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**The conservation of native New Zealand butterflies in the  
ecologically enhanced farming landscape of  
Waipara, northern Canterbury**

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A thesis  
submitted in partial fulfilment  
of the requirements for the Degree of  
Doctor of Philosophy

at  
Lincoln University  
by

Mark Gillespie

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Lincoln University  
2010

Abstract of a thesis submitted in partial fulfilment of the  
requirements for the Degree of Ph.D.

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by

**Mark Gillespie**

Conventional farming landscapes suffer from low levels of biodiversity, and therefore a paucity of nature's services – the products and services that ecosystems provide humans. This is largely due to the fragmentation and loss of habitats caused by intensive farmland management. However, attempts have been made recently to combine the theoretical principles of agricultural production and biodiversity conservation as they can be mutually beneficial. In the northern hemisphere, this relationship is formalised in the form of agri-environment schemes with varying degrees of success. Such compensatory schemes are not available in New Zealand so the incorporation of environmentally benign techniques that promote nature's services is driven by research institutions and landowners themselves. The objective of this thesis was to quantify the effects of one such project on a group of non-target organisms in an intensively managed landscape in Canterbury. The native New Zealand butterfly fauna has not been studied in detail to date, but future conservation of these species may rely on less intensive farming methods. Enhanced butterfly populations may also act as an indicator of environmental health and subsequently assist in the marketing of wine. Measures were therefore also sought to enhance butterfly populations in such landscapes.

Initial survey work showed that Waipara vineyards are poor habitats for butterflies. Planting plots involved in the "Greening Waipara" project did not support a large number of butterflies, probably because they were not designed with butterflies in mind and were too isolated from remnants. The habitat factors that were most important in explaining variation in butterfly populations were native shrub cover, legume cover (the host plant family of the most abundant butterfly species, *Zizina oxleyi*) and flower abundance. Enhancing these features in future conservation efforts is likely to enhance butterfly populations, although many other aspects of butterfly conservation are discussed.

Subsequent experiments sought to identify specific conservation issues in the Waipara region which the Greening Waipara project could help to address in future. In molecular and

morphological analyses of the two *Zizina* species in New Zealand, the endemic *Z. oxleyi* and the introduced *Z. labradus*, contrasting results were found concerning their distributions. Such incongruence is not rare in the literature and may indicate recent speciation or hybridisation. These hypotheses are discussed in relation to the evolutionary history of the two species in New Zealand and the conservation of *Z. oxleyi*.

Field and laboratory experiments supported the finding that nectar source provision may be suboptimal for butterflies in Waipara. In field survey work, perennial ‘weed’ species were the most visited flower species by adults of the common copper (*Lycaena salustius*). However, subsequent field and laboratory work showed that native *Hebe* spp. and *Fagopyrum esculentum* are more attractive and impart greater fitness benefits to adults than some of the ‘weed’ species currently available.

In addition, host plant provision for *L. salustius* may not be optimal in Waipara. In laboratory performance experiments, larvae performed poorly on common host plant species. Conversely, females laid most eggs on *F. esculentum*, a novel host for this species on which larvae also developed poorly. Explanations for this choice by females may relate to the chemistry of *F. esculentum* relative to ancestral hosts. In the absence of *F. esculentum*, females preferred *Muehlenbeckia astonii*, a rare ancestral species which scored highly in larval performance assays.

This thesis represents a relatively rare investigation into butterfly ecology in New Zealand and demonstrates the complexity in conserving butterfly populations. While research based recommendations are tentatively suggested for the enhancement of populations using inferences from northern hemisphere studies, further study is evidently required and the species encountered provide excellent opportunities for doing so.

**Keywords:** *Zizina oxleyi*, *Z. labradus*, *Lycaena salustius*, butterfly conservation, butterfly transect, habitat, COI, host plant, oviposition preference, larval performance, fitness, nectar, hybrid, speciation, evolution, choice, *Fagopyrum esculentum*, *Hebe*

