Habitat requirements, translocation and management of the critically endangered Cromwell chafer beetle *Prodontria lewisii* Broun

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Emma Rachel Curtin
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Abstract

Translocation is an important tool for the conservation of endangered species with threatened habitats and low population numbers. Without high habitat quality, translocations have low chances of success, regardless of how many organisms are released or how well they are prepared for the release. It is therefore crucial to be able to identify sites in which translocations are most likely to be successful based on key environmental characteristics specific to the species and habitat in question. Species information is also needed to determine critical life history traits and minimum habitat fragment sizes. The Cromwell chafer beetle *Prodontria lewisii* Broun is an ideal candidate for translocation because it has a very limited habitat range, being entirely confined to the 81 ha Cromwell Chafer Beetle Nature Reserve (CCBNR) in Cromwell, Central Otago. The entire population is estimated to contain about 3,000 individuals. This study aimed to identify key plant and soil sites for optimum larval and adult survival by using a combination of field and laboratory-based studies. Larvae survived significantly better on the cushion plant *Raoulia* and on the grass *Festuca rubra* than on silver tussock *Poa cita*, despite this being the plant with which they are traditionally associated. Plant and soil surveys were conducted both within the existing reserve and in a potential new site at the Lindis Crossing. Soil pH, density and particle size were measured, but were not significantly related to chafer beetle survival. However, both larvae and adults survived significantly better when raised in soil from the CCBNR sites than from the experimental Lindis translocation site. Survival varied within the different soil sites of the beetles' current range, with survival increasing significantly from south to north within the reserve. Results are discussed in the context of their management implications and a set of recommendations are presented. The approach taken here presents a model that could be applied to help identify suitable habitat for the translocation of other invertebrate species.

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Chapter One: General Introduction

The vast majority of life on earth consists of invertebrates. Yet, while there is a wealth of information regarding the conservation and translocation of vertebrates, the invertebrates remain woefully neglected. Most invertebrates can survive without vertebrates, but vertebrates cannot survive without invertebrates. Vertebrates are dependent on invertebrates for direct sources of food, pollination of food crops and cycling of nutrients. Many vertebrate herbivores eat plants whose survival depends on pollination by invertebrates. Some vertebrates rely on these herbivores for their own existence. The cycling of nutrients critical to all food chains is dependent on the breakdown of plant and animal matter by invertebrates. It seems that in trying to preserve the pyramid of life, conservationists have focused almost exclusively on preserving the very tip, giving no regard to the base upon which that tip must rest in order to survive. Nature built the pyramid of life by focusing the majority of her efforts on the invertebrates. There is a desperate need for conservationists to follow suit.

Insects make up 80 percent of all living animal species currently in existence (Samways, 1993). With almost 10,000,000 species, they are the most speciose class in the animal kingdom (Mora, *et al*., 2011). They are the most diverse group of organisms in the history of life (Grimaldi & Engel, 2005). Yet only 7 – 10% of all insect species are estimated to have been scientifically described (Samways, 1993). Out of 9522 animal species listed as 'Threatened' (that is, 'Critically Endangered', 'Endangered' and 'Vulnerable') in 2011 by the IUCN, just 741 were insects. This equates to 7.78% of all species listed as threatened. Just 37 insect species are listed as endangered or threatened under the Environmental Protection Agency in the United States, out of a total of 1179 species (Redak, 2000). If we assume that the same proportion of invertebrates is threatened as for vertebrates, that figure should be almost 30,000 in North America alone (Dunn, 2005). Even if insect taxa are shown to be less at risk of extinction than other taxa, this discrepancy remains enormous. Dunn (2005) speculated that insects may be more at risk of extinctions than other taxa, because they occupy smaller habitats spatially and tend to be more specialised to those smaller habitats than is typical for the larger vertebrates (Dunn, 2005). Head for head, insect populations also have a smaller geographic range, due to the fact they are able to fit more individuals into a smaller area than can most vertebrates. Clearly the

insects are a group that is extremely important yet tragically threatened and woefully understudied.

Conservation biology is the application of science to conservation problems with the aim of addressing the biology of species, communities and ecosystems that are perturbed, either directly or indirectly, by human activities or other agents (adapted from Soulé, 1985). Its goal is to provide principles and tools for preserving biological diversity (Griffith *et al*., 1989). Translocation is an integral part of conservation biology and is becoming a more widely used and valued conservation tool in New Zealand and around the world. Translocation is defined as the intentional release of animals to the wild in an attempt to establish, re-establish, or augment a population (IUCN, in Griffith *et al*., 1989). Translocation then, by definition, can be seen as a vital and integral tool in the implementation of conservation biology to preserve a given species.

From 1973 to 1986, almost 700 translocations were performed each year in New Zealand, Australia, the United States of America, Hawaii and Canada alone (Griffith, *et al*., 1989). That number has increased since. Of those ~700 translocations, 90% were of native game species. Only 46% of those translocations involving threatened, endangered or sensitive species were successful (Griffith, *et al*., 1989). Sherley (*et al*., 2010) summarised all known native bat, reptile, amphibian and terrestrial invertebrate translocations undertaken in New Zealand. They found that of all known translocations (>905 translocations), only 41 involved arthropods, despite the fact that there are at least 1000 threatened invertebrate species in New Zealand (McGuinness, 2001). This figure seems grossly disproportionate when compared with the >723 translocations undertaken involving birds, even though there are just 170 bird species in New Zealand. This means that there is an average of ~10.5 translocations for each of the 69 'Threatened' bird species in New Zealand (IUCN, 2011). Conversely, the number of translocations per invertebrate species known to be threatened is 0.08 translocations per species. For all invertebrate species suspected to be under threat, the same figure is just 0.04 translocations per species. Clearly the area of invertebrate translocations is severely lacking and drastically underrepresented.

New Zealand has around 80,000 invertebrate species, of which an estimated 20,000 are beetles (McGuinness, 2001). In comparison, there are 2,000 endemic vascular plants (Dopson *et al*., 2000; Mark, 1985) and around 350 terrestrial vertebrate species (Watt,

1976). McGuinness (2001) lists around 500 invertebrate species in New Zealand which are *known* to require conservation research – just 0.6% of the total number of invertebrate species estimated to exist in New Zealand. The same author lists a further ~500 invertebrate species that are believed to be of conservation concern, but about which so little is known that they have not yet been allocated a conservation status. Of the 1,000 or so invertebrate species listed as threatened in McGuinness (2001), just 31 species have protection under the Wildlife Act (1953).

Griffith (*et al*., 1989) considered that a translocation could be deemed a success if it resulted in a self-sustaining population. A self-sustaining population would be one in which the population was able to find and/or provide all the resources that it required to maintain or increase its numbers over time. This would inevitably include the production of offspring. Thus one way of measuring the success of a translocation would be to measure the number of offspring surviving. Since the young of many species often have different habitat requirements from their parents (Rueda, *et al*., 2008; Simonov, 2009), measuring offspring growth and survival will involve identifying environmental factors that are specific to survival of the young of a species. Measuring juvenile growth and survival will therefore be particularly important for species which undergo any form of metamorphosis during their lifecycle (e.g.: amphibians, insects, and many other invertebrates), as these species will have highly stage-dependent requirements. For example, for insects with subterranean larval such as the Cromwell chafer beetle (*Prodontria lewisii* Broun), understanding the influence of different soil and host plant species on larval survival, coupled with complementary knowledge for the adults, would make ex situ conservation more likely to succeed, as ideal habitat matches for both adults and larvae can be found.

The Cromwell chafer beetle is listed as critically endangered by the IUCN Red List of Threatened Species. It was formerly distributed over about 500 ha of the Cromwell Basin, Central Otago, but much of this habitat was destroyed with the erection of the Clyde dam, construction of which began in 1979, and the subsequent flooding of the Clyde River and adjacent sand dunes. The flooding lead to the formation of Lake Dunstan, which covers much of the beetle's former habitat range (Watt, 1979). Watt (1979) reports that the densest population of the beetle formerly occurred within the Cromwell township, on a block of untouched land. When the land was to be levelled for housing, two conscientious

locals, Mrs R. Olds in 1976 and Miss S. Connelly in 1985, laid pitfall traps on the block and transferred the captured beetles onto the proposed Cromwell Chafer Beetle Nature Reserve (CCBNR). To date, this is the only known translocation of the Cromwell chafer beetle.

Due to the extremely restricted range and cryptic, nocturnal nature of the Cromwell chafer beetle, little research has been done on the species and there is a lack of knowledge in keys areas such as larval feeding preferences and development. To this date, pupae of the Cromwell chafer beetle have never been found (Ferreira & McKinlay, 1999). Watt (1979) was the first person to undertake a scientific study of the species and record that it is entirely restricted to the sand dunes of the Cromwell basin, of which only those comprising the CCBNR remain suitable for chafer beetle survival. Having a small, localised range is a characteristic that in part defines the genus *Prodontria* (Barratt, 2007). It is possible that at least one other species in the genus has become extinct due to habitat loss (Barratt, 2007) and it is therefore fortunate that the Cromwell chafer beetle has a reserve dedicated to the preservation of the species and of its habitat.

The activity patterns and population characteristics of the Cromwell chafer beetle have been described by Ferreira & McKinlay (1999). Using pitfall traps over six years, they found that in some years, adult male beetles emerged earlier in the season and remained active for longer than female beetles, although not significantly so. On average, males and females had the same number of 'active' nights. The activity patterns they observed were positively related to temperature and to humidity, although these relationships only explained 27% of the observed variation in activity levels. Significantly, the authors found that the activity patterns of the Cromwell chafer beetle differed from those of the striped chafer *Odontria striata*, which demonstrated a peak in activity during the productive summer months. *O. striata* is a pasture pest which occurs commonly throughout Otago, Canterbury and Southland (Barratt, *et al*., 1988). Unlike the Cromwell chafer, *O. striata* can fly. Barratt, *et al*. (1982) concluded that *O. striata* had a more flexible life cycle than that of *Costelytra zealandica*, or grass grub, a wide spread and commercially important pasture pest in New Zealand. The life cycle of the Cromwell chafer beetle appears to be longer than that documented for *O. striata* and *C. zealandica*, with larvae taking over a year to develop (Ferreira & McKinlay, 1999). This slow development, coupled with an inability to fly, a small population size and a highly

restricted and specialised range, make the Cromwell chafer beetle an ideal candidate for intensive management and translocation.

Species with restricted ranges and small population sizes, such as the Cromwell chafer beetle, are at a greater risk of extinction. Without high habitat quality, translocations have low chances of success, regardless of how many organisms are released or how well they are prepared for the release (Griffith *et al.*, 1989). It is therefore crucial to be able to identify sites where translocations are most likely to be successful. Suitable habitat can be found only by combining both species and ecosystem approaches. Ecological information is necessary to determine aspects such as species interactions and factors determining habitat quality; species information is needed to determine critical life history traits and minimum habitat fragment size (Griffith *et al.*, 1989). In the case of the Cromwell chafer beetle, larval and adult plant food requirements must be determined, along with the ideal soil site. Cromwell chafer beetles spend a high percentage of their life cycle underground, with larvae potentially being entirely subterranean for up to two years and adults only emerging for a few hours a night during the spring and summer months from August to March to feed and mate when weather conditions are suitable (Watt, 1979).

The aim of this study was to investigate the habitat requirements and translocation potential of the Cromwell chafer beetle. Habitat variations within the beetles' current range and at a potential new site were examined, along with the tolerance levels of the adults and larvae to different soil and plant types. In this study, four translocation sites were identified based on current and historical data regarding the location of subpopulations of chafer beetles at the CCBNR. A fifth experimental translocation site was identified at the Lindis Crossing. This site was chosen as the nearest geologically similar site with a similar plant community composition to the CCBNR. In order to determine the ideal food plant and soil site combinations for optimal chafer beetle growth and survival, adults were placed in enclosures at the five translocation sites. Surviving adults and larvae were then excavated from these soil sites as a measure of survival and breeding success at each site. In order to determine larval soil and feeding preferences, larvae were raised in one of the five different soil sites with one of three different plant species as a food source. Growth and survival were measured over time. A comprehensive soil and plant analysis was then undertaken at the CCBNR and the Lindis

Crossing and the results used to explain the variances seen in the growth and survival data from the translocation attempts.

A Note On Chapter Organisation

Each of the three main chapters in this thesis has been written as an individual scientific paper. Because of this, some overlap in material does occur between chapters. Each chapter focuses on a specific aspect of the habitat requirements and translocation of the Cromwell chafer beetle. The chapters are presented in a logical order, such as they might occur when planning a translocation. Chapter 2 focuses on habitat analysis, something which must precede all translocations; Chapter 3 addresses the translocation and breeding of adult chafer beetles; Chapter 4 deals with the rearing and habitat requirements of the chafer beetle larvae. The General Discussion combines the results from all three chapters and discusses them in terms of their management implications and applicability to other invertebrate translocations.

References

Barratt, B.I.P. (1982). Biology of the striped chafer, *Odontria striata* (Coleoptera: Scarabaeidae) II. Larval development. *New Zealand Journal of Zoology*, 9, 267-277.

Barratt, B.I.P. (2007). Conservation status of *Prodontria* (Coleoptera: Scarabaeidae) species in New Zealand. *Journal of Insect Conservation*, 11, 19-27.

Barratt, B. I. P., Ferguson, C. M., Jones P. A., Lauren, D. R. (1988). Control of striped chafer, *Odontria striata* White (Coleoptera: Scarabaeidae), in pasture using lindane. *New Zealand Journal of Agricultural Research*, 31, 205-210.

Dunn, R. R. (2005). Modern Insect Extinctions, the Neglected Majority. *Conservation Biology*, 19, 1030-1036.

Ferreira, S.M., McKinlay, B. (1999). Conservation monitoring of the Cromwell chafer beetle (*Prodontria lewisii*) between 1986 and 1997. *Science for Conservation, 123*.

Griffith, B., Scott, J.M., Carpenter, J.W., Reed, C. (1989). Translocation as a species conservation tool: Status and strategy. *American Association for the Advancement of Science*, 245, 477-480.

McGuinness, C.A. (2001). The Conservation Requirements of New Zealand's Nationally Threatened Invertebrates. *Threatened Species Occassional Publications No.20, Department of Conservation*.

Mora, C., Tittensor, D.P., Adl, S., Simpson, A.G.B., Worm, B. (2011). How many species are the on earth and in the ocean? *PLoS Biology*, 9, 1-8.

Redak, R.A. (2000). Arthropods and multispecies habitat conservation plans: are we missing something? *Environmental Management*, 265, 97-107.

Samways, M. J. (1993). Insect in biodiversity conservation: some perspectives and directives. *Biodiversity and Conservation*, 2, 258-282.

Sherley, G., Stringer, I., Parrish, G. R. (2010). Summary of Native Bat, Reptile, Amphibian and Terrestrial Invertebrate Translocations in New Zealand. *Science for Conservation 303*, Department of Conservation, Wellington.

Watt, J.C. (1979). Conservation of the Cromwell chafer *Prodontria lewisii* (Coleoptera: Scarabaeidae). *New Zealand Journal of Ecology*, 2, 22-29.

Chapter Two: Analysis of the Current and Potential Habitat of the Cromwell Chafer Beetle

Abstract

Determination of habitat suitability is the first crucial step in the translocation process. The aim of this chapter was to analyse two habitat variables known to be crucial to the survival of the critically endangered Cromwell chafer beetle (*Prodontria lewisii* Broun) – plant type and soil. The analyses took place at four sites within the chafer beetles current habitat range and at one experimental translocation site. Plants were surveyed in seventy-five 1 m x 1 m quadrats, fifteen at each of the five sites. The total number of plant species and the total number of plants per species was recorded. Samples of each plant species found were collected, preserved and later identified to species level where possible. Plant ground cover estimates were made from photographs taken of each quadrat. Non-native plants dominated the plant survey, with *Rumex acetosella* being the most common plant species across all five sites. Plant cover did not vary significantly between sites. Eight soil core samples were collected per site: four sampling the top 0-10 cm of soil and four sampling soil 10-20 cm below the surface. Particle size distribution, pH, and soil density were determined for each site. There was no significant difference in soil particle size, soil pH, or soil density at 0 – 10 cm, but there was a significant difference in density at 11 – 20 cm. There was a significant difference in overall soil density (0 – 10 cm and 11 – 20 cm combined) across all five sites. The results are discussed in the context of their management implications and what they mean to the success of any potential future translocations.

Introduction

The flightless Cromwell chafer beetle, *Prodontria lewisii* Broun, is a Category 'A' threatened species, with an historic distribution of no more than 500 ha of rare South Island inland sand dunes (Ferreira & McKinlay, 1999; Watt, 1979). It is now restricted to an 81 ha reserve in Cromwell, Central Otago (Ferreira, *et al.*, 1999; Hamilton, 1999). The genus comprises 14 species, all of which are restricted to the lower South Island, specifically Otago and Southland (Ferreira & McKinlay, 1999). Adult Cromwell chafer beetles are nocturnal and emerge to feed at night during the spring and summer months from August to March. They are known to eat a variety of plants, including the cushion plant *Roaulia australis*, sheep's sorrel *Rumex acetosella*, speedwell *Veronica arvensis*, and various lichens. In contrast, almost nothing is known about what plant species the larvae eat (Barratt, *et al.*, 2006; Ferreira & McKinlay, 1999), though historically it has been assumed that the larvae are associated with the roots of silver tussock, *Poa cita* (Watt, 1979). This assumption appears to have been based on this being the dominant native grass in the area at the time (Barratt, *et al.*, 2006). Knowledge of the plant host requirements of the larvae is crucial to conservation efforts of this critically endangered species (Barratt, *et al.*, 2006; Ferreira & McKinlay, 1999) and essential if translocations are to become part of the management strategy.

Translocation is defined as the intentional release of animals to the wild in an attempt to establish, re-establish, or augment a population (IUCN, 1987). Without high habitat quality, translocations have low chances of success, regardless of how many organisms are released or how well they are prepared for the release (Griffith *et al.*, 1989). It is therefore crucial to be able to identify sites where translocations are most likely to be successful. Suitable habitat can be found only by combining both species and ecosystem approaches. Ecological information is necessary to determine aspects such as species interactions and factors determining habitat quality; species information is needed to determine critical life history traits and minimum habitat fragment size (Griffith *et al.*, 1989).

Cromwell chafer beetles spend a high percentage of their life cycle underground, with larvae potentially being entirely subterranean for up to two years and adults only

emerging for a few hours a night to feed and mate when weather conditions are suitable during spring and summer (Ferreira & McKinlay, 1999; Watt, 1979). This, coupled with them being entirely restricted to inland sand dune systems, suggests that soil composition is likely to be an important determinant of suitable habitat. Different soils may well determine the species of plants that are able to grow on inland sand dune systems, and hence the potential food sources available to the beetles. Soil parameters may also influence the behaviour of the beetles. For example, larvae may have to burrow deeper underground away from the sun and wind to prevent desiccation in soils that do not hold moisture well. Some soil sites may be easier to dig through than others, thus aiding or restricting chafer beetle larvae access to a variety of different food sources, as well as restricting adult access to day time burrowing sites and potentially exposing them to stressors such as increased heat and wind, as well as predation. Soil particle size and composition may therefore be limiting factors in chafer beetle survival and distribution, and they are the defining factors in all of the above potentially crucial environmental variables.

This study aimed to investigate the ecological factors necessary for effective translocation of the critically endangered Cromwell chafer beetle, with emphasis on plant type and soil site.

Methods

Plant survey

Plant surveys were undertaken at four sites within the Cromwell Chafer Beetle Nature Reserve (CCBNR), and one at the Lindis Crossing site. The sites surveyed were the same as those used for translocation of adult Cromwell chafer beetles and excavation of larvae (see Chapter 3). These sites were chosen using distribution data for Cromwell chafer beetle (personal communication Anderson, 2008; Hunt, 2007) and apparent potential suitability. The Cromwell Middle (CM) site was selected because the beetles have always been there in substantial numbers, both historically and today; Cromwell Bannockburn (CB) was selected because the beetles were found there historically, but are no longer found there; Cromwell Worm farm (CW) has a self-introduced beetle population today,

but this population has not been recorded in previous beetle population surveys of the CCBNR and is therefore estimated to have established around 2008; Cromwell Roadside (CR) has never been known to have had a beetle population, past or present. The Lindis site (L) was selected because it was the only other inland sand dune deemed potentially suitable for beetle translocation. The beetles have never been found at the Lindis site (Ferreira, *et al*., 1999). All five sites are on loess (wind-blown), shallow loamy sand dunes deposited by the Clutha River (McKinlay, 1997).

Fifteen 1 x 1 m quadrats were sampled per site, giving 75 quadrats in total. The plant survey took place over a week-long period in early May, 2009. Quadrats were placed next to each of the 15 translocation enclosures at each site. The enclosures were randomly distributed within each site (see Chapter 3). To simplify processing, the quadrat was divided with string into 16 equal squares.

The total number of plant species and the numbers of plants per species within each quadrat were recorded. Where it was not possible to differentiate between one individual plant and another (such as for certain types of lichen), that species was recorded as being present only. Very small and numerous plants (e.g. *Cerastium* sp.) were also recorded as present but were not counted individually. Samples of each plant species encountered were taken, stored, and later identified to species level were possible.

