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Investigating the poor performance of heather beetle, *Lochmaea suturalis* (Thompson) (Coleoptera: Chrysomelidae), as a weed biocontrol agent in New Zealand: Has genetic bottlenecking resulted in small body size and poor winter survival?

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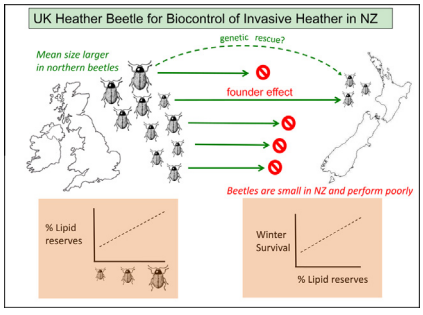
HIGHLIGHTS

- Heather beetle released in New Zealand (NZ) to control heather is underperforming.
- NZ heather beetles are small cf. UK beetles, and are genetically bottlenecked.
- Small NZ beetles have proportionally less lipids and lower winter survival.
- Northern UK beetles are larger and genetically distinct from southern UK beetles.
- Large UK heather beetles could be used to genetically rescue the NZ populations.

GRAPHICAL ABSTRACT



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ABSTRACT

Heather beetle (*Lochmaea suturalis*), has underperformed as a biocontrol agent when compared with the damage it does to native heather in Europe. Mean heather beetle body size, measured by elytron area, was 10% smaller in NZ populations compared with beetles from northern UK where the NZ beetles originated. Previous research in Europe showed that small beetles suffer higher winter mortality. Field-collected heather beetles in NZ show a positive relationship between body size and the proportion of pre-overwintering food reserves (lipids) they contained. Beetles that died in an overwintering experiment had lower proportional lipid reserves, and a smaller mean body size, than surviving beetles. Smaller body size in NZ is probably mostly due to a severe founder effect: line-rearing of beetles in NZ to eliminate a microsporidian disease, and poor establishment success, resulted in NZ beetles being derived from one or two field-collected females from one UK site. Several measures of genetic variability in NZ beetles compared with beetles from the UK indicated severe genetic bottlenecking. In particular, reductions in heterozygosity in NZ versus UK beetles were a close match to theoretical heterozygosity

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after a severe bottleneck. Heather beetle populations from southern UK were genetically distinct from those sampled from northern UK, and previous collecting showed higher microsporidian infestations in beetles from southern UK compared with northern beetles. Mean elytron area was 2.2% smaller in the southern UK population compared with the northern population. Genetic rescue of NZ heather beetles could use beetles from the northern UK that have slightly larger body size and lower levels of microsporidian infection.

1. Introduction

Heather, *Calluna vulgaris* (L.) Hull, was deliberately introduced into Tongariro National Park (TNP) in New Zealand (NZ) in the early 20th century as a precursor to the release of game birds from Europe (Williams and Keys, 1994). By the 1980s it was dominating over 50,000 ha of native tussock grassland and subalpine scrub, and in 1990 the Department of Conservation initiated a biocontrol program (Williams and Keys, 1994). The obvious choice of agent was the heather beetle, *Lochmaea suturalis* Thompson, (Coleoptera: Chrysomelidae), which is a pest of valued native heather in Europe (Brunsting, 1982; Cameron et al., 1944; Pakeman et al., 2002).

Host range testing confirmed that the heather beetle was sufficiently host-specific for release in NZ (Syrett et al., 2000). However, pre-release screening revealed a microsporidian gut parasite in the beetles (Wigley, 1997). The microsporidian was eliminated by quarantine-rearing of iso-female lines, with lines being terminated if offspring tested positive for the disease (Fowler et al., 2008; Smith et al., 1998). From 1995 to 1999, 17 releases totaling 5700 beetles were made, with most comprising mixtures of the iso-female lines to restore genetic variability (Fowler et al., 2008). Establishment was only achieved at one site, and this was from only two iso-female lines (Fowler et al., 2008). By 2001 the beetles had killed 0.03 ha of heather at this site, and the program appeared highly promising and environmentally safe, with field surveys confirming the beetles' host specificity (Paynter et al., 2004). However, in 2003 this beetle population collapsed despite plentiful live heather at the periphery of the site, and the beetle now appears to be locally extinct (Peterson et al., 2011).