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# Chapter 1 Introduction

## 1.1 GENERAL INTRODUCTION

Wide scale anthropogenic changes in land use and the intensification of agricultural practices to maximise production are widely regarded as one of the key drivers behind biodiversity loss (Robinson and Sutherland 2002, Tilman *et al.* 2002). Although during previous agricultural eras so-called ‘traditional’ human land uses which stall vegetation succession created a heterogeneous landscape of benefit to many groups of species (WallisDeVries *et al.* 2002, Schmitt and Rakosy 2007), post-war ‘modern’ agriculture has been responsible for large scale species diversity decline (Warren 1993a,b, van Swaay and Warren 1999, Thomas *et al.* 2004). Local causes of decline have included shortened crop rotation cycles and the use of agrochemicals, while at the landscape scale the amalgamation of homogenous fields has led to both uniform, simple landscapes that characterise modern agriculture, and isolated and fragmented natural habitats that are unproductive. The result is reduced biodiversity and altered community structure (Steffan-Dewenter and Tscharniske 2002, Tscharniske *et al.* 2002b, Ockinger and Smith 2007, Tylianakis *et al.* 2007), typified by the release of herbivores from natural enemy control for example (Tscharniske *et al.* 2005, 2007).

Agriculture is managed in this way in order to maximise the production of food and fibres, and these are just two of the services that nature provides to society (Fiedler *et al.* 2008). However, the range of ecosystem services (ES), the means by which nature “sustains and fulfils human life” (Daily 1997), that biodiversity can provide are much wider and the role of agro-ecosystems to facilitate their delivery could be much greater (Tilman *et al.* 2002, Swinton *et al.* 2006). Ironically though, current ES such as pest and weed suppression, soil nutrient cycling and clean air and water provision are threatened by modern agriculture (Costanza *et al.* 1997, Landis *et al.* 2000).

Despite the threats to and the intrinsic importance of biodiversity to the global food supply and society in general (Costanza *et al.* 1997, Daily 1997), agricultural land use and biological conservation have traditionally been studied separately due to perceived incompatibility. However, recent work has combined the issues (Pimental *et al.* 1992; Bengtsson *et al.* 2003, Diaz *et al.* 2006, Zehnder *et al.* 2007) and sustainable ‘habitat management’ techniques are considered important tools in enhancing ecosystem services in agricultural landscapes (Landis *et al.* 2000, Reid *et al.* 2005). The conservation of fragments of natural systems is no longer considered enough to maintain or improve biodiversity levels (Novacek and Cleland 2001, Merckx *et al.* 2009), and recognition is increasing that

agriculture and conservation can be mutually beneficial: agricultural conservation management can make improvements to landscape biodiversity, which can simultaneously provide benefits to production through valuable ecosystem services without jeopardising profit (Daily 1997; Kremen 2005; Tscharntke *et al.* 2005; Zehnder *et al.* 2007). With escalating energy costs and demand for food caused by population increase, the production and security of food will increasingly rely on natural ecosystem services in future (Tilman *et al.* 2002, Diaz *et al.* 2006, Tscharntke *et al.* 2007).

This relationship has been formally recognised by many countries through schemes that involve “payments for ecosystem services” (PES) (FAO 2007) where farmers are given financial incentives to enhance ES provision on their land. In Europe and the US these take the form of agri-environment schemes (AES), and in countries such as Kenya, Tanzania and Costa Rica government backed projects tackle local problems (FAO 2007). However such centrally governed plans do not exist in countries such as New Zealand and the adoption of beneficial practices are often driven by university or research institution led initiatives. Nevertheless, research into the benefits of combining the fields of agriculture and biodiversity is gathering pace in the southern hemisphere, and interest in the benefits of habitat management practices to overall biodiversity is growing (Wade *et al.* 2008, Fiedler *et al.* 2008).

This thesis aims to contribute to research surrounding the importance of habitat manipulation efforts to incidentally enhancing ES not directly targeted, with specific emphasis on native butterfly species, a group of organisms thought to act as indicators of overall biodiversity as well as being of general conservation value. While in New Zealand the benefits of certain flower species have been studied in relation to single ecosystem services such as pest biological control (Tylianakis *et al.* 2004, Berndt *et al.* 2006, Lavandero *et al.* 2006, Robinson *et al.* 2008), little work has been conducted concerning other services emerging from habitat management (Fiedler *et al.* 2008). If additional benefits to general biodiversity can be shown to accrue from the application of habitat management techniques, such projects are likely to appeal to a broader range of funding agencies and farmers alike (Rosenzweig 2003, Fiedler *et al.* 2008).