Plant species were ranked first by the number of sites out of 5 at which they were present, then by the number of quadrats out of 75 in which that species was recorded, then by the total frequency within all quadrats. Species for which only a presence or absence was recorded were eliminated from ranking analysis.

Simpson's and Shannon-Wiener indices values were calculated for all species at each site (excluding those for which only presence or absence were recorded).

Plant ground cover estimates

Photographs were taken to enable estimation of percentage ground cover. A camera was positioned directly above the centre of each quadrat and the top and bottom edges of the quadrat frame were lined up with the edges of the camera view to ensure continuity between photos. Four close-up photos of each quadrat quarter were also taken. Whole quadrat photos were identified using a labelled piece of paper in the top right hand corner of each photo: CM, CB, CR, CW or L, followed by a number 1-15 for each site. Labels for each close-up photo of each quarter-quadrat were marked with an A, B, C or D. Ground cover estimates were made using the four quarter-quadrat photos for each enclosure site. A proportion of the total ground cover of each quarter was estimated. For example, a ground cover estimate of 75% was recorded as 0.75. The four proportions were then added to give a total for each entire quadrat. This value was then converted to percentage total cover by multiplying by 100.

Soil particle size

Approximately 30 g of soil from each soil sample (two samples taken from a randomly selected location within each of the five sites) was placed in a 400-600 mL beaker. Beakers of this size were large enough to ensure no overflow occurred during the following hydrogen peroxide reaction.

A solution of 35 % hydrogen peroxide was added to each beaker to just cover the sample. This was done to dissolve all organic matter within the samples. The beakers were placed on a hot plate at 30 °C and covered with paper towels until all signs of reaction had ceased. During the reaction, the samples were stirred twice every half hour for the first hour, then once every day until cessation of fizzing. Further hydrogen peroxide was then added to each beaker and the samples stirred using a clean metal spatula. The reaction was deemed to have finished when no fizzing occurred even when additional hydrogen peroxide was added. This took upwards of 24 h in most cases, depending on the amount of organic matter in the sample. One sample took over two weeks to finish reacting.

Once the reaction was complete, each sample was covered with deionised water, divided equally into six centrifuge tubes and centrifuged at 2000 x g min^{-1} for six minutes. The clear liquid on top of each tube was decanted into a beaker and discarded. Great care was taken not to tip any cloudy water off the sample, as this may have contained silt necessary for complete particle size analysis. Additional deionised water was added to each centrifuge tube and the samples were centrifuged again. This process was repeated a minimum of three times per sample until the liquid on top of each tube was completely clear. This process removed any remnant hydrogen peroxide from the samples.

Once clean, all six centrifuge tubes containing one sample were emptied into a clean 400-600 mL beaker. The sides of each tube were washed thoroughly with deionised water and the liquid added to the beaker to ensure no sample remained behind. The beakers were placed in a 50 °C oven for two days or until completely dry.

Dried samples were tipped into a stack of sieves with a collection pan underneath and placed in an industry-standard "Endecott sieve shaker" for 30 min. The mesh sizes of the sieves were 4 mm, 2.8 mm, 2.0 mm, 1.4 mm, 1.0 mm, 710 µ, 500 µ, 355 µ, 250 µ, 180 µ, 125 µ, 90 µ, and 63 µ. Everything in the collection pan was combined to form a < 63 µ category.

Once sieving was complete, all of the material remaining in each sieve was transferred to a zip lock plastic bag, marked with the sample site and particle size, and weighed (after taring for the weight of the plastic bag).

Once all of the size categories for each sample had been weighed, the percentage weight distribution across the particle size categories was calculated. Particle size distributions were assessed using Gradistat version 4.0. Samples were categorised into gravel, sand or mud based on the universally accepted definitions in Folk (1968). Gravel is defined as having a particle size of great than 2 mm. Sand is divided into the categories of very course sand: 1 – 2 mm; course sand: 500 µ - 1 mm; medium sand 250 µ - 500 µ; fine sand: 125 µ - 250 µ; and very fine sand: 62.5 µ - 125 µ. For the purposes of this study, mud is not divided into categories but instead is considered to be anything smaller than 63 µ.

Soil bulk density

Eight soil cores were collected per site: four sampling the top 0-10 cm of soil and four sampling soil 10-20 cm below the surface. This provided data over the full range of depths to which both larvae and adult chafer beetles are known to burrow to (Barratt, *et al.*, 2007). The volume of the soil corers was 89.7 mL. The eight samples from each site were taken from within the area in which the adult beetle translocation enclosures were placed (see Chapter 3).

Before coring, moss and other plant material was scraped off the surface of the soil to be sampled, leaving a flat surface exposed. The soil corer was placed on this flat surface, with a block of wood on top of it, and pressed into the ground. The soil around the sides of the corer was carefully dug away. A wide, flat cheese slicer was then slid carefully underneath the corer, keeping it flush with the end, and the corer lifted out. This ensured that the soil sample exactly filled the corer and that no soil fell out the bottom. Each full corer was then laid gently on its side on a piece of tin foil, wrapped tightly, and placed in a labelled zip lock plastic bag. This process was repeated for 10-20 cm samples, using the same hole as was dug for the 0-10 cm samples. A ruler was used to determine the depth at which each sample was taken. An additional small zip lock bag (approx 200 g) was filled with soil from each site for soil pH tests and particle size analysis. Core samples were dried and weighed and their bulk density calculated using the volume of the corer.

Soil density was analysed using SAS general linear modelling tests. All particle size definitions were based on those given by Folk (1968).

Soil pH

Three 10.0 g samples from each of the five sites were placed into clean glass beakers. All samples had 25 mL of deionised water added to them and the samples were then mixed thoroughly using a clean metal spatula. A pH meter was then inserted into a beaker and the pH recorded. The pH meter probe and the metal spatula were rinsed thoroughly in deionised water samples to avoid contaminating subsequent samples.

Results

Plants

Rumex acetosella was the dominant plant across the entire study area (Table 2.1), being present at all five sites and recorded in all but four of the 75 quadrats. *R. acetosella* was approximately five times more abundant than the next most abundant species, *Agrostis capillaries*. Although *A. capillaries* was the second most abundant species, it is ranked 11th out of 26 species because it was found at just one site. Likewise, the ranking position of *Echium vulgare* is not representative of its overall importance, because although it was found at all five sites, only 30 individual plants were recorded in total. *R. acetosella*, and *Anthoxanthum odoratum* were found in 71 of the 75 quadrats. Only *R. acetosella*, *A. odoratum*, *Hypochaeris radicata*, *Festuca rubra*, and *Echium vulgare* were found at all five sites. In contrast, there was a total of 16 species which were found at only one site. Of these, 12 were found only at the Lindis site. Just five species recorded in Table 1 are native (*Raoulia australis*, *Carex breviculmis*, *Celmisia gracilenta*, *Poa cita* and *Luzula celata*) and only one of these, *R. pumilum*, was found in any great numbers. Of these, *L. celata* is the only species known to be in decline (de Lange, 1999).

A one-way ANOVA demonstrated that plant ground cover did not vary significantly between soil sites ($P = 0.2166$) (Figure 2.1). Bartlett's test for equal variances showed that there was no significant difference in ground cover variation between any of the soil sites ($P = 0.1355$).

Table 2.1: Plant species ranked according to the number of sites at which they occurred, the number of quadrats in which they were recorded, and their frequency within all quadrats. Plants marked with an asterisk were found at the Lindis site only.

Rank	Scientific name	Common name	No.sites/5	No.quadrats/75	Total no.
1	*Rumex acetosella*	Sheep's sorrel	5	71	5109
2	*Anthoxanthum odoratum*	Sweet vernal	5	71	1114
3	*Hypochaeris radicata*	Cat's ear	5	39	842
4	*Festuca rubra*	Red fescue	5	36	458
5	*Echium vulgare*	Viper's bugloss	5	13	30
6	*Hypericum perforatum*	St John's wort	4	39	598
7	*Raoulia australis*	Vegetable sheep	4	14	42
8	*Carex breviculmis*	Grassland sedge	3	17	85
9	*Hypochaeris sp.*	None	3	14	37
10	*Hieracium pilosella*	Mouse-ear hawkweed	3	9	472
11	*Agrostis capillaris**	Browntop	1	15	1890
12	*Vulpia bromoides*	Vulpia hair grass	1	12	12
13	*Aphanes arvensis**	Parsley piert	1	3	102
14	*Veronica arvensis**	Speedwell	1	2	170
15	*Eschscholzia californica*	Californian poppy	1	2	3
16	*Polygonum arenastrum**	Small-leaved wire weed	1	1	25
17	*Sedum acre**	Stone crop	1	1	13
18	*Acaena agnipila*	Sheep's bur	1	1	11
19	Unidentified puffball*	Puffball	1	1	5
20	Unidentified grass A*	None	1	1	4
21	*Celmisia gracilenta**	Common mountain daisy	1	1	2
22	*Poa cita*	Silver tussock	1	1	1
23	Unidentified grass B*	None	1	1	1
24	Unidentified grass C*	None	1	1	1
25	*Verbascum thapsus**	Great or Common mullein	1	1	1
26	*Luzula celata**	Dwarf woodrush	1	1	1

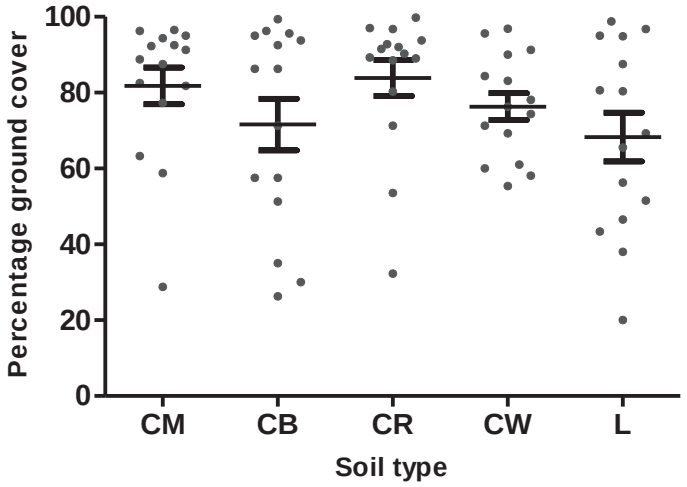
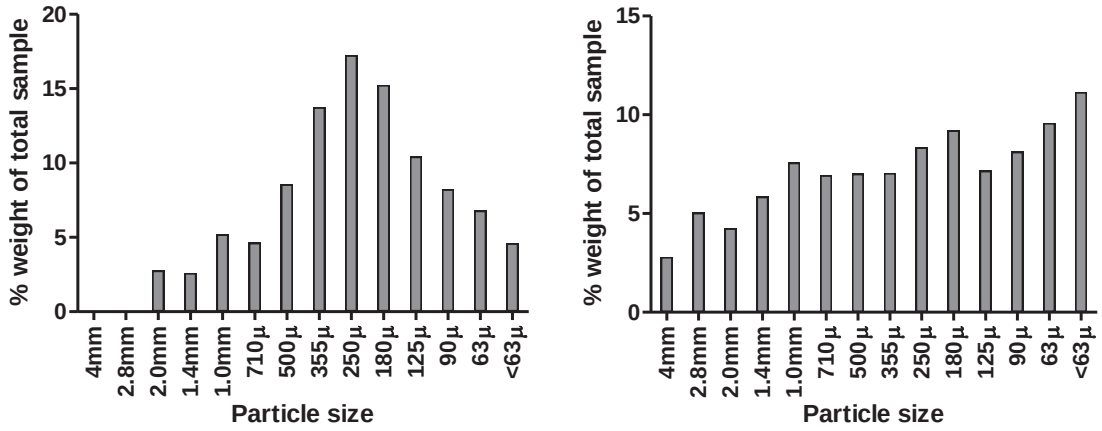


Figure 2.1: Percentage ground cover within 15 quadrats at each soil site, showing mean and +/- SE of the mean (Cromwell Middle, Cromwell Bannockburn, Cromwell Roadside, Cromwell Wormfarm, and Lindis).

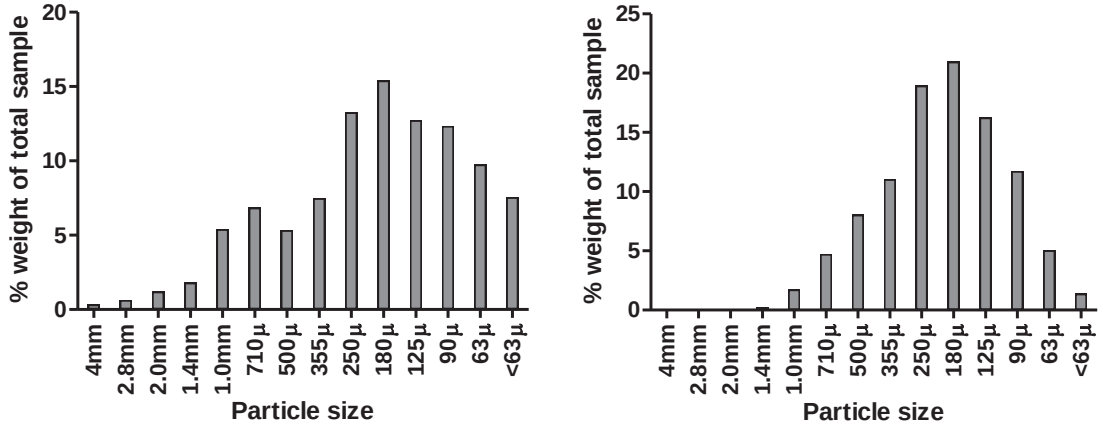
Soil

Site Cromwell Middle (CM) 1 produced a unimodal spread of particle sizes (Figure 2.2). The dominant particle size was 250 μ. The particle size distribution and particle size variation of both samples means that they fall within the universally accepted definition of 'sand'. The particle size which made up the greatest percentage weight of sample CM 1 is 250 μ, placing it into the subcategory of 'medium-fine sand'. The dominant particle size for sample CM 2 is <63 μ. This grouping includes 'very fine sand' and 'mud'. Mud includes the groups 'course silt', 'medium silt', 'fine silt', 'very fine silt', and 'clay'. Gradistat analysis describes the composition of sample CM 1 as 92.7% sand, 4.6% mud, and 2.8% gravel. The Gradistat composition of sample CM 2 was 76.8% sand, 12.1% gravel and 11.1% mud. The percentage weight distribution graph for site CM 2 (Figure 2.3) is slightly unusual in appearance, but still has a unimodal spread and displays an expected and acceptable particle size distribution for sand (J. Palmer, personal communication).



Figures 2.2 and 2.3: Percentage weight distributions of different particle sizes from sites Cromwell Middle 1 (left) and Cromwell Middle 2 (right).

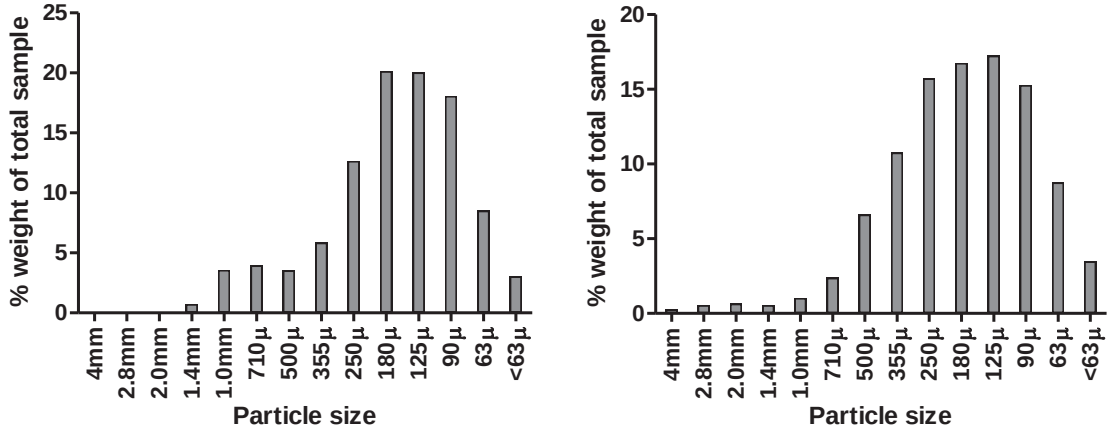
Both Cromwell Bannockburn (CB) sites 1 and 2, exhibited unimodal spreads (Figures 2.4 and 2.5). Sample CB 2 was slightly better sorted than CB 1, being more centrally gathered around a narrower particle size range. However, the dominant particle size for both samples was 180 µ, or 'fine sand'. Gradistat sample composition analysis described sample CB 1 as having 90.3% sand, 7.5% mud and 2.2% gravel. Gradistat analysis of CB 2 revealed the sample was composed of 98.6% sand, 1.4% mud and 0.0% gravel.



Figures 2.4 and 2.5: Percentage weight distributions of different particle sizes from sites Cromwell Bannockburn 1 (left) and Cromwell Bannockburn 2 (right).

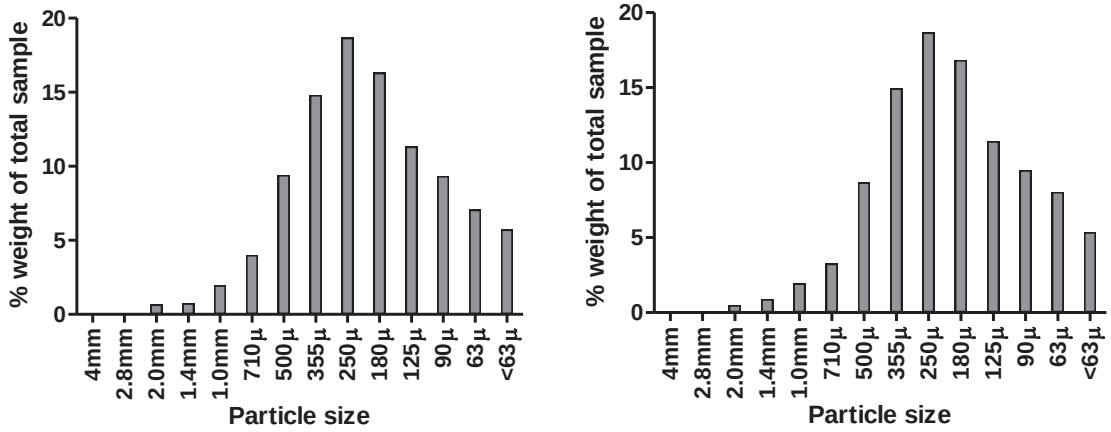
Both samples from site Cromwell Roadside (CR) were unimodal (Figures 2.6 and 2.7). Sample CR 1 comes under the definition of 'fine sand', with the dominant particle size being 180-125 µ. Sample CR 2, with a dominant particle size of 125 µ, falls on the border

between ‘fine sand’ and ‘very fine sand’. Gradistat analysis described sample CR 1 as being composed of 97% sand, 3% mud and 0% gravel, and sample CR 2 as being composed of 95.1% sand, 3.5% mud and 1.4% gravel.



Figures 2.6 and 2.7: Percentage weight distributions of different particle sizes from sites Cromwell Roadside 1 (left) and Cromwell Roadside 2 (right).

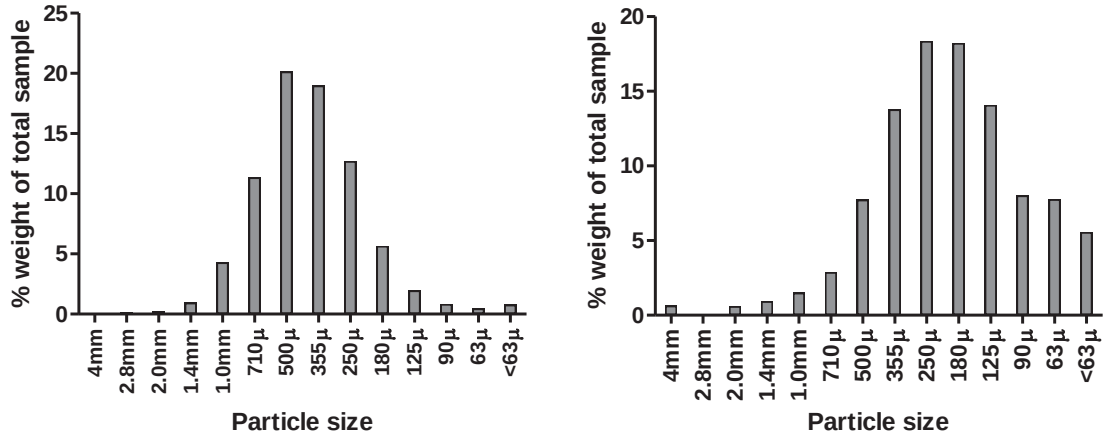
The two samples for site Cromwell Wormfarm (CW) were almost identical in terms of their particle size percentage weight compositions (Figures 2.8 and 2.9). Both were unimodal, and both shared the dominant particle size of 250 µ, placing them in the ‘medium-fine sand’ subcategory. Both samples were almost identical in their Gradistat analyses. Sample CW 1 was comprised of 93.6% sand, 5.7% mud and 0.7% gravel; sample CW 2 is comprised of 94.2% sand, 5.3% mud and 0.5% gravel.



