insufficient to fully test this hypothesis. A visit to Poronui Station on 17 November 2010 revealed only 1% parasitism, although the rate of parasitism rose to 26% on a later visit on 10 December 2010. A much more thorough study would be required at Poronui Station to establish if the Florentine Valley strain and hybrids show any different phenology than the Western Australian strain.

The collection and spread of *E. nassau* from Poronui Station to other sites in New Zealand is not recommended at this stage. It is important to first establish whether the Florentine Valley strain and the hybrids detected at Poronui Station show any increased advantage for biological control, such as a greater oviposition activity at lower temperatures or better over-wintering survival.

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