This introduction provides a context for the subsequent chapters which detail the laboratory and field studies conducted to meet the goals briefly outlined above. Below, the relationship between agriculture and biodiversity is explored in more detail, before a brief review of agri-environment schemes. The region of the field studies is then introduced, followed by a summary of recent butterfly research in relation to the New Zealand fauna and to agriculture in New Zealand, Europe and the USA. The chapters that follow are written in

the style of scientific journal articles and are therefore self-contained. In including an introduction and discussion, each chapter contains a specific review of the literature, while the thesis introduction and conclusions chapters aim to synthesise all experimental chapters. Where possible, repetition has been avoided.

## **1.2 AGRICULTURE AND BIODIVERSITY**

### **1.2.1 Ecosystem services: The benefits of conservation to agriculture and society**

The role of biodiversity in providing benefits to humans was first raised by Potts and Vickerman (1974) who linked invertebrate biodiversity to crop pest population dynamics. Since then, work on the relationship between functional biodiversity and ES has found a number of benefits which can be categorised as provisioning, regulatory, supporting or cultural services (Fiedler *et al.* 2008) depending on their dominant function.

In addition to basic provisioning services of food and fibres, regulatory services such as biological pest control (Potts and Vickerman 1974, Landis *et al.* 2000, Lee and Heimpel 2005), disease reduction (Jacometti *et al.* 2007), and improved crop pollination (Tscharnkte *et al.* 1998, Bosch and Kemp 2001) are functions that have the potential to be enhanced on farmland. Supporting services include soil fertility and moisture maintenance and nutrient cycling, both of which can be enhanced with well placed habitat management (Nicholls and Altieri 2004). Finally, cultural services are the spiritual values attached to aspects of biodiversity, the recreational benefits provided by natural areas and the aesthetic value that people attach to recognisable facets of the natural world (Costanza *et al.* 1997, Fiedler *et al.* 2008). Cultural services are more difficult to quantify but studies have discussed the visual appeal of flowering plants in agricultural landscapes (Frank and Shrewsbury 2004, Shrewsbury *et al.* 2004, Ellis *et al.* 2005) and the ‘willingness to pay’ of the general public for general and specific environmental improvements (Takatsuka *et al.* 2005, Forbes *et al.* 2009).

Although the relationship between ecosystem function and biodiversity is still ambiguous (Hooper *et al.* 2005), and increasing biodiversity in agriculture *per se* may in some cases be counterproductive (Baggen and Gurr 1998, Landis *et al.* 2000, Lavandero *et al.* 2006), Cardinale *et al.* (2006) suggested the most common relationship was positive but decelerating with increasing species richness. ES have also been calculated as having an annual average global economic value of US\$33 trillion (Costanza *et al.* 1997). Clearly, the conservation of a wide range of plant, insect and animal species can contribute to agricultural production and cost effectiveness, while ignoring declines is likely to lead to escalating costs and agricultural product shortages in the future.

### 1.2.2 The role of agriculture in conservation

The benefits or resulting enhancements to ES of habitat management are usually studied individually, but in theory farm-scale improvements are likely to lead to ‘stacked ecosystem services’, a suite of beneficial outcomes beyond the targeted result (Fiedler *et al.* 2008). If stacked services can be demonstrated, agriculture can be shown to benefit the conservation of non-target and often threatened species without jeopardising productive output. For example, Schmitt *et al.* (2008) found that 8 out of 34 butterfly species associated with vineyard-fallow mosaics (a patchwork of vineyard blocks and fields left unseeded and unploughed for one or more growing seasons) were listed in the German Red Data Book. Similarly, the addition of unsprayed and unmowed ‘beetle banks’ on British farms provide an alternative refuge for arthropods ensuring the persistence of natural enemies from one season to the next (Thomas *et al.* 1991). The banks have subsequently resulted in increased populations of birds such as the skylark (*Alauda arvensis* L.) and grey partridge (*Perdix perdix* L.) (Thomas *et al.* 2001) and mammals such as the harvest mouse (*Micromys minutus* Pallas) (Boatman 1999) by providing nesting sites and an important source of winter food. ‘Conservation headlands’ which are designed to provide winter food for gamebirds are also beneficial to a number of butterfly species (Dover *et al.* 1990, Dover 1991).