Figures 2.8 and 2.9: Percentage weight distributions of different particle sizes from sites Cromwell Wormfarm 1 (left) and Cromwell Wormfarm 2 (right).

Both Lindis (L) samples are unimodal in their distribution (Figures 2.10 and 2.11). Sample Lindis 1 was slightly more skewed to the courser end of the particle size scale than the other samples in this study, though this difference is not significant. Gradistat analysis showed that while sample Lindis 1 had a normal composition break-down (98.6% sand, 1% mud and 0.5% gravel), a much higher proportion of the sand in the sample was composed of course sand: 40.2% as compared to an average of 11.6% course sand across the other nine samples. For comparison, the next highest percentage composition of course sand was sample CM 2, with 13.9% course sand. The dominant particle size of sample Lindis 1 falls into the 'coarse-medium sand' category.

The dominant particle size of 250 μ in the Lindis 2 sample places it in the 'medium-fine sand' category. Gradistat analysis showed sample Lindis 2 to be composed of 93.2% sand, 5.6% mud and 1.2% gravel.



Figures 2.10 and 2.11: Percentage weight distributions of different particle sizes from sites Lindis 1 (left) and Lindis 2 (right).

There was no major difference in particle size between any of the ten sites. All were unimodal and the means were centred on similar particle sizes. The most common dominant particle size was 250 μ, which prevailed in four out of the eight samples.

Very little difference existed between the average soil densities at 0-10 cm (Figure 2.12) for any of the five soil sites (one-way ANOVA: $P = 0.1343$, $F_{1,4} = 3.342$).

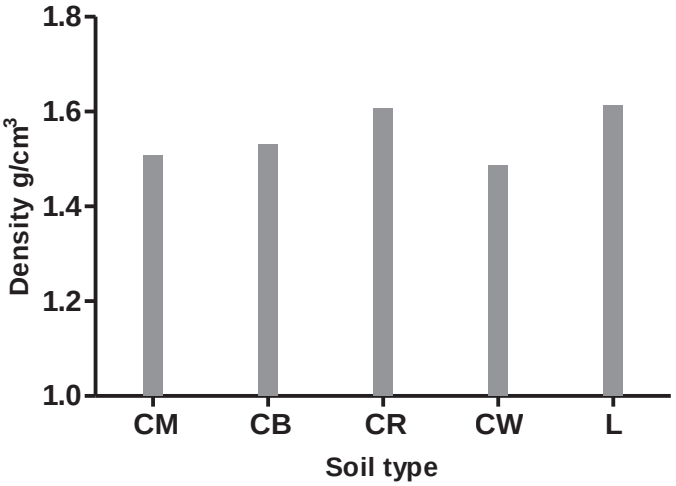


Figure 2.12: Average soil bulk densities across all five soil sites (Cromwell Middle, Cromwell Bannockburn, Cromwell Roadside, Cromwell Wormfarm, and Lindis) at 0-10 cm.

The densities of the soil at all five sites increased with increase in depth (Figure 2.13), with the Lindis site being significantly denser than the four CCBNR sites ($P = 0.0443$, $F_{1,4} = 3.872$).

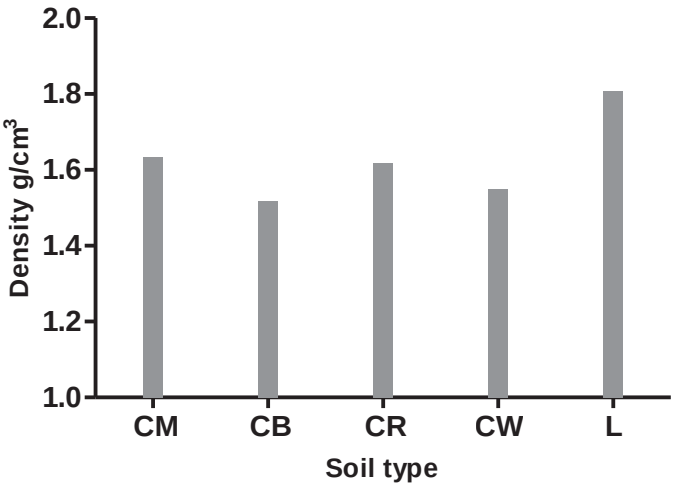


Figure 2.13: Average soil densities across all five soil sites (Cromwell Middle, Cromwell Bannockburn, Cromwell Roadside, Cromwell Wormfarm, and Lindis) at 11-20 cm.

Figure 2.14 shows the average soil density from each of the five soil sites, calculated by combining the raw data of both 0-10 cm and 11-20 cm density values. There was an almost imperceptible difference in soil density between the CCBNR sites. Site CW was slightly, though not significantly, denser than the other CCBNR sites. A one-way

ANOVA detected no significant difference between the means of the CCBNR samples (P = 0.998). A second one-way ANOVA showed that the mean soil density of the Lindis site varied significantly from those of the CCBNR sites (P = 0.021).

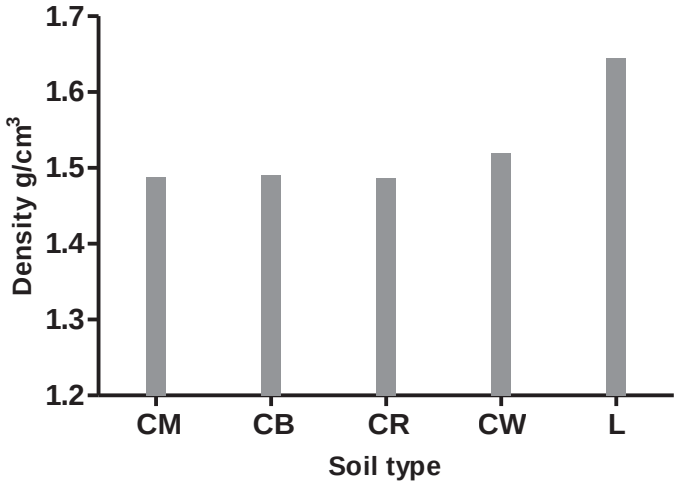


Figure 2.14: Average soil density of all samples from each soil site (Cromwell Middle, Cromwell Bannockburn, Cromwell Roadside, Cromwell Wormfarm, and Lindis).

A SAS general linear model showed that soil density varied across both site and strata, but with no significant site*strata interaction (Model $F_{9,28}$ = 3.10, P = 0.0104; Site $F_{4,28}$ = 4.23, P = 0.0084; Strata $F_{1,28}$ = 6.84, P = 0.0142). Soil samples taken from the 10-20 cm depth range were always denser than those taken from the 0-10 cm depth range, irrespective of which sites the samples were taken from. The Lindis site differed significantly from those of the CCBNR (LSD critical value of t = 2.048, df = 28, Lindis differed from all other sites by ≤ 0.05.).

Table 2.2: The pH levels of three different samples from each of the five soil sites, Cromwell Middle (CM), Cromwell Bannockburn (CB), Cromwell Roadside (CR), Cromwell Wormfarm (CW) and Lindis (L).

	CM	CB	CR	CW	L
Sample 1	6.64	5.90	6.15	6.30	6.20
Sample 2	6.66	5.97	6.19	6.30	6.07
Sample 3	6.20	5.98	6.01	6.17	5.97
Average	**6.50**	**5.95**	**6.12**	**6.26**	**6.08**

Unpaired t-tests showed that site CB had a significantly lower pH level than sites CM (P = 0.023, t = 3.614, df = 4) and CW (P = 0.004, t = 6.120 df = 4). There was no significant

difference between any of the other pairs of sites. The two sites with the lowest pH levels – CB and L – are also the two with the lowest adult survival, though these survival differences were not significantly lower than the other sites. However, sites CB and L did have significantly lower numbers of excavated larvae than the other three sites (see Chapter 3 for adult and larvae survival data). All pH levels observed (Table 2) are within the normal and expected range for sandy soil sites in the Central Otago area (A. Palmer, personal communication).

Discussion

Plant survey

Rumex acetosella and *Anthoxanthum odoratum* were strongly associated with all five sites. This is interesting because *R. acetosella* has not previously been associated with Cromwell chafer beetles. Although little is known about what larvae or adults eat, previous research papers have presumed that both may be associated with native and introduced grasses. *R. acetosella*, however, is neither native nor a grass. *A. odoratum* is a grass but is not native and is not nearly as abundant in this survey as *R. acetosella*. While these two plants appear to be strongly associated with sites where chafer beetles do well, they are equally strongly associated with sites where the beetles do not do well. Some further research into whether chafer beetles can and do actually consume these plant species would help to resolve the question of whether or not they are able to be used as potential indicators of appropriate habitat.

Although *Agrostis capillaris* was the second most abundant species, it ranked just 11th out of 26 species because it was found at just one site. Thus this plant's abundance cannot explain the variation in beetle survival numbers seen across the five sites. Furthermore, the only site at which *A. capillaris* was found was the Lindis site – the site at which the beetles exhibited the poorest survival rates. It may be that this plant is negatively associated with chafer beetles, although further study into whether it is able to be consumed by the beetles is needed to confirm this. The presence of *A. capillaris* may be an indicator of soil type rather than of a potential food source for chafers. Given that the Cromwell Chafer Beetle Nature Reserve (CCBNR) and the Lindis site are geographically

close, it is surprising that this plant was recorded at the Lindis site only. *A. capillaris* is a nationally common species found in a variety of habitats. The fact that it appears not to occur at the CCBNR may be indicative of a soil characteristic which the plant finds undesirable. Given that chafer beetles do significantly better at the CCBNR than at the Lindis site (see Chapters 3 and 4), this same soil characteristic may be something that is beneficial to the beetles. Identifying this characteristic, assuming it exists, may provide a valuable indicator for suitable chafer habitat.

St John's wort, *Hypericum perforatum*, was the fifth most prolific plant found throughout the plant survey. This plant has not previously been reported to be associated with chafer beetles. However, the author has observed and filmed several adult Cromwell chafer beetles consuming the leaves of this plant in what appeared to be large quantities (videos available upon request). St John's wort was not found at site CB. This may be due to the fact that this site had a significantly lower pH level than two other sites and the lowest pH level overall. Cultivation of St John's wort in a variety of different soil pH levels would confirm this theory. If a low pH is indicative of poor growing conditions for St John's wort, it would also provide an indicator of the suitability of the site for chafer beetles, in that by placing them there they would be deprived of at least one known food source. Similarly, if St John's wort is proven to have a similar range of pH tolerances as chafer beetles, the presence or absence of the plant could be used as a quick visual assessment of whether a particular site may or may not be worth investigating for the purposes of establishing a chafer population there. Likewise if a potential site has been identified, planting St John's wort and measuring its success over time could provide a cheap and risk-free test for whether that site is likely to be suitable for chafers. Such tests should be undertaken prior to any actual beetle translocations, thus minimising the associated risks of translocation to the chafers themselves.

It is interesting that the plant historically thought to be associated with chafer beetles, *Poa cita* or silver tussock, occurred just once throughout the entire plant survey. Clearly this plant is not required for chafer beetle survival. Previous planting of *Poa cita* at the CCBNR by the Department of Conservation appears to have been in vain, as this plant is neither prolific nor essential. This finding is supported by the results in Chapter 3 which show that larval survival rates when fed only silver tussock were significantly worse than when fed other plant species.

The vast majority of plant species listed in Table 2.1 are non-natives. Non-natives dominate the CCBNR. It would appear from these results that Cromwell chafer beetles eat a much wider range of plant species than was previously thought.

Ground cover estimates

Ground cover estimates do not appear to explain the observed variation in survival numbers between sites. The site at which both larvae and adult survival was worst - site L – had the greatest spread of percentage ground cover estimates. Site CW, while the best for survival, had the smallest range of percentage ground cover estimates and the third lowest average percentage growth. However, sites CM and CR also had good survival rates, but the spread of percentage growth rates for these two sites was much closer to those observed for sites L and CB than to CW. Sites L and CB both exhibited poor survival rates. Percentage ground cover estimates appear to be a poor predictor of site suitability.

Ferreira & McKinlay (1999) found that the amount of bare ground present at various quadrats and within chafer beetle population areas (equivalents of CB, CM and CW) provided a very poor explanation for the observed variances in beetle pitfall trap catch numbers between sites and quadrats.

Some ground cover estimates recorded bare patches of soil. This may not necessarily affect the beetles, since they can move to other areas within each site. More likely, it would only affect them if there were vast tracks of bare sand which would be difficult for them to cross. Small bare patches can easily be skirted just like a rock would be. Likewise, eggs are not going to be laid there and larvae are unlikely to stray too far into areas with no roots.

Soil particle size

The lack of any significant difference in the distributions of particle size between any of the five sites indicates that differences in adult and larval survival in soil sampled from these sites (see survival data in Chapters 3 and 4) are unlikely to be due to particle size distribution per se. It would appear that the soil particle sizes measured in this study have no significant impact on adult or larval chafer beetle survival.

However, while there was no significant difference found between samples from the Lindis sites and those from other sites, it is probable that if further soil textural tests were carried out, a difference would be detected because the Lindis soil felt coarser in hand than samples from CCBNR. It was also much more difficult to dig through when burying enclosures and excavating larvae, to the extent that continued excavation by hand caused many skin abrasions, something not experienced whilst working on the CCBNR, even for prolonged periods and across four times as many sites. When examined under a microscope, Lindis soil granules appeared to be less polished and rounded overall than soil granules from the CCBNR sites. Lindis granules also appeared to have more sharp angles than CCBNR granules. When rolled between the fingers, Lindis soil granules were less easily rolled than those from the CCBNR sites. When moved around in a petri dish during examination under the microscope, Lindis granules made scratchy, abrasive noises on the surface of the dish, something that the CCBNR granules did not do.

This apparent roughness may have been a significant factor in the ease with which adults and larvae were able to burrow for food and shelter and thus may have contributed to the poor survival rates seen at the Lindis site (see Chapters 3 and 4). Larvae and adults may find it easier to move through sand with a finer, smoother texture. If it is easier for the beetles to move around, they will have greater access to more food sources, possibly allowing them to feed on more than one food type. Results of feeding experiments show that certain plant types are much better for larvae survival than others (see Chapter 4). It would seem that larvae may benefit from being able to access specific beneficial food plant sources. An investigation into how easily or otherwise larvae are able to move through different particle size spectrums and through different textures of sand may prove beneficial to understanding their soil habitat requirements. Sand granules that are rough in

texture may also be more abrasive. Abrasiveness could be a barrier to survival, particularly for the larvae, which have soft exoskeletons.

Adults may also be affected by the ease with which they can move through different textures of sand. While adult beetles have an advantage over larvae in that they are able to come above ground to feed and can travel greater distances on foot than larvae can underground, adults must still burrow into the sand during the day. If it is easier for adults to burrow into softer sand, for example, they are going to be better covered during the day and thus more likely to be able to avoid stressors such as increased heat, wind, and predation.

Soil density

Another factor which may influence the ease with which chafer beetle larvae and adults can move through soil is its density. In this study, soil from the Lindis site was found to be significantly denser overall than those soils from the CCBNR sites. All soil sites became denser with increasing depth from 0-10 cm to 10-20 cm. The Lindis site is also the site at which larvae survival was the worst (see Chapter 4). Adult survival at the Lindis site was the second-worst behind site CR, but this difference was not significant (see Chapter 3). Soil density may influence the numbers of surviving adults and larvae both by restricting their ability to move through the soil and by being more energetically costly to do so. This study did not investigate the mobility of chafer beetle larvae and there is very little in the literature regarding the underground mobility of other beetle larvae species.

A rare example of the underground movements of an invertebrate species being studied comes from Castello & Lazzari (2004), who documented the subterranean host-seeking behaviour of parasitic larval robber flies, *Mallophora ruficauda*. This species has very specific host requirements and the larvae are thus highly mobile in order to meet this requirement. In the wild, larvae of the species search for their host underground. The host is the scarab beetle *Cyclocephala signaticollis* Burmeister, which is also highly mobile. This implies that larvae must often travel quite some distance in order to locate a host beetle, although no actual travelling distances have been recorded in the field. Since it is

highly possible that Cromwell chafer beetle larvae eat a variety of plant species (see Chapter 4), it would seem reasonable to assume that they too need to move a considerable distances underground, perhaps towards specific plant targets. Further experimentation to determine how far the larvae are able to travel and whether they are able to target specific plant species could be conducted by placing larvae in plain sand at a known distance from a host plant, or variety of host plants, and determining the directness and time taken for them to reach a plant. Plant preferences could also be determined from this experiment. Once this had been undertaken, the experiment could be repeated using a preferred plant type, if found, with varying soil densities. If larvae are found to be significantly slower in reaching a host plant, particularly if they had previously shown a preference for that host plant species, then soil density could be said to negatively impact on larvae mobility. This would add further support to the findings of this study, ergo that the site with the highest density had the lowest chafer beetle larvae survival rates (Chapter 4).

pH

Site CB is located at the southern-most point of the CCBNR. It is bordered on two sides by pine tree plantations. Pine needles are known to increase the acidity of soil (Lodhi & Killingbeck, 1980). Site CB had a significantly lower pH than sites CM and CW and the lowest pH level overall. Given that the pine trees are immediately behind site CB with regards to the prevailing southerly wind, it is entirely likely that pine needles are blown onto this site. In 1998, site CB was recorded as having a healthy-sized subpopulation of beetles, with around 52 recorded on one night search (Hunt, 2007). Aerial photographs of the CCBNR taken in 1998 show that the pine plantation to the east of site CB was newly planted and still in a very juvenile state, whilst the pine plantation to the west of site CB was not yet in existence. Examination of aerial photos from 2006/07 shows the east and west pine plantations to have both grown considerably and now resembling near fully grown plantations. Subsequent night surveys in 2006 and 2007 failed to find any beetles at site CB. It may be that the presence of the pine trees after 1998 is the reason for the significant drop in pH seen at this site. This drop in pH may also have been a contributing factor to the low survival rates of beetles seen at this site, although it is unlikely to have been the sole contributor.

Scientific soil tests have three components – chemical, compositional and textural. This study investigated the chemical component in the form on pH tests and the textural component in the form of particle size analysis. For compositional analysis, it was necessary to turn to geological maps to determine the underlying geology and origin of the soil region. The five soil sites measured in this study are from two distinct sand dune regions, the Cromwell Chafer Beetle Nature Reserve and the Lindis Crossing. Both contain alluvial sands originating from the Lowburn and Deadman's Point terrace faces, which were originally deposited by the Clutha River (McKinlay, 1997). The two areas appear to have very similar underlying soil compositions, being primarily comprised of tills, tills referring to soil comprising of many different particle sizes which was deposited by glacial activity (Turnbull, 2000). There is very little compositional difference in the surrounding hillsides

Abiotic factors such as soil pH can have a significant impact on the physiology of soil invertebrates (de Boer, *et al.*, 2010). A low pH level was shown to be very important to the nest presence of leafcutter ants, *Atta sexdens* (van Gils, *et al.*, 2010). Interestingly, Cornelisse & Hafernik (2009) found that pH had a significant effect on the choice of oviposition site by females of a locally common tiger beetle species *Cicindela oregona*, but not on a locally threatened tiger beetle, *C. Hirticollis*. They found that *C. oregona* had a preference for oviposition sites with a mildly acidic pH of 5.5. Since there are very few studies into the effects of soil pH on insect oviposition preferences (Cornelisse & Hafernik, 2009), there is little information as to why these tiger beetles should prefer soil with a high pH. Soils with higher pH levels have lower nutrient levels (Cornelisse & Hafernik, 2009) and it may therefore seem counter-intuitive for insects to prefer such soils when selecting oviposition sites. However, the authors postulated that acidic soils may offer eggs and larvae a reprieve from harmful fungi and bacteria. It may be worthwhile to study the growth of fungi and bacteria known to be harmful to different insect species under different pH conditions.

Sastrodihardjo & van Straalem (1993) investigated the responses of five different isopod species, *Oniscus asellus*, *Trichoniscus pusillus*, *Porcellio scaber*, *Philoscia muscorum* and *Armadillidium vulgare* to varying pH levels. They found that while all of the species had a broad tolerance range to pH levels ranging from 2 to 5 or 7 depending on the species. Of the five, only *A. vulgare* showed a distinct preference for alkaline soils. It

would seem that the pH tolerance spectrums and pH preferences of invertebrates can and do vary widely.

van Straalen & Verhoef (1997) developed a bioindicator system for soil acidification based on the pH preferences of a variety of 20 different soil invertebrates, including species of springtails, mites and woodlice. There was a broad range of pH tolerance levels, and so the bioindicator system was based on the median preferences of each species tested. Species with high or low pH preferences were classed as acidophilous or alkalophilous accordingly. The 'arthropod acidity index' allows the median preferred pH of an arthrodpod community to be estimated from these indicator values. Such a scale can be used to help measure soil acidification in response to changes in land use, for example.