Before the population collapse, beetles were collected from this site and used to make 43 releases (totaling over 10,000 beetles) in and around TNP (Peterson et al., 2007). Once again, establishment success was extremely poor with just four of these releases producing field populations (Peterson et al., 2011). Three releases were also made at lower elevation sites near Rotorua, and all of these established, with two showing rapid population growth and achieving high levels of damage in just 2 years (Peterson et al., 2007).

However, the main invasion of heather in NZ is in TNP, and at the best performing site in TNP it has taken 9 years to achieve heather death over a 17 ha area (Peterson et al., 2011). This is slow compared with heather beetle outbreaks in the UK that can build up and die out all within 2–3 years (Pakeman et al., 2002). Damage from heather beetle outbreaks in the UK typically comprises patches that can be small (0.25 ha) to large (1000 ha) but can extend over huge areas: the extensive outbreaks in northern England and southern Scotland in 1997–2001 severely damaged about 350,000 ha of heather (Pakeman et al., 2002). So why has heather beetle performed comparatively poorly as a biocontrol agent in TNP, New Zealand? Predators and parasites can often reduce the effectiveness of introduced biocontrol agents (Goeden and Louda, 1976) but Peterson et al. (2004) show that natural enemies are not responsible for the poor performance of heather beetle in NZ.

Heather beetle outbreaks in Europe have been linked to unusual weather such as warm wet springs (Cameron et al., 1944; Rosenburgh and Marrs, 2010), and one Danish study showed that heather beetles with smaller than average body size showed poor survival if winter temperatures were warm or fluctuating (Jensen and Nielsen, 1985). In Europe, beetle body size, and perhaps other factors affecting population growth such as fecundity, may also be linked to increased plant organic nitrogen levels caused by increases in air pollution (Brunsting and Heil, 1985; Power et al., 1998; Rosenburgh and Marrs, 2010). Nutritional and climate issues relevant to heather beetle performance in NZ are being investigated and will be reported elsewhere. This study tests (i) whether NZ heather beetles have smaller body size than their native counterparts; (ii) if low proportional lipid reserves, and hence low winter survival, could be the cause of poor performance of this biocontrol agent in NZ; (iii) whether the smaller body size could have been caused by founder effects as a result of iso-female line-rearing and poor initial establishment rates.

The findings are then used to assess whether importing additional heather beetles into NZ could genetically rescue the NZ heather beetle populations, and improve the performance of this biocontrol agent.

2. Methods

2.1. Field collections, morphometrics and lipid extraction

In NZ (southern hemisphere seasons), a total of 544 heather beetles were collected for measurement in spring 2005, autumn 2006, spring 2007, spring 2008 and spring 2010 from the two sites (TNP and Rotorua). In the UK (northern hemisphere seasons), a total of 954 heather beetles were collected for measurement in spring 2000, spring and autumn 2005 and spring 2010 at 25 sites including Oakworth, the site of origin of the NZ population (Fowler et al., 2008; Peterson et al., 2007). A sample size of at least 20 beetles per site per date was the target but at some sites in some years this was not possible because of beetle rarity, and collections were somewhat sporadic over the 5–10 years as dictated by field activity. Beetles were either stored in ethanol or frozen. An elytron was removed from each beetle and its area measured using Image-Pro software (Peterson et al., 2007). Elytron area was used as a measure of body size because it is independent of adult nutritive or reproductive state.

On 13 March 2006 (southern hemisphere autumn), 20 overwintering heather beetles were collected from each of the TNP and Rotorua sites. Beetles were stored at −80 °C before lipid extraction using the method described by Bligh and Dyer (1959). Briefly, this comprised thawing at room temperature for 10 min, oven-drying at 60 °C for 24 h, weighing, and then adding diethyl ether to individual beetles contained in 1.5-ml microcentrifuge tubes. For each of three rinses the diethyl ether containing dissolved lipids was removed using a Pasteur pipette. Beetles were dried for 48 h in a fume cupboard then oven-dried and weighed.

2.2. Overwintering experiment

Offspring from beetles collected from Rotorua and TNP in September 2005 (southern hemisphere spring) were reared at 20 °C 16:8 h (light:dark) in feeding cages consisting of