Improving the conservation importance of agricultural land can take various forms, usually involving reducing management intensity. Insect diversity can be increased by planting or allowing (by refraining from spraying herbicides) rich non-crop habitats and borders for example (Dyer and Landis 1997, Holland and Fahrig 2000, Gurr *et al.* 2003) and through organic management (Bengtsson *et al.* 2005, Zehnder *et al.* 2007). At the landscape scale, improving the connection between farmland and conserved natural habitats can increase population densities and dispersal of invertebrates, reducing extinction risk (Steffan-Dewenter and Leschke 2003, Tscharntke *et al.* 2007). While large conservation areas might be preferable to biodiversity conservation, a spread of several well connected small areas over a large region may also maximise biodiversity, as has been found for butterflies and grasshoppers in calcareous grasslands (Tscharntke *et al.* 2002a). Networks of connecting linear features like hedgerows, grass banks and field margins may be substandard habitat for many specialist species, but they can make positive contributions to farmland vertebrate and invertebrate diversity, pest suppression and to the buffering of the environmental impact of modern agricultural operations (Kinross *et al.* 2004). Increasing the heterogeneity of agricultural land in general can improve the biodiversity of relatively barren areas (e.g., Tscharntke *et al.* 2005; see also Tscharntke *et al.* 2007 for a review). For example, agroforests

have been shown to have similar species compositions of bees and wasps to semi-natural forests compared to monocultural plantations (Tylianakis *et al.* 2005), and in Europe many weeds in arable farmland are also threatened species (Roschewitz *et al.* 2005).

### **1.2.3 Schemes to promote widespread adoption: agri-environment schemes**

Despite the potential of improving individual fields and landscapes to the benefit of nature conservation while contributing to farming profits and reducing external costs (e.g., Jacometti *et al.* 2007, 2010), universal implementation by farmers remains a challenge (Warner 2006). In Europe and the US, adoption is being encouraged through agri-environment schemes (AES), instruments which provide incentives for farmers to use more environmentally friendly practices, by compensating them for any loss in income as a result. This is an example of ‘Payment for Ecosystem Services’ (PES), a recent approach to promoting the adoption of practices that enhance ES, where those that benefit from ES make payments to those that provide them (FAO, 2007). Twenty six European countries currently offer such compensation (Kleijn and Sutherland 2003), and their implementation is considered vital if biodiversity losses are to be reversed (Tschamntke *et al.* 2007, Warren *et al.* 2008). The schemes have had mixed results in practice (Herzog *et al.* 2005, Knop *et al.* 2006, Lovell and Sullivan 2006, Kleijn *et al.* 2006, Holzschuh *et al.* 2007). A recent meta-analysis of agri-environment scheme evaluations found that just over half of the taxa included in the study increased in richness or abundance compared with control sites (Kleijn & Sutherland 2003). Other schemes including the National PES Programme in Costa Rica and the Pro-Poor Rewards for Environmental Services in Africa scheme (PRESA) in Kenya and Tanzania are other examples of incentive schemes with focus on water quality, watershed function and carbon sequestration (FAO 2007).

PES schemes should probably be regarded as ‘works in progress’. Research into AES has frequently identified areas of improvement (Kuussaari *et al.* 2007, Rundlof *et al.* 2008, Merckx *et al.* 2009) and success often depends on external factors and the scale of AES in the surrounding landscape (Bergman *et al.* 2004, Whittingham 2007, Rundlof *et al.* 2008). In any case, it is important for governments to update schemes when new research is available (Merckx *et al.* 2009), and for research to help elucidate aspects of the schemes that can be modified to maximise the provision of services (Fiedler *et al.* 2008).

### **1.2.4 Schemes to promote widespread adoption: Greening Waipara, New Zealand**

Centrally administered compensation schemes like AES do not exist in countries such as New Zealand, making landowner adoption of habitat management practices all the more

difficult. However, in Canterbury a regional scale approach to agricultural land improvement is attempting to combine research outcomes with landowner enthusiasm. The Greening Waipara project (<http://bioprotection.org.nz/greening-waipara>) is led by Lincoln University and Landcare Research and funded by national and local government as well as individual landowners and a Japanese company. Waipara is a major wine growing region where highly modified 'colonial' landscapes are the result of having been largely stripped of native vegetation and are much simpler than their European counterparts (Tscharrntke *et al.* 2007). The project is a scheme that follows on from success in the Conservation Biological Control of the light brown apple moth, *Epiphyas postvittana* Walker (Berndt and Wratten 2005, Berndt *et al.* 2006). From the initial targeting of this one ecosystem service, the project has moved on with aims to provide a suite of services associated with the planting of native species within vine blocks and around vineyards that opt for participation (Fiedler *et al.* 2008).