The above authors were able to use species as pH change indicators, but only after rigorous testing to determine the very different pH preferences of the species' in question. It is therefore necessary to recommend that pH tolerance levels of invertebrates not be generalised and no extrapolations should be made. Because pH tolerance ranges and preferences vary so widely, any investigation into the effect of pH must be carried out on a species-by-species basis. pH levels alone are unlikely to be good indicators of whether a potential habitat is likely to be suitable for a given invertebrate species or not.

Conclusion and recommendations

There was a clear difference in the survival of chafer beetles, particularly larvae, between the five tested soil sites (see Chapters 3 and 4). Yet there was no significant difference in soil particle size or soil pH. It would seem from the results of this study that a soil variable other than these two is responsible for this discrepancy. The Lindis soil was found to be significantly denser than soil from the CCBNR sites. Further investigation into the effects of varying densities on chafer beetle adult and larvae mobility may shed light on the differing survival rates seen in this study (see Chapters 3 and 4). It is also the feeling of the author that soil textural components may have an effect of survival. The Lindis soil felt coarser and more abrasive and appeared under a microscope to be of a rougher texture than the CCBNR soils. Perhaps an investigation into the number of planes per sand granule would provide a measurement of roughness.

It would seem sensible to remove the pine trees close to the CCBNR and establish a larger buffer zone than the one that currently exist the trees and the boundary fence along the SW border of the CCBNR to prevent pine needles blowing onto the reserve and acidifying the soil. The current distance between the trees and the Reserve is about 3 m. The Reserve needs to be beyond the reach of windfall pine tree branches and of most wind-blown pine needles. In order to establish what distance this might be, typical movements of wind-blown pine needles would need to be assessed. As the trees growing closest to the SW border are sheltered from the prevailing wind by those tress growing behind (southern) to them, this distance may not need to be great.

Some further research into whether chafer beetles can and do consume the plant species that are most abundant in the plant survey would help to resolve the question of whether or not said plants are able to be used as indicators of potentially suitable habitat.

References

Barratt, B.I.P., Barton, D., Ferguson, C.M. (2006). *Prodontria lewisii* Broun *(Coleoptera: Scarabaeidae): Population monitoring; larval feeding preferences; predation by earwigs.* Report for the Department of Conservation.

Barratt, B.I.P., Barton, D., Ferguson, C.M. (2007). *Prodontria lewisii* Broun *(Coleoptera: Scarabaeidae): population monitoring; larval feeding preferences; rearing protocol*. Report for the Department of Conservation.

Barratt, B.I.P. (2007). Conservation status of *Prodontria* (Coleoptera: Scarabaeidae) species in New Zealand. *Journal of Insect Conservation*, 11, 19-27.

Castelo, M.K., Lazzari, C.R. (2004). Host-seeking behaviour in larvae of the robber fly *Mallophora ruficauda* (Diptera: Asilidae). *Journal of Insect Physiology*, 50, 331-336.

Cornelisse, T.M., Hafernik, J.E. (2009). Effects of soil characteristics and human disturbance on tiger beetle oviposition. *Ecological Entomology*, 34, 495-503.

de Boer, T.E., Holmstrup, M., van Straalen, N.M., Roelofs, D. (2010). The effect of soil pH and temperature on *Folsomia candida* transcriptional regulation. *Journal of Insect Physiology*, 56, 350-355.

de Lange, P. J.; Heenan, P. B.; Given, D. R.; Norton, D. A.; Ogle, C. C.; Johnson, P. N.; Cameron, E. K. (1999). Threatened and uncommon plants of New Zealand. *New Zealand Journal of Botany*, 3, 603-628.

Ferreira, S.M., McKinlay, B. (1999). Conservation monitoring of the Cromwell chafer beetle (*Prodontria lewisii*) between 1986 and 1997. *Science for Conservation, 123*.

Ferreira, S.M., Ravenscroft, N.O.M., McKinlay, B. (1999). Activity patterns and population characteristics of the New Zealand endemic Cromwell chafer, *Prodontria lewisii* (Scarabaeidae; Melolonthinae). *New Zealand Journal of Zoology*, 26, 229-241.

Folk, R.L. (1968). *Petrology of Sedimentary Rocks*. University of Texas, Geology.

Griffith, B., Scott, J.M., Carpenter, J.W., Reed, C. (1989). Translocation as a species conservation tool: Status and strategy. *American Association for the Advancement of Science*, 245, 477-480.

Grimaldi, D., Engel, M. S. (2005). *Evolution of the Insects*. New York USA: Cambridge University Press.

Hamilton, W.J. (1999). Potential threat of hedgehogs to invertebrates with a restricted range, Otago region. *Conservation Advisory Science Notes No. 254*, Department of Conservation, Wellington.

Hunt, D. (2007). Monitoring of the Cromwell Chafer Beetle (*Prodontria lewisii*) by Pitfall Trapping and Night Searches. *Department of Conservation, Dunedin* (internal report).

International Union for Conservation of Nature and Natural Resources (IUCN), "Position Statement on the Translocation of Living Organisms: Introductions, Re-introductions, and Re-stocking" (IUCN Council, Gland, Switzerland, 4 September 1987).

Lodhi, M.A.K., Killingbeck, K.T. (1980). Allelopathic inhibition of nitrification and nitrifying bacteria in a ponderosa pine (*Pinus ponderosa* Dougl.) community. *American Journal of Botany*, 67, 1423-1429.

McKinlay, B. (1997). Changes in Cromwell Terrace vegetation and soils. *Conservation Advisory Science Notes,* 163: 1-5.

Palmer, Julie (Sept, 2011). Personal communication regarding how to interpret the percentage weight distribution graphs, in particular what was considered a normal output or abnormal output.

Samways, M. J. (1993). Insect in biodiversity conservation: some perspectives and directives. *Biodiversity and Conservation*, 2, 258-282.

Sastrodihardjo, F.X.S., van Straalen, N.M. (1993). Behaviour of five isopod species in standardized tests for pH preference. *European Journal of Soil Biology*, 29, 127-131.

Turnbull, I.M. (compiler) (2000). Geology of the Wakatipu area. *Institute of Geological and Nuclear Sciences 1:250,000 geological map 18*.

van Gils, H.A.J.A., Gaigl, A., Gómez, L.E. (2010). The relationship between soil variables and leafcutter ant (*Atta sexdens*) nest distribution in the Colombian Amazon. *International Union for the Study of Social Insects*, 57, 487-494.

van Straalen, N.M., Verhoef, H.A. (1997). The development of a bioindicator system for soil acidity based on arthropod pH preferences. *Journal of Applied Ecology*, 37, 217-232.

Watt, J.C. (1979). Conservation of the Cromwell chafer *Prodontria lewisii* (Coleoptera: Scarabaeidae). *New Zealand Journal of Ecology*, 2, 22-29.

Chapter Three: Adult Translocation and Breeding of the Cromwell Chafer Beetle

Abstract

Ongoing survival and breeding of adults is crucial to the success of any translocation. The aim of this chapter was to measure the survival of adult Cromwell chafer beetles over time following translocation to five different soil sites. Male-female pairs were confined to fifteen separate enclosures at each of the five sites. Breeding success was measured by excavating the enclosures at the end of the breeding season and counting the larvae produced in each enclosure and comparing the results between sites. Adult survival did not vary significantly between translocation sites. Male survival was significantly correlated with female survival. The number of larvae produced at each site was correlated with soil site, with two sites producing significantly lower larval numbers than the site with the highest number of larvae. Although adult survival did not vary significantly with site, it was positively correlated with larval survival – higher adult survival rates led to higher numbers of excavated larvae and vice versa. The results are discussed in the context of their management implications and what they mean to the success of any potential future translocations.

Introduction

Translocation is a vital and integral tool in species conservation, but thus far has been applied primarily to vertebrates. Success rates of translocations, especially of rare species, is low (Griffith, *et al*., 1989), but the number of translocations being performed, including of rare species, are increasing. As the field of invertebrate translocations is a new and rapidly expanding one, there is a pressing need to develop reliable translocation methods.

The Cromwell chafer beetle, *Prodontria lewisii*, is as a 'Category A' threatened species, making it amongst one of the 'highest priority threatened species for conservation action' in New Zealand. The species is a nocturnal, flightless beetle of the family Scarabaeidae. The genus *Prodontria* is endemic to the lower half of New Zealand's South Island (Emerson & Wallis, 1994; Emerson & Barratt, 1997). *Prodontria lewisii* has a highly restricted and localised distribution, being limited to an 81 ha reserve in Cromwell, Central Otago. The reserve was gazetted in 1983 under the Reserves Act (1977) and is managed by the Department of Conservation.

Adult beetles emerge at night during the spring and summer months from August to March (Armstrong, 1997; Watt, 1979). Adults feed on a variety of native and introduced vascular and non-vascular plants such as the cushion plant *Raoulia australis*, sheep's sorrel *Rumex acetosella*, lichen (Ferreira, *et al*., 1999) and St John's wart *Hypercium perforatum* (personal observation). Although little is known about the larvae, past studies (e.g.: Ferreira, *et al*., 1999; Watt, 1979) have speculated that they may be associated with the roots of silver tussock *Poa cita*, and that they require more than one year to develop. No pupae of this species have yet been recorded (Watt, 1979; Ferreira, *et al*., 1999).

The Cromwell chafer beetle is an ideal candidate for translocation because it has a very restricted and localised range. The establishment of insurance populations could therefore be considered as an important tool in the management of this species. Unlike many other threatened invertebrates, the Cromwell chafer beetle has been the focus of conservation effort already. Thus, enough is already known about the distribution, abundance and lifestyle of the beetles to be able to attempt translocations.

The life cycle, adult morphological variations, activity patterns, population characteristics, conservation monitoring, and conservation status of the Cromwell chafer beetle, as well as the potential threat posed by hedgehogs and other predators have been described in Barratt (2007), Barratt *et al*. (2006; 2007), Ferreira *et al*., (1999), Ferreira & McKinlay (1999al; 1999b; 2001), Hamilton (1999), and Watt (1976). However, nothing has yet been done on the beetles' range of tolerance for different soil types, other than to acknowledge that they appear to be restricted to sandy soils. No translocations of the beetles, aside from the original supplementing of the Cromwell Chafer Beetle Nature Reserve (CCBNR) population in 1975-76, have yet been performed.

This study aims to identify the tolerance and breeding success of adult Cromwell chafer beetles to different translocation sites which vary in soil and plant community composition. The results are discussed in context of their management implications, with specific mention given to their relevance to future translocations.

Methods

Five sites were chosen as translocation sites for adult beetles - four within the Cromwell Chafer Beetle Nature Reserve (CCBNR) and one at the Lindis Crossing. Site selection within the CCBNR was based on the historical and current data regarding distribution of beetle populations within the reserve (DoC, 2007; Anderson, 2008; personal night time observations of areas of high activity of adult beetles). The Cromwell Middle (CM) site was selected because the beetles have always been there in strong numbers, both historically and today; Cromwell Bannockburn (CB) was selected because the beetles were found there historically, but are no longer found there; Cromwell worm farm (CW) has a self-introduced beetle population today, but has never been known to have one in the past; Cromwell Roadside (CR) has never had a beetle population, past or present, but has similar habitat to sites where the beetles already exist. Both the soil and plant composition at CR superficially appears to be similar to that found in areas of high adult beetle activity elsewhere on the Reserve.

The Lindis site was selected because it is the only intact inland sand dune system in the region with a similar plant composition to the CCBNR. The Lindis site lies on the Clutha

River, the same river that helped form the chafer beetles' current habitat (Kemp, 1955; Leamy & Saunders, 1957; Ferreira *et al*., 1999). Although the Lindis site appears to possess potentially suitable habitat, Cromwell chafer beetles have never previously been found at the Lindis site (Ferreira *et al*., 1999). The only other inland sand dune in the region, the Alexandra sand dune, was deemed unsuitable for translocation because it is planted in pine trees, which chafer beetles are not associated with.

A total of 75 galvanised metal enclosures, 15 per site, were used to hold the translocated beetles. Each enclosure was 400 mm deep and encompassed an area of 0.1 m². Enclosure design was based on that described by Barratt *et al*. (2006). The enclosures were dug into the ground in mid-late November 2008 to a minimum depth of 15 cm to minimise the risk of beetles digging their way out from under the enclosure (Barratt, *et al*., 2006). The enclosures were randomly distributed at each site, with the constraint that each enclosure had to contain part of a *Raoulia australis* plant, in addition to other plant species, as this is a known food of both the larvae and adults (Ferreira, *et al*., 1999). The placement of the enclosures was also limited by soil variations. On occasion small, localised areas of stony soil prevented an enclosure from being placed in a certain location. Each enclosure was covered with a fine mesh, held in place by a length of tire tube inner. The GPS coordinates of each enclosure were recorded using a Garmin 12 GPS, which is accurate to within 3 m.

One hundred and fifty adult Cromwell chafer beetles - 75 males and 75 females - were collected at night over a 10-day period in mid-late November 2008 and gender determined using a hand lens to examine the antennal clubs. The antennae of male Cromwell chafer beetles have four terminal segments (quadralamellate) each 1 - 1.5 mm in length. Female antennae are trilamellate and thus have three terminal segments which are much shorter - less than 1 mm - than those of the males (Ferreira, *et al*., 1999). Females occasionally have a fourth very small segment (Wilson, pers. comm., 2008). One male and one female were placed into each enclosure and the mesh cover replaced.

Plate 1: Photos showing female (left) with three small terminal club segments and one much smaller segment, and male (right) showing four large terminal club segments (photos taken by author).

The enclosures were excavated over a week-long period in late January - early February 2009. The plant species present inside each enclosure were recorded. Small plants (e.g. mosses, *Trifolium arvense, Rumex acetosella*) were carefully removed from the soil surface inside the enclosure and discarded. Larger plants (e.g. *Raoulia*, grasses) were removed, inverted over a bucket and the roots carefully separated and searched for larvae. The sand inside the enclosure was then removed a handful at a time and sifted into a bucket to extract larvae. Each enclosure was excavated to a minimum depth of 20 cm, or to just beyond the base of the enclosure. When a larva was found it was recorded and placed in a film canister three quarters filled with sand and containing a segment of plant roots. Each film canister had two air holes in the lid. At the end of each day the larvae were given a cube of carrot as a food source (Barratt, *et al*., 2007).

When an adult beetle was found it was recorded as alive or dead, sexed where possible (some had decomposed beyond sexing), and released alongside the enclosure. The number of live earwigs (*Forficula auricularia*) in each enclosure was also recorded, as these are potential predators of Cromwell chafer beetle eggs and first instar larvae (Barratt, *et al*., 2006).

Data were analysed using GraphPad Prism version 5.00 for Windows, Microsoft Office Excel 2007 and Minitab. Analyses performed were one-way ANOVAs, paired t-tests, and Dunn's multiple comparison tests.

Results

The total number of surviving adults per soil site for the Cromwell Middle (CM), Cromwell Bannockburn (CB) Cromwell Roadside (CR), Cromwell Wormfarm (CW) and Lindis (L) sites are presented in Figure 3.1. Soil site CW had the highest total number of surviving adults, while soil site CB produced the lowest, but these differences were not significant - one-way ANOVA (P = 0.2066). Dunn's multiple comparison showed that there was no significant difference between any given pair of soil sites.

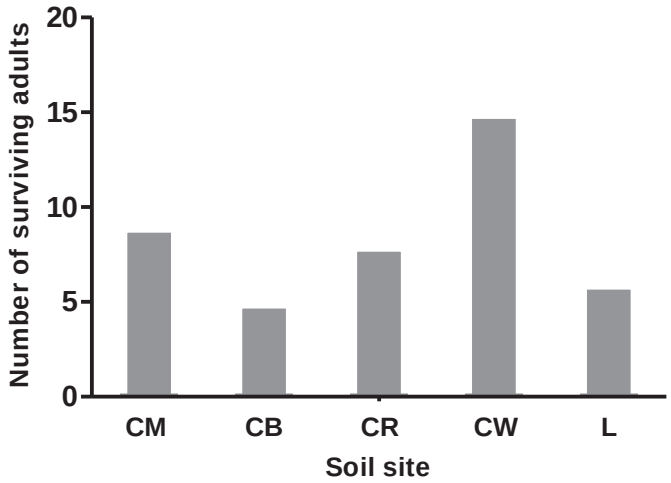


Figure 3.1: Total number of surviving adults for each soil site (Cromwell Middle, Cromwell Bannockburn, Cromwell Roadside, Cromwell Wormfarm, and Lindis).

Sites CM and CB had lower numbers of males surviving than females, whereas site CW had more females surviving than males (Figure 3.2). Sites CR and L had no difference between the numbers of surviving males and females. A paired t-test showed no significant difference between male and female survivorship between any of the sites (P = 0.6213, t = 0.5345, df = 4).

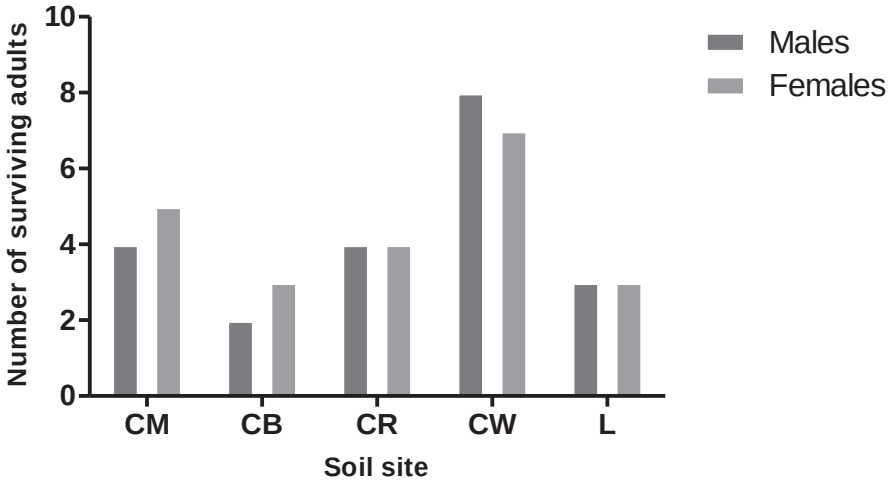


Figure 3.2: Total number of surviving adult males and females from each soil site (Cromwell Middle, Cromwell Bannockburn, Cromwell Roadside, Cromwell Wormfarm, and Lindis).

A total of 219 larvae were excavated across all five sites. Of the 75 enclosures, 37 failed to produce any larvae. Twelve of the enclosures that failed to produce any larvae were located in soil from the Lindis site, and eleven were located in soil from the CB site. Figure 3.3 shows a significantly higher number of larvae were excavated from the worm farm soil site than from any other soil site ($P = 0.0002$, $F = 6.318$, $df = 4$). The mean number of larvae excavated from soil site CW was 7.8, almost three times greater than the next highest mean number of larvae excavated, which was from soil site CR (mean = 2.733). Soil site CB had the lowest mean number of larvae excavated (0.6) as well as the smallest range in the number of larvae excavated (0 – 3). Soil sites CM, CR and L had similar mean numbers of larvae excavated, and similar ranges of larvae excavated. A one-way ANOVA showed that the means were significantly different ($P = 0.0041$, $F = 6.32$, $df = 4$). Dunn's multiple comparison test showed that the differences lay between sites CW and CB, and sites CW and L.

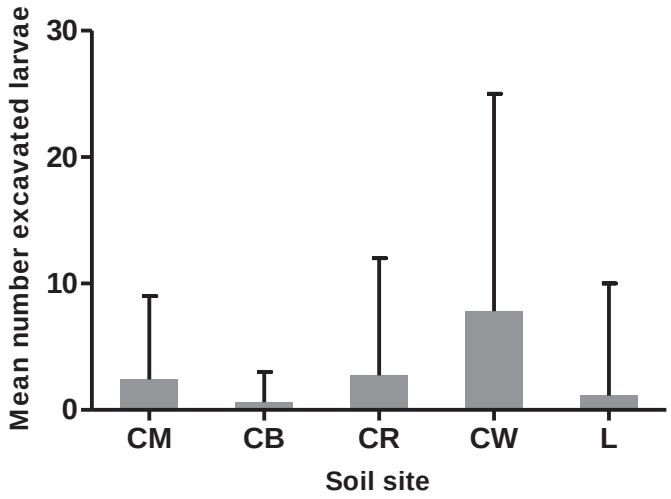


Figure 3.3: Mean and range of the number of larvae excavated from each soil site (Cromwell Middle, Cromwell Bannockburn, Cromwell Roadside, Cromwell Wormfarm, and Lindis).

No earwigs were found at site L (Figure 3.4). Paired t-tests showed there was no significant pairing in the number of surviving adults found in each enclosure and the number of earwigs found in that same enclosure at any site (P-values ranged from 0.4010 to 0.0584). Paired t-tests also showed that there was no significant pairing between the total number of surviving adults excavated from a site and the total number of earwigs excavated from a site, for any site (P = 0.3157).

The relationship between the number of surviving adults and the number of excavated larvae was significant at sites CM (P = 0.0149, t = 2.774, df = 14), CR (P = 0.0154, t = 2.757, df = 14) and CW (P = 0.0040, t = 3.439, df = 14). The relationship was not significant at site CB (P = 0.4027, t = 0.8629, df = 14) or site L (P = 0.2787, t = 1.127, df = 14), the sites which had the lowest number of surviving adults and the lowest number of excavated larvae.

Paired t-tests demonstrated that the relationship between the number of earwigs per enclosure and the total number of larvae excavated from that enclosure at any given site was not significant (P-values range from 0.2036 to 0.3209). However, the differences in the mean number of earwigs per site and the mean number of excavated larvae per site was significant at site CM and CW (P = 0.0241, t = 2.529, df = 14 and P = 0.0178, t =

2.683, df = 14, respectively). Both these sites had relatively small numbers of earwigs and comparatively large numbers of excavated larvae.

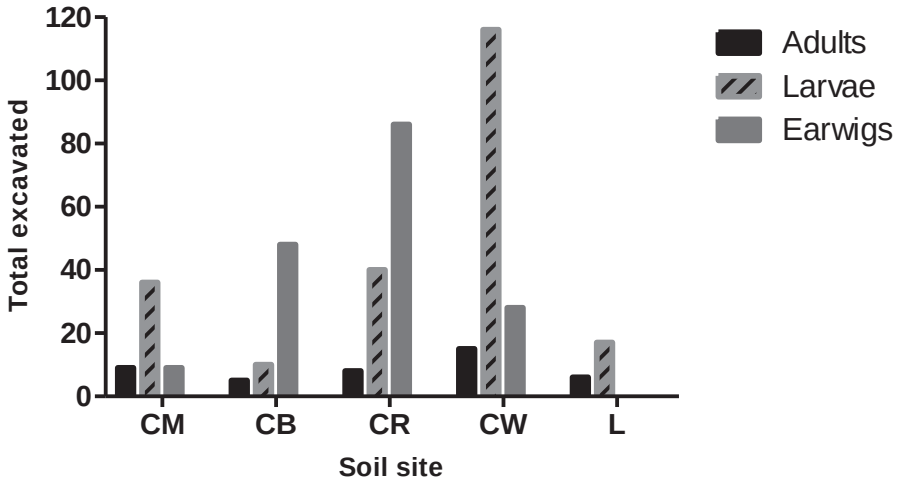


Figure 3.4: Total numbers of surviving adults, excavated larva and live earwigs per soil site (Cromwell Middle, Cromwell Bannockburn, Cromwell Roadside, Cromwell Wormfarm, and Lindis).

Discussion

Adult survival did not vary significantly between sites and there was no significant difference between the numbers of males and females excavated at any of the sites. However, the number of surviving males was significantly correlated with the number of surviving females. This means that if one sex is likely to survive at a given site, the other is too. Conversely, the opposite is true. If one sex does poorly at a given site, the other sex is likely to do the same. In this study, it would appear that both sexes react similarly to the specific features of the five different environment sites tested.

Adult beetles are active from August to March, with male beetles emerging before females and remaining active for longer (Ferreira, *et al*., 1999). Given that males emerge earlier in the season than females, it may be that they exhibit different rates of development. Where survivorship of males and females differs, it may be worth investigating the effects of different developmental rates and different activity patterns between the two sexes. Ferreira *et al*. (1999 found that 27% of the variation in activity during the spring and summer months could be explained by temperature and humidity.

Clearly other factors are also influencing activity levels, and discovery of these would be beneficial to understanding Cromwell chafer beetle population dynamics and survival rates.

If translocation to a poor quality site can reasonably be assumed to be a source of stress in this study, then adult breeding females at these sites can be expected to produce fewer eggs than those translocated to good quality sites. If this assumption is correct, then the poor adult survival rates at some sites in this study (site Cromwell Bannockburn (CB) in particular) should correspond to poor larval excavation numbers. While there was no significant difference in the total number of surviving adults between any of the five sites, site CB did have the lowest total number of surviving adults. This corresponded with the lowest number of excavated larvae being taken from site CB, although this number was not significantly different from site Cromwell Middle (CM), Cromwell Roadside (CR) and Lindis (L).

Adult survival was significantly correlated with the number of excavated larvae at all sites except for site CB. It would appear from this correlation that the number of larvae produced is positively influenced by the ongoing survival of the parents. It may be that adult health, as indicated by on-going adult survival at the time of excavation, is a predecessor for larval survival. Figures 3.2 and 3.3 produced very similar looking results between and across the five sites shown on both graphs. For example, where sites CB and L had low numbers of surviving adults, these two sites also had low numbers of excavated larvae. Similarly, site Cromwell Wormfarm (CW) had the highest number of surviving adults, and also the highest number of excavated larvae.

The variation in adult survival per site was not significant, but the difference in excavated larvae numbers was. This indicates either that adults are able to survive in conditions which are unsuitable for breeding, or that some sites are suitable for adult survival but not for larvae survival. To distinguish between the two, experiments in which an egg count was taken prior to larval hatching would need to be conducted. If the number of eggs produced was significantly higher than the number of larvae surviving (after accounting for a certain number of expected deaths per clutch), this may indicate that adults were able to breed but that the conditions were unsuitable for larvae survival. If the number of eggs produced was significantly lower than those that would be expected given normal

good breeding conditions, this may indicate that the site in question is not suitable for breeding. A further experiment regarding adult choice in breeding sites could be undertaken to determine whether adults chose to move away from sites that performed poorly in an egg-count experiment if left to their own devices.

The effects of parental health, particularly maternal health, on offspring health are well researched and well publicised in vertebrates (e.g. Visman, *et al*., 1996; Kunz & King, *et al*., 2007). Mileva *et al*. (2011) showed that elevated stress levels in females of the cichlid fish *Neolamprologus pulcher* resulted in longer intervals between spawning times, and eggs being smaller and fewer in number. There is a great deal less research in the field of invertebrate stress with regards to offspring health. Stefano *et al*. (2002), however, postulated that mammalian responses to stressors evolved from those already present in simpler organisms such as invertebrates. They therefore suggested that invertebrates would have similar responses to stressors as mammals. They demonstrated that invertebrates do display mammalian-like signal molecules and corresponding behaviours in response to stressors. It is therefore reasonable to assume that stressed invertebrate mothers, like their vertebrate counterparts, will exhibit poorer breeding results than non-stressed invertebrate mothers.

A significantly higher number of larvae were excavated from site CW than from any other site. CW also had the greatest range in the number of larvae excavated per enclosure. Site CW was chosen as a translocation site because it was one where Cromwell chafer beetles had not been found historically, but not in recent years (Anderson, 2008, personal communication; DoC, 2007; personal observations of night time activity of adult beetles). Site CW was so named because it of its proximity to the Cromwell worm farm. Also there is the potential for leeching of concentrated nutrients produced at the worm farm into the Cromwell Chafer Beetle Nature Reserve (CCBNR), the worm farm has the majority of its soil contained within sealed underground trenches and the farm is about 100 m from the translocation site and 20 m from the edge of the CCBNR. Any potential nutrient leeching is likely minimal, if at all. CW had many times more larvae excavated than any other site. It is clear that the breeding and larval development conditions at site CW are superior to those found at the other sites.

The adult survival results of this study are complementary to those of Ferreira *et al*. (1999). In that study, adult chafer beetle densities at three different sites were estimated using pitfall trap data collected during the beetle's active season across six sampling years. They found that there were no significant differences in densities between the two sites equivalent to those labelled CM and CW in this study. However, the highest densities were found at the site equivalent to CW. The equivalent site to CB yielded significantly lower densities than either the CM or CW equivalents. While the difference in adult survival found in this study was not found to be significant, the results to match with those of Ferreira *et al*. (1999) in that site CB produced the lowest survival rates as compared to other sites on the CCBNR.

One hundred percent of all larvae raised in laboratory conditions in soil from site CW survived (see Chapter 4). However, there was no significant difference in larval survival numbers between those raised in CW soil and those raised in any other CCBNR soil (all CCBNR soil sites had significantly higher rates of larval laboratory survival than the Lindis soil sites). Within the four soils from the CCBNR, soil site did not appear to significantly influence laboratory survival of larvae. However there was a significant difference in the numbers of surviving larvae based on which plant type they were fed. Those fed *Raoulia* did significantly better than those fed *Festuca rubra* or *Poa cita*.

Dune movement from one side of the CCBNR to the other may be a factor in the differences seen in historical and current populations of Cromwell chafer beetles across the reserve. Dunes are highly mobile environments (Navarro, *et al*., 2011). The drifting sands of the CCBNR originate from the alluvial terrace faces between Lowburn and Deadman's Point, originally deposited by the Clutha River (Ferreira & McKinlay, 1999; McKinlay, 1997).

Site CB produced the lowest number of larvae and also displayed the lowest range in the number of larvae excavated per enclosure. Site CB was historically a location in which Cromwell chafer beetles have been found, but they have not been seen there in recent years (Anderson, 2008; DoC, 2007; personal observations of night time activity of adult beetles). It would appear that the beetles have either died out in this area, or have moved elsewhere on the reserve in response to a change in conditions at the site.

Site CB is the least exposed of the four CCBNR sites, being bordered on its two most southern sides by mature pine trees. This site is thus more protected from prevailing southerly winds than other sites. When spring time night monitoring of adult beetle activity was undertaken in 1998, two clusters of beetles were found, totalling 22 adults. In a subsequent monitoring event in 2006, only one beetle was found at this site. No beetles were found in the 2007 monitoring event. In 1997 and 1998, when the first two night surveys were undertaken, the pine trees bordering this site were recently planted and as yet not tall enough to provide a significant wind barrier to this area of the CCBNR (see images in DoC, 2007; Hunt, 1998) . It may be that the growth of the trees over the past few decades has sheltered the sand dune systems from the wind and thus slowed their movement. This may have had an adverse effect on the suitability of the habitat for the beetles. The mobility, and thus habitat diversity, of sand dune systems is a significant factor in the on-going survival of some dune-based terrestrial invertebrate species (Howe, *et al*., 2009).

In contrast to site CB, site CW is one of the more exposed sites on the reserve. It is also the most successful site in this study for larvae excavation and adult survival. It may be that the openness of this site contributes to a healthier sand dune system due to increased sand dune movement via wind exposure. Site CM, the site at which there has always been a healthy Cromwell chafer beetle population, is also in an exposed area of the reserve. Data on the progression of the sand dunes across the reserve and any corresponding population movement of the beetles may well yield informative results. If beetle movement and population density are positively correlated with sand dune movement, then monitoring sand dune movement and progression over time could help predict where beetles are likely to move from and to. This could have important management implications in terms of surveys, population monitoring and localisation of conservation actions such as planting. Given that the CW site is almost at the border of the CCBNR, continued monitoring of sand dunes may even help predict whether that particular part of the sand dune system, a highly suitable chafer beetle habitat, is likely to move right out of the CCBNR and into the unsuitable industrial area beyond. However, data on sand dune movement may be difficult to obtain within time, budget and minimal dune disturbance policy constraints. Wind speed measurements at different points around the CCBNR, particularly in areas sheltered by the pine trees versus areas that are more exposed, might prove both easier to record and indicative of sand dune movement.

Another factor that may be potentially contributing to the poor survival results of the beetles at site CB is soil acidity. Site CB has a pine tree border in close proximity to two sides of the site area, which has been present since 1997/98. Site CB has a significantly lower pH than all the other sites (see Chapter 2, Table 2.2). Pine trees are known to lower soil pH (Lodhi & Killingbeck, 1980). However, site CR also has a pine tree border which has been present since 1997/98 and yet the survival of the beetles was high at this site. The difference between the two sites may be due to the prevailing winds. The border that lies adjacent to site CR and one side of site CB runs from south to north, parallel with the direction of the prevailing wind from the south. Pine needles from this border are thus more likely to be blown north, parallel with this side of the CCBNR, and not west onto the sites CR and CB. However, the second pine tree border near site CR runs across the prevailing wind, from south-east to north-west. Pine needles caught in the prevailing southern wind may be blown north onto site CB. This, coupled with the fact that site CB is more sheltered than the other sites, and thus more likely to experience dune stabilisation, may be contributing to the poor survival rates of the beetles in this location.

Earwigs have been identified as another potential threat to chafer beetle survival, possibly by preying on eggs and larvae (Barratt, *et al*., 2006). There were no earwigs at the Lindis site, which could be interpreted as a good thing for chafer beetle success. No significant correlation between the number of larvae excavated and the number of earwigs found was detected in this study at site CB and CR, and no significant correlation was found within any single enclosure from any site. Given that sites CB and CR had the greatest numbers of earwigs, and yet site CR still produced high numbers of excavated larvae, it would seem that the effect of earwigs on chafer beetle larvae survival is minimal.

Also, a great many of the larvae excavated from all the sites were still very early instar larvae. If predation was going to occur, this is the stage at which it would be most likely to do so, and yet there is little evidence for predation in this data. However, no predation of eggs was examined in this study. It may be that the numbers of offspring at site CR may initially have been higher, but was reduced by egg predation before larvae emerged. This would be something worthy of investigation under laboratory conditions.

While site CB also had no significant correlation between the mean number of earwigs found and the mean number of excavated larvae, it produced a much lower number of excavated larvae than site CR. It would appear from this data, and from other data gathered in this study (see Chapter 4) that the reason for poor breeding success at site CR is due to other variables and not related to the number of earwigs found at the site.

A significant difference in the mean numbers of earwigs per site and the mean number of excavated larvae per site was detected at sites CM and CW. Both these sites had small numbers of earwigs compared to the number of excavated larvae found there. From this, it would seem that larvae do better when earwig numbers are low. Given that the opposite cannot said to be true (that larvae do worse when earwig numbers are high) it appears that the effect of earwigs on larval populations is minimal.

Site L had no earwigs. Regardless of whether or not earwigs are detrimental to chafer beetle survival, site L still performed poorly in terms of the number of larvae excavated from it, the number of surviving adults remaining, and the long-term survival of larvae in soil from this site (see Chapter 4). An earwig deficiency cannot compensate for these failings. It is therefore not recommended that earwig numbers be used to determine whether or not a given site is suitable for translocation. It is recommended that soil site be used as the best indicator of Cromwell chafer beetle success. Earwig data may be useful as supplementary data, and may be constructive if potential egg predation is a concern.

To avoid the loss of potentially gravid females, and thus vital new members of the species, male beetles could be used to test the suitability of prospective new habitat. Male and female survival did not vary significantly, so it is reasonable to assume that the survival of males will be indicative of female survival. Because of the small population size, the loss of any adult beetle when testing potential new habitat should be minimised wherever possible. It may prove advantageous to establish small, captive populations within enclosures such as those used in this study and that of Barratt *et al*. (2006; 2007). This would allow maximum recapture of adult beetles if necessary and allow close monitoring of survival and breeding to take place.

Problems

I have no laying/excavation data for tussock, because none of the enclosures were placed over tussock. In future, it may be helpful to obtain data regarding adult survival and laying behaviour/larval excavation numbers around tussock plants, and combining this with larval survival when fed tussock.

It may be that some adults were coming to the end of their natural lives when translocated. The subsequent death of an adult may not have been due to the conditions within their particular enclosure. However, there is no way of distinguishing those who died naturally and those who died because of sub-standard conditions at their translocation site from the data available.

Recommendations

Habitat analysis is a much better predictor of translocation success than earwig numbers. Continued monitoring of sites CM, CR and CW would be highly valuable to determine population growth and movement over time. Surveys of the CR and CW sites in particular would be interesting, as these are both relatively new populations. The beetles moved to site CW of their own accord, but did not move to site CR. Yet site CR proved very successful in producing high adult survival and high larvae excavation numbers. It may be that the chafers are unable to access some areas of suitable habitat, such as site CR, because of the presence of natural barriers to movement on the CCBNR. There is a large track of stony, inhospitable ground between site CR and the rest of the reserve and chafer beetle migration across this area is likely to be minimal. Assessment of potentially suitable areas around the CCBNR could be undertaken, followed by assisted colonisation of these areas. Having many populations spread over the reserve could be a valuable survival and conservation strategy should disease, fire, or other hazards threaten the reserve.

Further study should be undertaken in a controlled situation to examine whether earwig predation of chafer beetle eggs occurs. A small amount of predation may be reasonable to allow, but if predation of eggs is high, earwig control methods may need to be employed in areas of high earwig concentration on the reserve. Alternatively, larvae could be

hatched and raised past the early instars in the lab, or in earwig-free enclosures on the reserve, before being released (similar to operation nest egg with kiwi, Colbourne *et al.,* 2005). This would be a labour intensive and costly undertaking, and so research into earwig predation of eggs, with a mind to perhaps setting an acceptable low predation tolerance threshold, should be undertaken first.

References

Department of Conservation (2007). 2006/2007 Cromwell Chafer Beetle transect survey, and a comparison of the results with the 1997/1998 survey. *Department of Conservation Internal Publication*.

Barratt, B.I.P., Barton, D., Ferguson, C.M. (2006). *Prodontria lewisii Broun (Coleoptera: Scarabaeidae): Population monitoring; larval feeding preferences; predation by earwigs.* Report for the Department of Conservation.

Barratt, B.I.P., Barton, D., Ferguson, C.M. (2007). *Prodontria lewisii Broun (Coleoptera: Scarabaeidae): population monitoring; larval feeding preferences; rearing protocol*. Report for the Department of Conservation.

Barratt, B.I.P. (2007). Conservation status of *Prodontria* (Coleoptera: Scarabaeidae) species in New Zealand. *Journal of Insect Conservation*, 11, 19-27.

Colbourne, R., Bassett, S., Billing, T., McCormick, H., McLennan, J., Nelson, A., Robertson, H. (2005). The development of Operation Nest Egg as a tool in the conservation management of kiwi. *Science for Conservation, 259.*

Ferreira, S.M., McKinlay, B. (1999). Conservation monitoring of the Cromwell chafer beetle (*Prodontria lewisii*) between 1986 and 1997. *Science for Conservation, 123*.

Ferreira, S.M., Ravenscroft, N.O.M., McKinlay, B. (1999). Activity patterns and population characteristics of the New Zealand endemic Cromwell chafer, *Prodontria lewisii* (Scarabaeidae; Melolonthinae). *New Zealand Journal of Zoology*, 26, 229-241.

Griffith, B., Scott, J.M., Carpenter, J.W., Reed, C. (1989). Translocation as a species conservation tool: Status and strategy. *Science*, 245, 477-480.

Howe, M.A., Knight, G.T., Clee, C. (2009). The importance of coastal sand dunes for terrestrial invertebrate in Wales and the UK, with particular reference to aculeate Hymenoptera (bees, wasps & ants). *Springer Science and Business Media B.V.* (published online).

Hunt, D. (1998). Monitoring of the Cromwell chafer beetle (*Prodontria lewisii*) by pitfall trapping and night searches. *Department of Conservation, Dunedin*.

Kemp, W.S. (1955). Cherry growing in Central Otago. *New Zealand Journal of Agriculture*, 90, 49-60.

Kunz, L.L., King, J.C. (2007). Impact of maternal nutrition and metabolism on the health of the offspring. *Seminars in Fetal & Neonatal Medicine*, 12, 71-77.

Leamy, M.L., Saunders, W.M.H. (1967). Soils and land use in the Upper Clutha Valley, Otago. *Soil Bureau Bulletin, N.Z.*, 28, 110pp.

Lodhi, M.A.K., Killingbeck, K.T. (1980). Allelopathic inhibition of nitrification and nitrifying bacteria in a ponderosa pine (*Pinus ponderosa* Dougl.) community. *American Journal of Botany*, 67, 1423-1429.

McGuinness, C.A. (2001). The Conservation Requirements of New Zealand's Nationally Threatened Invertebrates. *Threatened Species Occassional Publications No.20, Department of Conservation*.

McKinlay, B. (1997). Changes in Cromwell Terrace vegetation and soils. *Conservation Advisory Science Notes*, 163, 1.5.

Mileva, V.R., Gilmour, K.M., Balshine, S. (2011). Effects of maternal stress on egg characteristics in a cooperatively breeding fish. *Comparative Biochemistry and Physiology A – Molecular & Integrative Physiology*, 158, 22-29.

Navarro, M., Munoz-Perez, J.J., Roman-Sierra, J., Tsoar, H., Rodriquez, I., Gomez-Pina, G. (2011). Assessment of highly active dune mobility in the medium, short and very short term. *Geomorphology*, 129, 14-28.

Stefano, G.B., Cadet, P., Zhu, W., Rialas, C.M., Mantione, K., Benz, D., Fuentes, R., Casares, F., Fricchione, G.L., Fulop, Z., Slingsby, B. (2002). The blueprint for stress can be found in invertebrates. *Neuroendocrinology Letters*, 23, 85-93.

Visman, V., Pesant, S., Dion, J., Shipley, B., Peters, R.H. (1996). Joint effects of maternal and offspring sizes on clutch mass and fecundity in plants and animals. *Ecoscience*, 3, 173-182.

Chapter Four: Critically endangered Cromwell chafer beetle larvae have narrow tolerance limits for soil site and host plant species

Abstract

The ongoing success of any translocation depends on the survival of the young of the translocated species. The young of many insect species have very different habitat requirements to their adult counterparts and it is therefore important to address them separately. The aim of this chapter was to measure the growth and survival of larvae of the critically endangered Cromwell chafer beetle (*Prodontria lewisii* Broun) over time in different soil and plant combinations. Four of the soil sites used came from within the chafer beetles' current range and one was from an experimental translocation site outside of the beetles' current range. The three plant types used were the cushion plant *Raoulia australis*, the grass *Festuca rubra*, and silver tussock *Poa cita*. One hundred and fifty larvae were raised in tubes containing a combination of each of the soil and plant types. Larval growth was measured using before and after weights. There were no significant differences in larval survival across any of the soil sites from within the beetles' current range. Larval survival was significantly lower at the translocation site located outside of the beetles' current range. *Raoulia* yielded significantly higher survival rates than either grass or tussock, and grass yielded significantly higher survival rates than tussock. However, growth rates of larvae raised on grass were generally higher than those of *Raoulia*. The results are discussed in the context of their management implications and what they mean to the success of any potential future translocations.

Introduction

Translocation is a vital and integral tool in species conservation, but thus far has been applied primarily to vertebrates. Success rates of translocations, especially of rare species, is low (Griffith, *et al*., 1989), but the number of translocations being performed, including of rare species, are increasing. As the field of invertebrate translocations is a new and rapidly expanding one, there is a pressing need to develop reliable translocation methods.

The Cromwell chafer beetle, *Prodontria lewisii* Broun, is as a 'Category A' threatened species, making it amongst one of the 'highest priority threatened species for conservation action' in New Zealand. The species is a nocturnal, flightless beetle of the family Scarabaeidae. The genus *Prodontria* is endemic to the lower half of New Zealand's South Island (Emerson & Barratt, 1997; Emerson & Wallis, 1994). *Prodontria lewisii* has a highly restricted and localised distribution, being limited to an 81 ha reserve in Cromwell, Central Otago. The reserve was gazetted in 1983 under the Reserves Act (1977) and is managed by the Department of Conservation.

Adult beetles emerge at night during the spring and summer months from August to March (Watt, 1979; Armstrong, 1997). Adults feed on a variety of native and introduced vascular and non-vascular plants such as the cushion plant *Raoulia australis*, sheep's sorrel *Rumex acetosella*, lichen (Ferreira, *et al*., 1999) and St John's wart *Hypercium perforatum* (personal observation). Although little is known about the larvae, past studies (e.g.: Watt, 1979; Ferreira, *et al*., 1999) have speculated that they may be associated with the roots of silver tussock *Poa cita*, and that they require more than one year to develop. No pupae of this species have yet been recorded (Watt, 1979; Ferreira, *et al*., 1999).

The Cromwell chafer beetle is an ideal candidate for translocation because it has a very restricted and localised range. The establishment of insurance populations could therefore be considered as an important tool in the management of this species. Unlike many other threatened invertebrates, the Cromwell chafer beetle has been the focus of conservation effort already. Thus, enough is already known about the distribution, abundance and lifestyle of the beetles to be able to attempt translocations.

Barratt (2007), Barratt *et al*. (2006; 2007), Ferreira *et al*. (1999), Ferreira & McKinlay (1999a; 1999b; 2001), Hamilton (1999) and Watt (1976) have variously covered the life cycle, adult morphological variations, activity patterns, population characteristics, conservation monitoring, and conservation status of the Cromwell chafer beetle, as well as the potential threat of hedgehogs and other predators. However, nothing has yet been done on the beetles' range of tolerance for different soil sites, other than to acknowledge that they appear to be restricted to sandy soils. No translocations of the beetles, aside from the original supplementing of the CCBNR population in 1975-76, have yet been performed, and Barratt *et al*. (2007) stated that "a major constraint to understanding and managing the *P. lewisii* population in the CCBNR is a lack of knowledge of larval host plant associations."

This study aims to identify the tolerance of Cromwell chafer beetle larvae to soil and plant combinations compatible with growth and survival. The results are discussed in context of their management implications, with specific mention given to their relevance to future translocations.

Methods

Larvae were obtained using the enclosure excavation method described in Chapter 2. One hundred and fifty PVC tubes, each approximately 30cm long and 8cm in diameter were used to hold developing larvae. The five adult translocation sites from Chapter 2 were used as soil sources for the tubes: sites CM, CB, CR, CW, and L. One set of 30 tubes were used per site. Three plant species were used as larval food sources: *Raoulia australis*, *Festuca rubra* (red fescue), and *Poa cita* (silver tussock). All three plant species occur in significant numbers within the CCBNR (see Chapter 2). Ten tubes from each site were planted with one specimen of each of the three plant types. In the case of tussock, which was too large to fit in one tube, three large plants were divided into smaller sections and each section was planted in a tube. Large Raoulia plants (>2 m²) were used to provide up to six tube segments. Segments were taken from near the edges of the plants, but far enough in so as to avoid any deadened areas at the very edge. Segments taken were evenly spread around the edges of each plant in an attempt to lessen the damage. Up to four segments were taken from medium-sized plants (>1 m²). Smaller

plants had one to two segments taken. One plant per tube was used for *Festuca rubra*. A unique code was marked on the side of each tube with a permanent marker to indicate where the soil had come from, which plant type was growing in it, and which number from one to ten that tube represented. For example, CM/T4 = soil site CM, tussock tube number four.

Larvae were obtained by excavating enclosures in which male-female pairs of adult Cromwell chafer beetles had been contained for several months (see Chapter 2). Only larvae excavated from the CCBNR enclosures were used in the larval tubes in order to minimise any developmental differences between larvae from the CCBNR and the Lindis site, and also to minimise any environmental effects the source sites may have had on larval development. Larvae were weighed using a Sartorius balance scale (accurate to 0.0001 g) and deposited into a small hole dug into the surface of each tube. The allocation of larvae to each tube was random. The tubes were placed in a temperature and light controlled room at Massey University, Palmerston North in early January 2009. The temperature was set to 16°C, the average temperature on the Cromwell Chafer Beetle Nature Reserve over the summer months (Barbara Barratt, pers. comm.). The light regime was set to mimic the daylight hours on the reserve during summer, coming on at 6 am and switching off at 9 pm. The plants were watered lightly every two weeks.

The larval tubes were excavated over a one-week period in April 2009. The plants were removed from the top of the tube and their roots carefully searched for larvae. The sand remaining in each tube was sifted by hand to recover larvae. The average search time devoted to each tube was 12 minutes. In this time all of the sand in the tube could be thoroughly sifted through twice. Larvae were recorded as dead or alive and live larvae were weighed using a Sartorius balance scale (accurate to 0.0001 g). If no larva was found in a given tube after 20 minutes searching, it was assumed that that larva had died. Live larvae were placed in individual aerated film containers that were three-quarters fill with sand and with a carrot cube as a food source (Barratt, *et al.*, 2007) and returned to the CCBNR one week after all weighing was completed.

Data were analysed using GraphPad Prism 5 for Windows, Minitab, Program R, and Microsoft Excel. Three binary logistic regression tests were performed using Minitab. The SAS System was used to analyse the significance of the relationships between larval

end weight and soil/plant combinations. Only Type III was used because of the uneven sample size. SAS was also employed to test for any significant differences in the starting weights of larvae. Only the starting weights of larvae which survived were used in this analysis. While weight gain of the larvae was measured in grams, the results are discussed in terms of percentage weight gain (with the exception of Figure 4.3). This was done in order to standardise for variation in individual starting masses of larvae.

Results

Larval Survival

The total numbers of larvae that survived across all three plant types in soil from each site and the breakdown by soil site are shown in Figure 4.1. There was no difference in the total number of larvae surviving in any of the soil sites found within the CCBNR (i.e. soil sites CM, CB, CR and CW). There were significantly less surviving larvae in soil from the Lindis soil site, with only three larvae surviving (see Figure 4.1). However, the number of surviving larvae per plant type did differ. On average, *Raoulia* yielded significantly higher numbers of surviving larvae than grass or tussock across all five sites. All of the larvae surviving in the Lindis soil were grown on Raoulia. Tussock yielded significantly lower numbers of surviving larvae than *Raoulia* and grass across all five soil sites. Grass had equal or lower survival rates than *Raoulia*, but always produced higher survival rates than tussock. Soil sites CM, CB and CR had 66.6% survival rates each. Soil site CW produced a 100% survival rate. However, the Lindis soil site produced only a 10% survival rate.

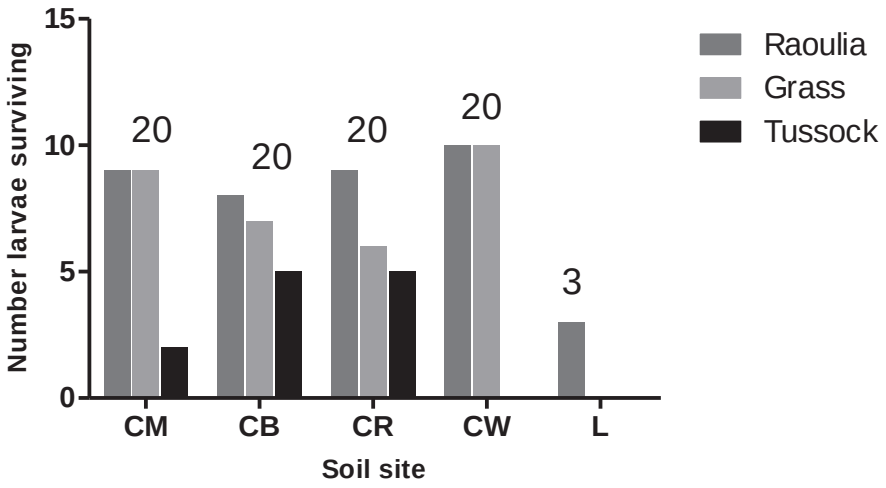


Figure 4.1: Total number of surviving larvae in soil from each site and total number of larvae surviving per plant species for each site (Cromwell Middle, Cromwell Bannockburn, Cromwell Roadside, Cromwell Wormfarm, and Lindis).

While *Raoulia* and grass produced the same number of surviving larvae at sites CM and CW, *Raoulia* yielded a greater number of surviving larvae at sites CB, CR and L, and a significantly higher total number of surviving larvae than grass (Figure 4.2). Grass yielded the second highest total of surviving larvae on average. No larvae fed on grass survived from the Lindis site. Both *Raoulia* and grass produced higher survival rates at every site than did their tussock counterparts. Where larvae fed on tussock survived, the survival numbers were lower across all sites. No larvae fed on tussock survived at the Lindis site. Of all the larvae fed on Raoulia, 78% survived in total. Of those fed grass, 66% survived, while only 30% of those fed on tussock survived.

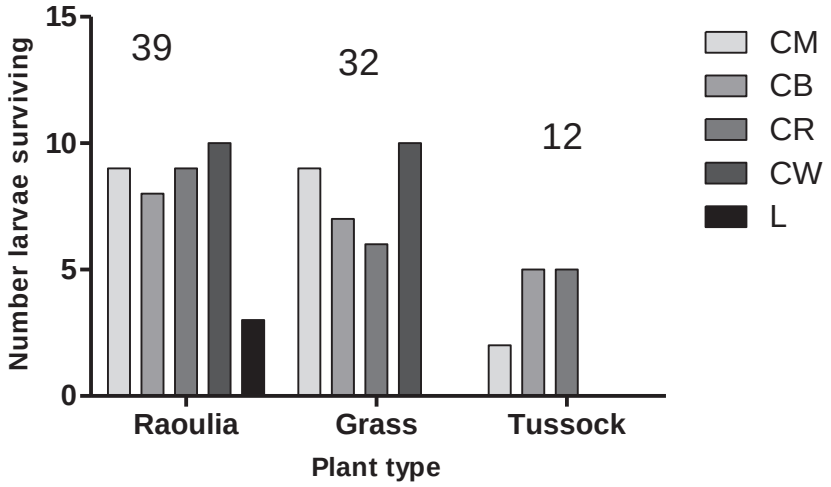


Figure 4.2: Number of larvae surviving per plant type across each of the five soil sites (Cromwell Middle, Cromwell Bannockburn, Cromwell Roadside, Cromwell Wormfarm, and Lindis).

Analysis of Larval Survival

Three binary logistic regression tests were performed to compare larvae survival in each trial (tube), with the plant type and soil in which each larva was growing.

In the first test, data were entered as events (surviving larvae = event; dead larvae = non-event), giving a total of 14 cases entered. Each case comprised one site-plant combination, for example CM *Raoulia*. The case "CW tussock" was excluded as this combination had no data. Soil site CB was chosen by Minitab as the comparison site. There was no significant difference in the number of surviving larvae between soil site CB and CM or CR (both P = 1.000), or CW (P = 0.997). There was a significant difference between CB and L (P = < 0.001, G = 52.460, DF = 5). Grass was chosen as the comparison plant. *Raoulia* yielded significantly higher survival rates than grass (P = 0.050, G = 52.460, DF = 5), while tussock yielded significantly lower survival (P = 0.013, G = 52.460, DF = 5).

In the second test, surviving larvae were entered as "1" and dead larvae were entered as "0", giving 140 cases, 10 for each site-plant combination for which there were data. The results did not differ from the first test. Using CB as a comparison soil again, no

significant differences were found in the number of surviving larvae in sites CM (P = 0.761), CR (P = 1.000), or CW (P = 0.997). Soil L again gave a significantly lower survival rate than any of the Cromwell soil sites (P = < 0.001, G = 52.460, DF = 5). *Raoulia* was again higher than grass (P = 0.050, G = 52.460, DF = 5) and tussock again lower (P = 0.013, G = 52.460, DF = 5).

Analysis of larval survival under each soil-plant combination was hampered slightly by the missing data set "CW tussock". Tussock was thus eliminated from one of the binary logistic regression analyses to check that the remaining two sets of tussock values were not giving an overly high/low representation of larvae survival or skewing the data in any other way. Missing values can cause the analysis to give misleading or incorrect results.

The third binary logistic regression test eliminated all larvae grown on tussock. This was done to ensure that the missing value was not impeding the running of the tests. Because the first two tests did not differ significantly in their results, data were entered only in '1/0' format. The results remained consistent with those of the first and second tests. In comparison to site CB, sites CM, CR and CW were not significantly different (P = 0.214, 1.000 and 0.997 respectively). Site L was significantly lower (P = < 0.001, G = 49.804, DF = 5). *Raoulia* was significantly higher than grass (P = 0.050, G = 49.804, DF = 5).

Growth Rates of Larvae

All larvae grown in *Raoulia* had relatively high growth rates, with the span of growth across all five soil sites ranging from 56.1% to 902.5% (Figure 4.3). *Raoulia* also had the highest number of larvae surviving in total – 39 out of 50. Soil CM had the most variation in percentage growth, while CR had the lowest. CM also had the highest median percentage growth (518.6%) and the highest average percentage growth (458.1%). The only three larvae to survive in the Lindis soil tubes were fed on *Raoulia*. The differences in the mean before and after weights of larvae fed on *Raoulia* were significant for all larvae raised in soil from site CM (P = 0.0149, t = 3.090, df = 8), CB (P = 0.0324, t = 2.661, df = 7), CR (P = 0.0024, t = 4.373, df = 8) and CW (P = 0.0228, t = 3.596, df = 4), but were not significant for larvae raised in soil from site L (P= 0.1731). The average

weight gain of larvae fed on *Raoulia* across all five sites was 0.0351g (a 327.1% increase in weight).

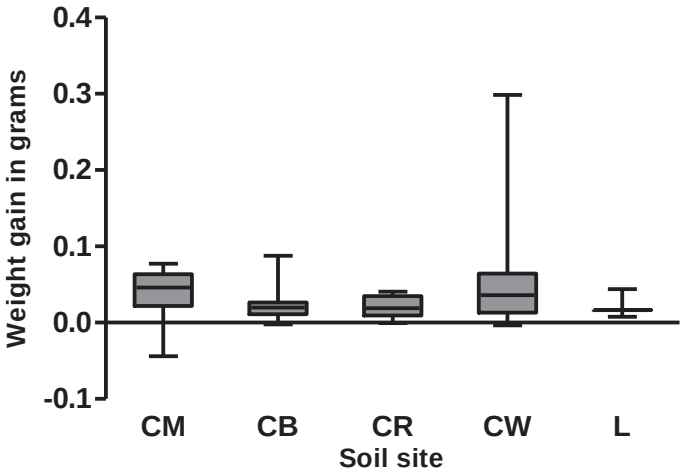


Figure 4.3: Weight gain (g) of larvae fed on *Raoulia australis* across all five soil sites (Cromwell Middle, Cromwell Bannockburn, Cromwell Roadside, Cromwell Wormfarm, and Lindis).

No larvae from the Lindis tubes survived when fed on *Festuca rubra* (Figure 4.4). Both the minimum (168.8%) and the percentage growth spread of larvae fed on this grass (168.8% to 2762.5%) were greater than those of larvae fed on *Raoulia*. However, survival overall was lower than for *Raoulia*, with 32 out of 50 larvae surviving. Overall, larvae fed on *Festuca rubra* did significantly worse than those fed on *Raoulia* ($P = 0.050$). Soil CW had the greatest spread in percentage growth (172.3% - 2762.5%). CR had the highest median percentage growth of 930.4%. CB had both the least spread and the lowest median percentage growth. The mean differences in the before and after weights of all larvae fed on *Festuca rubra* were significantly different for soil site CM ($P = 0.0357$, $t = 2.521$, $df = 8$), CB ($P = 0.0087$, $t = 3.824$, $df = 6$), CR ($P = 0.0032$, $t = 5.286$, $df = 5$) and CW ($P = 0.0099$, $t = 3.506$, $df = 7$). The average weight gain of larvae fed on grass across all five sites was 0.0903g (a 696.1% increase in weight).

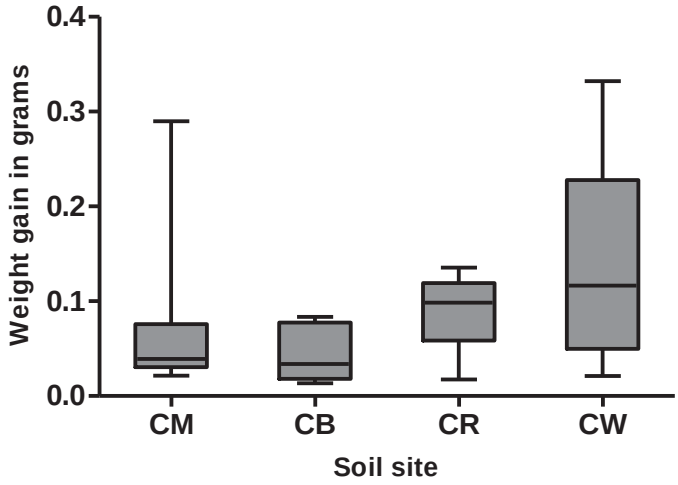


Figure 4.4: Weight gain (g) of larvae fed on grass (*Festuca rubra*) across four soil sites (Cromwell Middle, Cromwell Bannockburn, Cromwell Roadside, and Cromwell Wormfarm).

No larvae from the Lindis soil survived when fed *Poa cita* (silver tussock). No data were obtained for the CW soil. Tussock had the lowest survival of all three plant types, with only 12 of the 50 larvae surviving. Tussock also produced the lowest spread of growth rates of all three plant types, ranging from 131.0% to 790.9% (Figure 4.5). Overall, larvae fed on tussock did significantly worse than larvae fed on either Raoulia or *Festuca rubra* ($P = 0.013$). Only two larvae from CM soil survived. Of the five larvae that survived in each of the CB and CR soil tubes, those in CR tubes did better overall. The mean difference in the before and after weights of larvae raised on tussock in CM soil was not significant ($P = 0.3207$). The differences in the mean weights of larvae fed on tussock and raised in CB and CR soil were significant ($P = 0.0244$, $t = 3.523$, $df = 4$, and $P = 0.0228$, $t = 3.596$, $df = 4$ respectively). The average weight gain of larvae fed on tussock was 0.0341g (a 341.9% increase in weight).

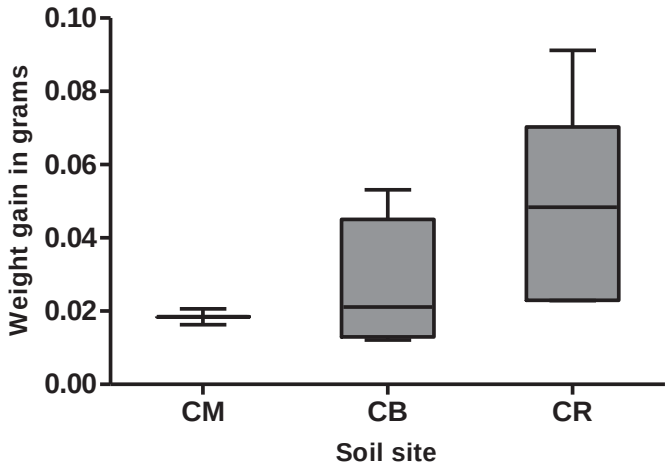


Figure 4.5: Weight gain (g) of larvae fed on silver tussock (*Poa cita*) across three soil sites (Cromwell Middle, Cromwell Bannockburn, and Cromwell Roadside).

Of the 20 larvae that survived in CM soil, only two of those were fed tussock (Figure 4.6). Nine larvae survived in *Raoulia* and in *Festuca rubra*. The median percentage growth for *Raoulia* and grass were almost identical (518.6% and 488.1% respectively). The spread of percentage growth was less for *Raoulia* (56.1% - 902.5%) than for grass (168.8% - 1528.1%). Larvae grown in tussock tubes had lower percentage growth rates than either *Raoulia* or grass.

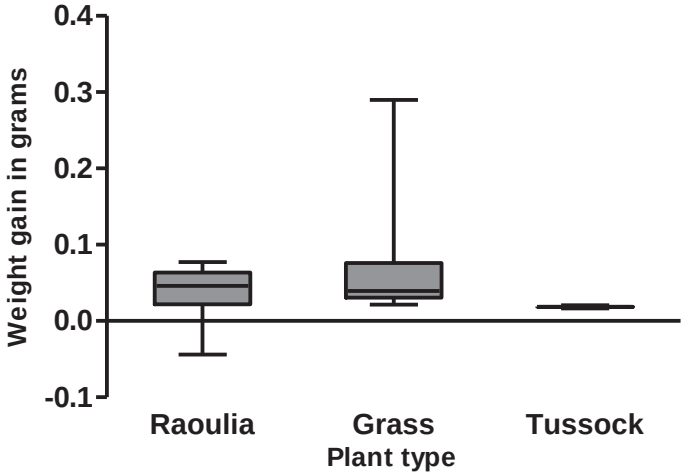


Figure 4.6: Weight gain (g) of all surviving larvae raised in soil from the Cromwell Middle site across all three plant types.

Twenty larvae also survived in CB soil (Figure 4.7). However, in this soil site the numbers of larvae surviving on each plant type were more evenly divided. Eight larvae

survived on *Raoulia*, seven on *Festuca rubra*, and five on tussock. Percentage growth rates in this soil site were low overall in comparison to other soil sites, ranging from 89.8% to 455.1%.

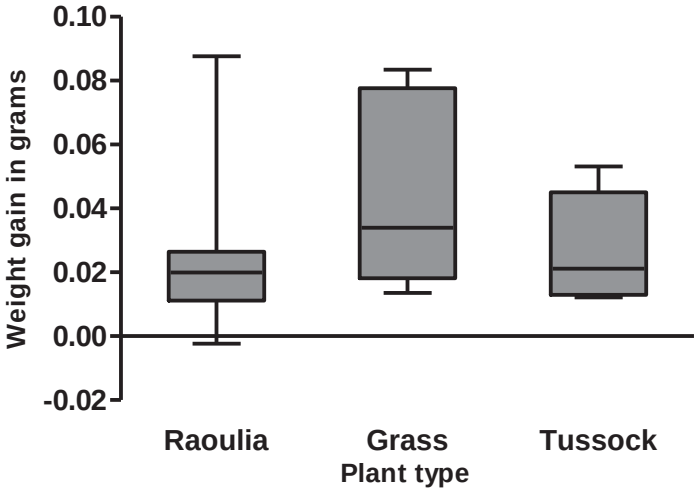


Figure 4.7: Weight gain (g) of all surviving larvae raised in soil from the Cromwell Bannockburn site across all three plant types.

Soil site CR also produced 20 surviving larvae; nine on *Raoulia*, six on grass and five on tussock (Figure 4.8). In terms of percentage growth, larvae fed on grass did better than those fed on *Raoulia* at this site, as did larvae fed on tussock. Percentage growth at this site ranged from 95.6% to 973.8%.

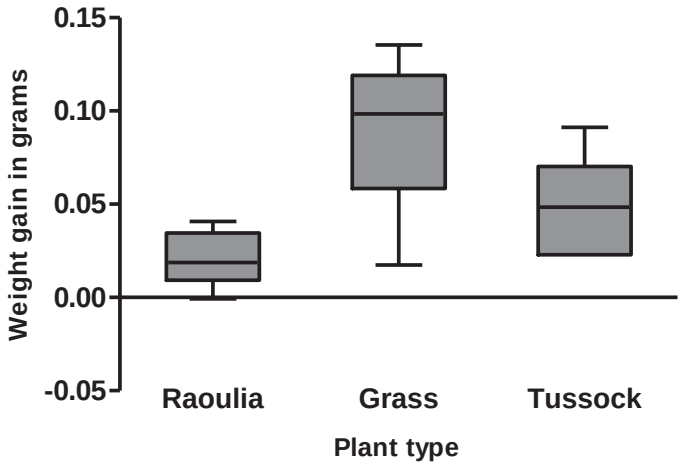


Figure 4.8: Weight gain (g) of all surviving larvae raised in soil from the Cromwell Roadside site across all three plant types.

All ten larvae from both *Raoulia* and grass survived in soil from site CW (Figure 4.9). Those fed on grass did better overall, with higher percentage growths than those fed on *Raoulia*. The spread of percentage growths was also wider for grass than for *Raoulia*. No data were available for the combination CW-tussock.

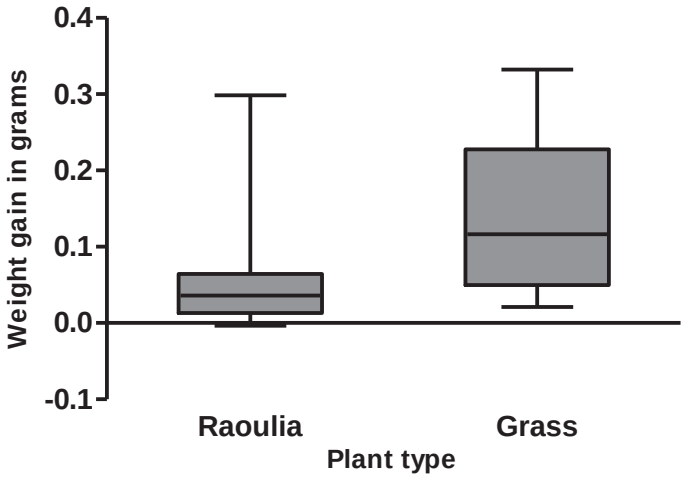


Figure 4.9: Weight gain (g) of all surviving larvae raised in soil from the Cromwell Wormfarm site across two plant types.

The only three larvae to survive in the Lindis soil were fed *Raoulia*. Percentage growth ranged from 161.1% to 702.7% (Figure 4.10).

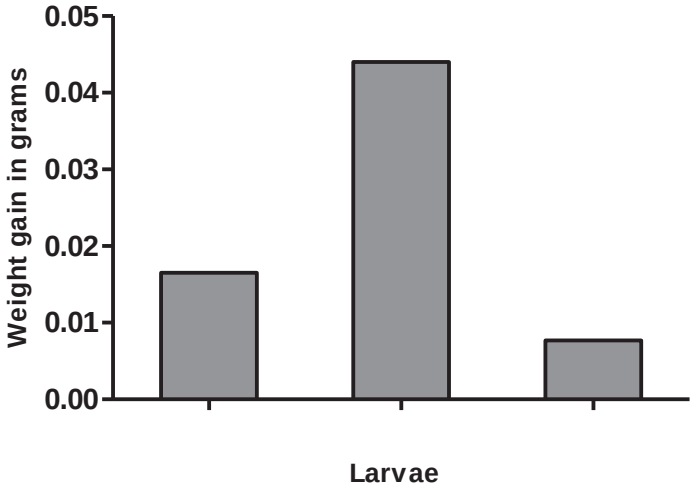


Figure 4.10: Weight gain (g) of the three surviving larvae raised in soil from the Lindis site.

Figure 4.11 is a summary of the medians for figures 2-9. While *Raoulia* produced significantly higher survival rates than grass or tussock (see Table 1, and text), grass produced the highest percentage growth rates in soil from all sites except CM, where *Raoulia* was marginally better, and L, were the only three larvae to survive were fed on Raoulia. While the survival numbers of larvae fed on tussock were significantly worse than those of larvae fed on *Raoulia* or grass, the percentage growth rates of tussock were higher than *Raoulia* in soil from sites CB and CR.

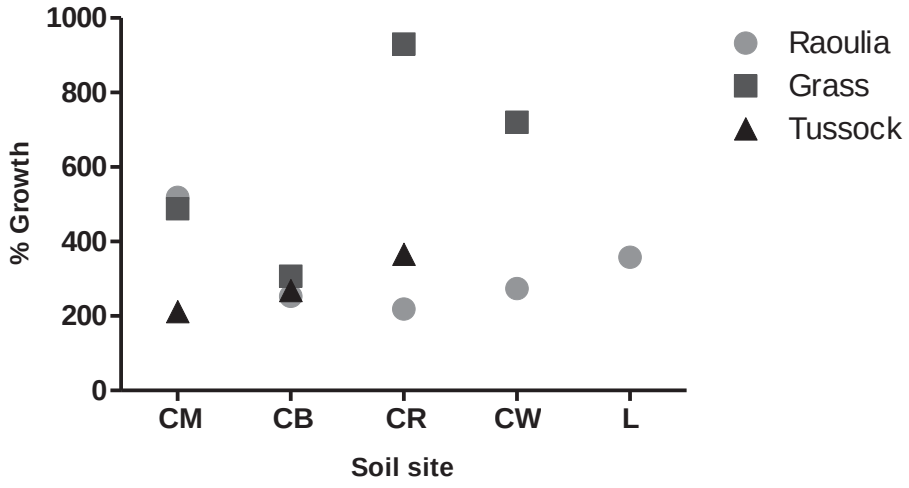


Figure 4.11: Median percentage growth of larvae fed on each of the three plant types for each of the five soil sites (Cromwell Middle, Cromwell Bannockburn, Cromwell Roadside, Cromwell Wormfarm, and Lindis).

Grass produced the highest median growth within soil from sites CR, CB and CW (Figure 4.12). However, no larvae raised in soil from the Lindis site survived when fed grass. *Raoulia* produced the highest median growth rate for soil site CM, but the lowest for soil site CR. Overall, tussock produced low median growth rates across the three soil sites for which data was available and larvae survived in.

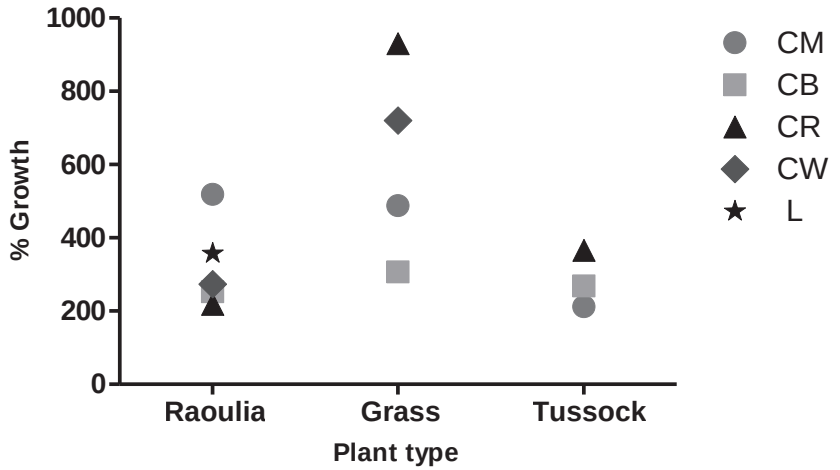


Figure 4.12: Median percentage growth of larvae raised on each of the three plant types across all five soil sites (Cromwell Middle, Cromwell Bannockburn, Cromwell Roadside, Cromwell Wormfarm, and Lindis).

SAS Analysis

The Type III GLM model used to test the significance of the differences in final weight of the surviving larvae gave the following results: $F_{11,82} = 2.82$. P = 0.0293. This test showed that although soil and plant had a significant impact on larval weight gain, the interaction between them was not significant (Table 4.1). The success of a larvae in a given soil site does not depend on what plant it is fed. If the larva does well, it is because the soil site is good. Similarly, a larva will do better if given the best plant type, regardless of what soil it is in. Conversely, the effects of a less suitable soil cannot be countered by providing a high quality food plant.

Table 4.1: GLM for Weight Change of Surviving Larvae

	Type III Sum of Squares	F Value	P Value
Soil	0.04312177	2.82	0.0293
Plant	0.04451662	5.92	0.0042
Soil*Plant	0.01346492	0.72	0.6137

Discussion

The growth and survival of Cromwell chafer beetle larvae varied significantly with different plant types and soil sites. Larvae survived best when fed *Raoulia* (39 out of 50 larvae survived), but grew best when fed grass *Festuca rubra*. The only larvae to survive from the Lindis site were those fed on *Raoulia*. Grass had the second highest survival rate, with 32 larvae surviving. However, the median growth rates for grass were significantly higher than *Raoulia* at sites CR and CW. Tussock produced significantly lower survival numbers (12 out of 50) than *Raoulia* or grass and lower than average growth rates. Surprisingly, the growth rates for tussock marginally outstripped those for Raoulia at site CB and significantly outstripped those for *Raoulia* at site CR.

The best soil site for larvae survival was any of the C-site soils - those from the beetle's current range on the Cromwell Chafer Beetle Nature Reserve. All four Cromwell soil sites produced the same number of surviving larvae. L-site soils, those from an experimental translocation site at the Lindis Crossing, are unsuitable for chafer beetle larvae to grow or survive in. The Lindis soil site produced very poor larvae survival results.

There was no interaction between soil site and plant type. Larvae fed on a good plant type will nevertheless do poorly if they are housed in soil from an unsuitable site. The effects of poor soil and/or plant type cannot be improved by the addition of a good soil and/or plant type. Similarly, soil from a good soil site will always produce better growth rates than a soil from a poor soil site, regardless of the suitability of the plant types growing in them. Thus, the best combination of plant and soil for the greatest larvae growth and survival can be found by combining the individual plant types and soil sites that produced the highest growth and survival. In the case of this study, the optimum combination for survival would be any of the four Cromwell soil sites planted with *Raoulia*. The optimum combination for growth would be any of the four Cromwell soil sites combined with the grass species used in this study, *Festuca rubra*. Conversely, the poorest combination for both growth and survival would be the Lindis soil site planted with silver tussock (*Poa cita*).

Larval growth and survival on each plant type

Barratt *et al*. (2006) fed Cromwell chafer beetle larvae on sections of root from different plants, including *Poa cita* and *Raoulia australis*. They found that while the larvae consumed roots from all experimental plant types, they did not consume the inner 'core' of *Poa cita* roots. Feeding on this plant was confined almost exclusively to the outer epidermis layer. In contrast, roots from *Raoulia australis* were entirely consumed. It may be that while the larvae can and do eat the roots of silver tussock, they are unable to eat the entire root, or find certain parts of it unpalatable and harder or less easy to consume in some way. It may be that the roots of silver tussock are nutritionally or edaciously incomplete. The results of Barratt *et al*. (2006) and of this study suggest that silver tussock alone is not a preferred diet for chafer beetle larvae, and nor is it a successful one in terms of survival rates. While growth rates for larvae fed on tussock marginally exceeded those for *Raoulia* at site CB and significantly exceeded those for *Raoulia* at site CR, the growth rates for tussock overall were poor. Tussock yielded the lowest weight gains overall out of the three plant types used in this study. This result, coupled with the poor survival numbers for tussock (12 out of 50), means that any small potential advantage in growth gained by feeding larvae exclusively on tussock is likely to be vastly overshadowed by poor survival rates. Providing silver tussock as a sole food source for Cromwell chafer beetle larvae is highly unlikely to be advantageous for the growth and survival of the population in the long run.

These results are in conflict with the original assumptions of Watt (1979), who suggested that the Cromwell chafer beetle is associated primarily with silver tussock, *Poa cita*. In this experiment, the survival of larvae grown on silver tussock was significantly worse than the survival of larvae grown on alternative food sources. There may be several reasons for this. Firstly, it may have been assumed, without thorough searching to confirm the assumption, that the beetles were associated with the predominant native grass in the area, which at the time was silver tussock (Barratt, *et al*., 2006). Secondly, when the larvae were first found and described by Watt in 1979, they were recorded as being associated with silver tussock roots. It may be that the only larvae found in that search were on silver tussock, or that a comprehensive search of other plant types was not conducted. Silver tussock is the tallest and most conspicuous plant growing on the

Cromwell Chafer Beetle Nature Reserve, and it may be that searchers gravitated towards these plants as likely areas for larvae to be found, dismissing automatically areas which appeared barren and assuming that they containing no larvae. If this was the case, this historical error may have been accepted as the truth. No further comprehensive larval searches under different plant types have been undertaken since. The results of this study indicate that Cromwell chafer beetle larvae are unlikely to be associated with silver tussock, at least not as a primary food source. It may be that larvae can and do eat silver tussock, but as an accompaniment to other, main food sources. Further study to determine how far larvae can travel, and whether or not they eat a variety of food plants in the wild would be interesting.

In contrast to the results from silver tussock, the *Raoulia* plants used in this study yielded the highest survival rates. Given that Barratt *et al*. (2006) found that the roots of this plant species also appear highly palatable to the larvae of the Cromwell chafer beetle, it appears that the species may be more readily associated with this plant type than with *Poa cita*. Interestingly, while *Raoulia* yielded the highest survival rates, the grass species used in this study (*Festuca rubra*, or red fescue) yielded the best growth rates. This would indicate that there is a trade-off in the choice of host plant between fast growth and long-term survival. It would be interesting to couple these data with information about female oviposition behaviour. It may be that the best diet for larvae is not simply one species of plant, but a combination of several. This could be determined if a method of tracking beetle larvae underground was employed. If larvae moved far enough to be able to access the roots of two or more species of plant, this would provide evidence in support of the hypothesis that larvae do best on more than one species of plant. Further laboratory studies could aim to determine the ideal combination of plants for optimal growth and survival by providing larvae with different combinations of plant types.

One issue that may be present in this study is the fact that all the tussocks used in the laboratory study were sourced from just three original plants. Each large plant was carefully separated into smaller sections and replanted in the larvae tubes. In the week following replanting, the tussock sections did appear to wilt somewhat. However, within two weeks and with regular watering, all tussock segments improved in appearance and survived the duration of the experiment. Although the greatest care was taken to ensure that the roots of each segment were separated gently and remained intact, it is possible

that the separation process had a detrimental effect on the tussock segments. Thus, those larvae that were fed on these plants may have been at a disadvantage from the outset. In addition, this small selection of source plants means that there is a conceivable possibility that all three were not healthy specimens to begin with. Care was taken to choose source plants which did appear to be in good condition prior to harvesting. However, given the great difference in the significance of larval survival fed solely on *Poa cita* in this study, and the feeding behaviour observations in Barratt *et al*. (2006), it seems likely that larvae fed on tussock did poorly simply because of the species of plant on which they were fed, and not because of the health of the plant itself.

Larval Survival in Different Soil Sites

Survival rates did not vary between any of the CCBNR sites, with 66% of all larvae from these four sites surviving. Soil from the Lindis site resulted in much lower survival numbers than the CCBNR sites, with only 10% survival. While it seems clear that the Lindis soil is not suitable for Cromwell chafer beetle larvae, it would appear that all of the CCBNR soil sites tested are suitable for larval growth. However, not all of the soil sites available on the Reserve were tested in this study. There is a barren, stony area near the middle of the Reserve which was not able to be used as a test site because of the practical issues involved in setting enclosures into stony ground. Thus, the selection of soil sites may have played to the Chafer beetle's advantage, as only sites with enough sand to bury enclosures in could be used.

One surprise of these results was the success of the site CR (Cromwell roadside). As its name suggests, this site was located right next to Bannockburn Road. The site was across the central rocky area from the main Chafer beetle population. No adult beetles had previously been seen at this site during any night searches (personal observation). In addition, because of the proximity of this site to the road, it appeared to have a higher weed count than the other sites (see Chapter 2). It would seem that the soil here is of the same good quality for Chafer beetle growth as the sandy areas across the rest of the Reserve. It is likely that the reason Chafer beetles have not been located here previously is to do with the isolation of this area from the main, central population. Unlike the rest of the Reserve, there is no sandy corridor linking this site with the central population.

Because of the narrow nature of this site and its close proximity to a roadway, it may be advisable to take precautions before translocating a population of beetles there. For example, a more solid fence could be installed to prevent beetles from moving onto the road. This could be done on an economical basis by testing how high the flightless beetles can climb and perhaps installing a low plank of wood, for example, along the bottom of the existing fence. Fence line vandalism is sadly a common occurrence at the CCBNR and it may be unwise to attract attention to an easily accessible part of the fence. However, if a barrier could be installed discretely, it may be worthwhile if an additional Chafer beetle population was to be established here.

Soil-plant interactions and their effect on larval growth and survival

Binary logistic regression tests determined that there was no interaction between soil site and plant type. This means that the survival success of a larva in a given soil site does not depend on what plant type it is fed on. If a larva does well, it is because the soil site is good. It follows that combining a soil site that is known to produce high larval growth and survival rates with a plant type that is also known to produce high larval growth and survival rates will produce the best possible larvae growth and survival rates. Combining a good soil or plant type with a mediocre soil or plant type will produce mediocre larvae growth and survival. Combining a poor plant type and a poor soil site will yield poor larvae growth and survival. To use an example from this study, larvae translocated to the Lindis site are always going to do badly regardless of what plant they are fed on, because the soil there is not suitable. Planting the Lindis site with *Raoulia* and red fescue, the two best food sources, will not improve larvae survival because plant type does not affect soil site. The quality of the poor soil cannot be countered by adding good plants to it. For this reason it is recommended that no attempt to translocate Cromwell chafer beetles to the Lindis site should be undertaken.

While the majority of the larvae tubes used in this study were able to provide enough data to accurately identify good soil and plant types, one data set was missing - the soil/plant combination CW/Tussock. In contrast to all of the other sites, site CW had 100% larvae survival in all tubes for which there was a value obtained. Survival was low (50% or less) for all tussock tubes from other sites. If values had been obtained for the combination

CW/tussock, tussock survival may have been greater at this site than others. However, this is merely speculation and cannot be predicted using the available data.

The results of this study have helpful management implications when it comes to the CCBNR, particularly the discovery that the soil and plant types used in this study have no interaction. Larvae will survive in any of the four CCBNR soil sites identified in this study. However, they will do best if those soil sites are also home to the best plant types for growth and survival: *Raoulia* and grass. The most cost effective way for the Department of Conservation to manage the CCBNR would be to identify those areas of the reserve that provide the ideal plant and soil match identified here and to focus planting and translocation efforts there. As plant type is more readily identified in the field than differing soils, the quickest way for staff to identify suitable sites for Chafer beetles may be to search by eye for areas in which *Raoulia* and red fescue are growing.

Problems

All the larvae in this study were subjected to only two variations in their environment – soil site and plant type. Temperature, humidity, light and water levels were kept constant. This enabled the effects of plant and soil on larvae growth and survival to be analysed effectively. The larvae were growing without any predation or competition in a laboratory environment. Therefore, the results may not be directly indicative of results which may occur in the wild.

Only three tussock plants were used to supply all of the larval rearing tubes containing tussock. Each tussock plant was split into smaller sections containing roots and leaves and each of those small sections was used to populate a larvae tube. With such a small selection of different plants, it could be that all three were not in prime condition and thus affected the development of all larvae. However, every effort was made to select healthy looking tussock from an area next to the CCBNR where a large population of tussock grows well. In future, it may be worthwhile noting which tubes had tussock from a certain plant and analysing the growth and survival of larvae fed on those different plants to see if host plant has an effect on larval development.

The splitting process could also have had a detrimental effect on the tussock, regardless of its original health status. Great care was taken to ensure that the roots of each segment of tussock remained intact during separation. However, tussock does not normally grow in such small pieces. It was noted that immediately following separation and replanting, all the tussock segments appeared to wilt and lose some colour. They regained their healthy appearance over the course of the experiment. This initial decline in health was not observed amongst the other plant types and may have put the tussock-fed larvae at a slight disadvantage to begin with.

The 50 tubes containing *Raoulia* also had their plants sourced from less than 50 individual plants. Large *Raoulia* plants (>2 m²) were used to provide up to six tube segments. Up to four segments were collected from medium-sized plants (>1 m²), using the same technique as for large plants. Smaller plants had 1-2 segments taken. The same issues describe for tussock may also be applicable in this case, although the relatively high survival rates of larvae fed on *Raoulia* would indicate that if there was an effect, it was small.

It is possible that some adults were coming to the end of their natural lives when translocated. The subsequent death of an adult may not have been due to the conditions within their particular enclosure. However, there is no way of distinguishing those that died naturally and those that died because of sub-standard conditions at their translocation site from the data available (see Chapter 3). It follows from this that a lack of larvae may not be due to poor conditions.

There were four instances where both adults we confirmed dead. One of these enclosures produced a single larva; the rest did not produce any larvae. There may have been more instances of adult death, but without recovery of a body, this could not be confirmed. It may be worth investigating whether adult survival is significantly linked to larvae survival in future experiments and translocation situations where adult bodies can be recovered.

It would have been useful to have assessed whether there was a significant relationship between the weights of larvae immediately after excavation and the site that they came

from. This could be a good indication of whether larvae from a certain site were suffering developmentally, a possible indicator of a poor site for larval development.

Recommendations

In light of this study I would recommend that Cromwell chafer beetle larvae be assisted to attain access to all areas of the CCBNR which contain the soil sites tested. I further recommend that any planting efforts on the CCBNR centre on areas that provide a match with these soil sites. Planting efforts should include *Raoulia australis* and *Festuca rubra*, as these are the two plant types that result in the best larvae growth and survival. Conversely, sites that do not provide a soil match with the four sites identified in this study should not be a focus of any planting or translocation efforts. Plant types are more easily identified in the field than soil sites. *Raoulia* and red fescue are both easy species to identify by sight.

McGuinness (2001) lists 'surveying distribution and abundance' as the action required for the majority of threatened species listed in his document. This is because only by obtaining basic information of the abundance and distribution of a given species can it be given threatened status with any certainty. In the case of the Cromwell chafer beetles, surveying of distribution and abundance has been undertaken. It is recommended that comprehensive surveys, such as that undertaken by Hunt (2007) continue to take place on a regular basis in order to maintain a close understanding of this mobile population. This will enable conservation efforts to be applied more effectively and more economically.

Barratt *et al*. (2007) stated that "a major constraint to understanding and managing the *P. lewisii* population in the CCBNR is a lack of knowledge of larval host plant associations." In an ideal world, nutritional analyses of all plants types found on the reserve, especially those plants which are particularly associated with the beetles, historically or currently, such as *Poa cita* and *Raoulia australis*, would be conducted. This would provide the ideal complement to the growth and survival data of this study, and the behavioural feeding observations of Barratt *et al*. (2006).

Haight *et al*. (2000) stated that "Managers planning a translocation must decide on the number of individuals to introduce, the number and timing of introduction, the method of introduction, how to closely monitor the results of the translocation, and the allocation of a limited budget among these activities." Based on the results of this study, enclosures as a means of population establishment appear to fulfil several of these requirements. Firstly, they are easy to monitor. Adult beetles can be seen moving about in the enclosures at night and eggs and larvae can be predictably found within, or at least very nearby, enclosures. Secondly, enclosures are cost-effective as they require only an initial construction cost and very little additional equipment or maintenance each year.

References

Dopson, S.R., de Lange, P.J., Ogle, C.C., Rance, B.D., Courtney, S.O., Molloy, J. (2000). The conservation requirements of New Zealand's nationally threatened vascular plants. *Threatened Species Occasional Publication No. 13*, Department of Conservation, Wellington.

Griffith, B., Scott, J.M., Carpenter, J.W., Reed, C. (1989). Translocation as a species conservation tool: Status and strategy. *American Association for the Advancement of Science*, 245, 477-480.

Haight, R.G., Ralls, K., Starfeild, A.M. (2000). Species translocation strategies when population growth and future funding are uncertain. *Conservation Biology*, 14, 1298-1307

Hunt, D. (2007). Monitoring of the Cromwell Chafer Beetle (*Prodontria lewisii*) by Pitfall Trapping and Night Searches. *Department of Conservation, Dunedin* (internal report).

International Union for Conservation of Nature and Natural Resources (IUCN), "Position Statement on the Translocation of Living Organisms: Introductions, Re-introductions, and Re-stocking" (IUCN Council, Gland, Switzerland, 4 September 1987).

Mark, A.F. (1985). The botanical component of conservation in New Zealand. *New Zealand Journal of Botany*, 23, 789-810.

Siminov, E. (2009). Differences in habitat use, daily activity patterns and preferred ambient temperatures of adult and neonate *Gloydius halys halys* from an isolated population in southwest Siberia: preliminary data. *Herpetology Notes*, 2, 1-7.

Stevens, G., McGlone, M., McCulloch, B. (1988). *Prehistoric New Zealand*. Reed, Auckland.

Rueda, M., Rebollo, S., Bravo, L.G. (2008). Age and season determine European rabbit habitat use in Mediterranean ecosystems. *Acta Oecologica – International Journal of Ecology*, 3, 266-273.

Soulé, M. E. (1985). What is Conservation Biology? *Bioscience*, 35, 727-734.

Watt, J.C. (1976). A biological survey of New Zealand? *New Zealand Entomologist*, 6, 138-143.

Watts, C., Stringer, I., Sherley, G., Gibbs, G., Green, C. (2008). History of weta (Orthoptera: Anostostomatidae) translocation in New Zealand: lessons learned, islands as sanctuaries and the future. *Journal of Insect Conservation*, 12, 359-370.

Chapter Five: General Discussion

Traditionally, it has been thought that Cromwell chafer beetles have a close association with silver tussock (*Poa cita*). However, a plant survey conducted in this study found only one individual silver tussock in 75 quadrats placed in known beetle hot-spots (Chapter 2). It is clear from this result that the beetles can and do exist without the presence of this plant. Adult breeding experiments show that adults breed successfully without silver tussock (Chapter 3). Fifteen adult breeding enclosures were randomly distributed at each of the five sites on the Reserve. None of these enclosures contained silver tussock and yet larvae were produced at every site. Larval feeding experiments also support this conclusion. Larvae fed only silver tussock did significantly worse in terms of growth and survival than larvae fed on *Raoulia* or grass (Chapter 4). Not only do chafer beetles appear to be indifferent to the presence of silver tussock when it comes to breeding, they appear to be hampered by it when feeding. Any lingering idea that Cromwell chafer beetles may be associated with silver tussock can be well and truly dispensed with.

These results beg the question 'what do chafer beetles feed on, if not silver tussock?' Larval feeding experiments showed that larvae fed solely on *Raoulia* have higher survival rates than larvae fed on grass and significantly higher survival rates than larvae fed on silver tussock. However, larvae fed solely on grass have higher growth rates than those fed on *Raoulia* or tussock (Chapter 4). It may be possible that chafer beetles do best when grazing on a variety of different plants. A nutritional analysis of different plant species found on the Reserve, coupled with larval and adult beetle preference experiments would go a long way to answering this question. Plant survey data found in this study (see Chapter 2) indicates that a wide variety of both native and introduced plants grow in close quarters across the entire Reserve. It would seem that the beetles are either naturally adept at surviving within this botanic diversity, or else they have adapted to it. Certainly the adults eat a variety of plants, including the relatively recently introduced St John' wort (personal observation; feeding videos are available from the author upon request). This observation gives support to the theory that the beetles are not overly selective eaters and are able to consume a wide variety of plant food sources. This is good news from a management perspective, as it means that no specialised planting need take place. The

botanic diversity of the Cromwell Chafer Beetle Nature Reserve (CCBNR) appears to have established of its own accord and is therefore likely to remain without human aid.

The closely packed and highly variable nature of the plants surveyed in this study would suggest that the root systems available for larvae to feed on below ground are similarly variable. Given that larvae have been shown to be able to grow and survive across several different plant types, it would seem that they too are adaptable to a variety of feeding conditions. Certainly a larva which was selective in the roots that it fed on would have to travel further than a larva which was able to feed on the majority of roots it came across. It would thus seem that a non-selective larva would be at an advantage compared to a selective larva when confronted with a variable root system, as is likely on the Reserve. It may be that larvae do best when given a variety of food sources. For example, if the high survival rates observed in larvae fed on *Raoulia* were combined with the high growth rates observed in larvae fed on grass in this study, perhaps larval success could be improved overall. This experiment could be conducted relatively easily in a controlled laboratory setting.

Plant ground cover estimates give further weight to the argument that a hypothetical non-selective larvae may do better than a selective one. There was no significant difference in percentage ground cover across any of the sites sampled. Highly varied plant communities are packed closely together across all sites. A larva that is able to make the most of this variable food source would be at an advantage.

Soil particle size is also fairly uniform across all sites. Because survival varied between sites but particle size did not, it is clear that a factor other than particle size is responsible for this discrepancy. The fact that the soil particles sizes did not vary significantly between any of the sites does not allow any conclusions to be drawn about the tolerance of chafer beetles to differing soil particle sizes. It may be that the uniformity of the particle sizes across the only site at which chafer beetles are currently found – the CCBNR – indicates that the beetles are highly adapted to this particular size of soil particle. It would be interesting to sample the particle sizes of soil in the surrounding area and of other potential habitat areas and ideally couple these data with experimental translocation results in order to answer this question. If soil particle size in the

surrounding areas differs significantly from that of the Reserve, this may indicate that soil particle size is a barrier to population expansion.

One soil variable that did differ between sites was soil density. The Lindis soil site was significantly denser than those of the CCBNR at a depth of 11-20 cm and significantly denser overall. An increase in soil density may result in increased energy expenditure by both adults and larvae in order to burrow for shelter during the day in the case of adults, and locate plant roots to feed on in the case of the larvae. More densely packed soil may also limit oxygen level beneath the surface, which in turn may inhibit larval development. Denser soil may also impact on plant growth, which in turn may impact of chafer beetle feeding. It may be that plant root masses vary in their spread and depth within the soil column when soil density varies. The effects of increased soil density on chafer beetle survival may be many and varied and further testing would need to be conducted before these effects can be separated. In the meantime, soil density alone is a reasonable indicator of chafer beetle survival and can be employed when testing potential translocation areas.

The Lindis site had significantly lower adult and larvae survival. Although the plant types at the Lindis were similar to those of the CCBNR, the soil varied significantly. Because the relationship between soil site and plant type did not impact on chafer beetle survival across any of the sites, the survival of larvae at the Lindis site cannot be improved by the addition of known chafer beetle food plants. It appears that the Lindis site is unsuitable for chafer beetle survival. It is not recommended that chafer beetles be established at the Lindis site.

Soil texture also differed between the Lindis site and those of the CCBNR. Despite the author performing four times more enclosure excavations at the CCBNR than at the Lindis site, skin abrasions were only ever encountered during excavation at the Lindis site. Initial examination by feel and with the use of a microscope indicated that there was a discrepancy between the coarseness of the Lindis and the CCBNR soils. Further scientific examination of the granules from each site may go some way to explaining this difference. It is possible that chafer beetles prefer sand with a greater number of edges per sand granule, thus less acute angles on each edge, resulting in a smoother texture.

Counting the sharp edges of sand granules could provide a simple and cheap test to determine whether a soil site could provide suitable habitat for chafer beetles.

Aside from the Lindis site, the only other inland sand dune system in Central Otago region is located in Alexandra, 30 km south of Cromwell. This sand dune has been planted in *Pinus radiata* and is used as a recreational area for walkers, cyclists and motorcyclists. At first glance, this area appears to be unsuitable for chafer beetles, given the extreme differences in dominant vegetation type between Alexandra and the CCBNR and the use of the former as a recreational area. However, some of the plants found on the CCBNR are also found at the Alexandra sand dune (author's personal observation). The major vegetative difference is the presence of pine trees in Alexandra. Given that chafer beetles appear to be tolerant to a wide variety of plant food sources, it may be worth testing their tolerance to *Pinus* sp. as a food source. However, if chafer beetles are tolerant to *Pinus* sp., this begs the question why have they not moved into the pine tree areas adjacent to the CCBNR? It may be that beetles are located in these areas and no searches have yet been conducted to locate them. However, given the poor survival of chafer beetles at the CB site, which was in close proximity to pine trees, it is possible that chafer beetles are not tolerant of pine trees. Nevertheless, given the extremely limited current range of chafer beetles, it seems foolhardy to completely disregard a potential new habitat before conducting conclusive tests to rule it out.

Given that chafer beetles are tolerant to a wide range of plant species, it would seem that the simplest way to test the suitability of a potential new habitat is to focus on the soil. Chafer beetles appear to have a low tolerance for dense soil and possibly a low tolerance for coarse soil. If the Alexandra sand dune is to be considered as a potential new habitat, it is recommended that the soil be the focus of any pre-translocation tests. Recreational use may have compacted the Alexandra sand dune to the point where it is not suitable for chafer beetle habitation. However, not all areas of the dune are used for recreation and some may remain un-compacted. There are also some areas which are devoid of pine trees. These areas may be suitable for chafer beetle translocation should the soil be deemed suitable. Preliminary examination of the Alexandra soil by hand suggested that it is closer to the soil found on the CCBNR than it is to that of the Lindis.

While habitat analysis is a vital part of translocation preparation, the animals themselves are equally important. In this study, adult survival was significantly correlated with larval survival at all sites except for CB. Adult survival could therefore be used as an indicator of larval survival when testing potential translocation sites. Adult beetles are much easier to locate than larvae and would therefore be less time consuming and less expensive to translocate. The reduced nutritional demands of adults compared to those of growing larvae may mean that they are able to survive longer in experimental habitats. They can also move greater distances than larvae in a short period of time by travelling over the ground at night. Recording this movement of adult beetles in itself may provide a useful indicator of suitable habitat. As well as being positively correlated with larvae survival, adult survival of one sex is correlated with the survival of the other sex. It may be prudent to use only male beetles for experimental translocations in order to avoid losing potentially gravid females. If similar correlations between adult/larvae and male/female survival are found in other long-lived New Zealand invertebrates species, this method may prove useful for translocating a variety of species.

As well as the endangered species in question, other species need to be considered when planning translocations. The discovery in this study that adult chafer beetles consume the leaves of St John's wort plants raises the issue of competition. St John's wort beetles (*Chrysolina hyperici*) are present in very large numbers on the CCBNR and are capable of reducing entire plants to stalks in one night (author's personal observation). Since St John's wort beetles are a valuable biological control tool for agricultural systems, their complete removal via biological or chemical control is not practical. However, their presence is certainly something to bear in mind when making management decisions regarding the conservation of the chafer beetle. Any translocations to areas where St John's wort beetles are prolific may mean that the available food sources for the adult beetles are reduced and this should be taken into account when selecting translocation sites and placing breeding enclosures.

Another species that has been identified as a potential threat to chafer beetles is earwigs (Barratt, *et al*., 2006). This study found no evidence of negative interactions between earwigs and chafer beetles. However, only larvae and adult beetles were investigated in this study. Eggs were not monitored for earwig predation and it may be that this occurs. The question of earwig predation on chafer beetle eggs could be answered in a laboratory

experiment. However, given the value of chafer beetles, there may be ethical concerns surrounding the deliberate destruction of eggs. The eggs of a substitute species such as the common grass grub (*Costelytra zealandica*) could be used to determine the extent to which earwigs predate Scarabaeidae eggs.

The factors affecting the success of translocations are many and vary widely between species. This study goes some way to identifying the factors necessary for the successful translocation of the critically endangered Cromwell chafer beetle. Soil, in particular soil density and soil texture, appear to significantly affect chafer beetle survival. Plant type, while still important, is less well defined in terms of how it is likely to affect the outcome of a translocation. However, two plant species have been positively identified as good food sources for larvae (*Raoulia australis* and the grass *Festuca rubra*), and one plant species that traditionally has been thought to be associated with the beetles (silver tussock, *Poa cita*) has been identified as actually being detrimental to larvae survival. The management implications and suggested habitat tests for potential translocation sites identified within this study may also be applicable to other members of the genus and indeed to other invertebrate species. The genus *Prodontria* comprises 16 formally identified species (Barratt, 2007), all of which are endemic to the southern regions of New Zealand and many of which have restricted habitat ranges and are facing threatening habitat loss and degradation as well as predation. The methods used in this study could be amalgamated with the recovery plans, where present, of other species in the genus and used to create a more widely applicable '*Prodontria* recovery plan'.

New Zealand has a very large number of endangered invertebrates (McGuinness, 2001). Worldwide, the number of translocations that have focused on invertebrates is extremely low. Bajomi *et al*. (2010) found that a mere 3% of the 3,826 publications they examined targeted invertebrates. This is grossly disproportionate to the overwhelming majority of the animal kingdom that the invertebrates comprise. 'Taxonomic chauvinism', where the probability of a manuscript being published depends on the study species favoured by reviewers, has been identified as a potential source of this discrepancy (Bonnet *et al*., 2002). If this is the case, surely the solution is to actively encourage invertebrate studies and to consciously select and promote invertebrate papers for publication. The taxonomic bias present in the academic literature must translate into a taxonomic bias in the focus of research grants, teachings at universities and schools and, ultimately, within our society.

Given the dependence of so many food chains on the survival of invertebrate species, we continue to ignore their importance at our own and other species' peril. The sooner we understand more about the animals that comprise the vast majority of life on earth, the better equipped we will be to conserve all life.

General References

Bajomi, B., Pulin, A.S., Stewart, G.B., Takacs-Santa, A. (2010). Bias and dispersal in the animal reintroduction literature. *Fauna & Flora International, Oryx*, 3, 358–365.

Barratt, B.I.P. (2007). Conservation status of *Prodontria* (Coleoptera: Scarabaeidae) species in New Zealand. *Journal of Insect Conservation*, 11, 19-27.

Barratt, B.I.P., Barton, D., Ferguson, C.M. (2006). *Prodontria lewisii Broun (Coleoptera: Scarabaeidae): Population monitoring; larval feeding preferences; predation by earwigs.* Report for the Department of Conservation.

Bonnet, X., Shine, R., Lourdais, O. (2002). Taxonomic chauvinism. *Trends in Ecology & Evolution*, 17, 1–3.

McGuinness, C.A. (2001). The Conservation Requirements of New Zealand's Nationally Threatened Invertebrates. *Threatened Species Occasional Publications No.20, Department of Conservation*.

Redak, R.A. (2000). Arthropods and multispecies habitat conservation plans: are we missing something? *Environmental Management*, 265, 97-107.