Currently over 50 vineyards and other properties are taking part in the six-year government-funded project. Native shrubs and trees planted within and surrounding the properties are aimed at restoring services provided by previous ecosystems and improving habitat for other native species (Wade *et al.* 2008). Subsequent tourism and marketing benefits are added to the ecological benefits of this conservation (McKinnon 2005). For example, 'biodiversity trails' constructed close to wineries provide visitors with opportunities to learn about biodiversity via information boards on a path through large planting areas. Visitors are also greeted with the aesthetic value of a heterogeneous landscape and the perceived environmentally friendly farming practices.

Research under way in the region is focussed mainly on the recognisable economic values of heterogeneity such as supporting and regulatory services (Fiedler *et al.* 2008) in order to boost participant confidence. However, there have been no studies to evaluate the role of the planting scheme in improving cultural services of biodiversity, i.e. the aesthetic values attached to biodiversity conservation (Fiedler *et al.* 2008). Although studies using questionnaires of visitors show a positive attitude towards the Greening Waipara biodiversity trails (Forbes *et al.* 2009), Fiedler *et al.* (2008) found that only 2 out of 34 studies of ecosystem service improvement in ecological restoration projects looked for services beyond pest suppression in evaluating plant species to be used for restoration in agriculture. However, in a tourism-dependent region such as Waipara, the aesthetic connotations of conservation can be beneficial. A study of the extra benefits derived from habitat manipulation can increase awareness of the ability of agricultural conservation management to produce 'stacked ecosystem services' to provide multiple benefits to society (Fiedler *et al.* 2008).

A potential solution to providing the evidence of stacked ecosystem services is to quantify the effects of habitat management on overall biodiversity. Butterflies have long been considered to be an important group of animals in monitoring and environmental assessment (Erhardt 1985) and they provide a cultural service due to their iconic nature, popularity and general conservation value (Dover 1996). The study of butterflies in Waipara may therefore help to establish links between habitat management and biodiversity, while simultaneously providing important basic information to enhance their conservation.

### **1.3 CONSERVATION OF BUTTERFLIES ON FARMLAND**

#### **1.3.1 The importance of butterflies**

While improving butterfly populations may not have a recognisable economic value to a farmer, it can be seen as a cultural service with a societal role. Butterflies carry a general conservation value (Dover 1996), are featured icons in many marketing campaigns and have been frequently described as sensitive indicators of environmental change and ecosystem health (Erhardt 1985, Erhardt and Thomas 1991, Oostermeijer and van Swaay 1998, Pywell *et al.* 2004, Poyry *et al.* 2005). The imagines of butterflies are well described, easy to identify in the field and have been shown to respond to environmental change relatively rapidly (Erhardt 1985, Erhardt & Thomas 1991, Scoble 1992, Waltz 2004, Thomas *et al.* 2004). The broad niche occupation of the different life stages of an assemblage of butterflies has resulted in their recommendation as proxies for plant diversity (Scoble 1992), other invertebrates (Brown 1991, Beccaloni and Gaston 1992, Thomas 2005) and other groups of organisms (Wilcox *et al.* 1986, Carroll and Pearson 1998, Thomas 2005). Of particular interest to a study of landscape manipulation, butterflies are also thought to be sensitive to the degree of heterogeneity of the surrounding farmscape (Bergman *et al.* 2004, Rundlof and Smith 2006). With this in mind, it is reasonable to expect that targeting butterflies in conservation efforts will often benefit other animal and plant species (van Swaay and Warren 1999, Fleishman *et al.* 2000, van Swaay 2002).

Yet even for the most well known fauna, the well studied UK butterflies, much detail is required before it can be stated which particular ecological conditions and environmental changes butterflies are indicating (Dennis 2004). Thus, using butterflies as indicators is not just a case of determining the presence or absence of an adult, but understanding the requirements of individual species and of a certain assemblage in order to make judgements on what community data can reveal about an area of habitat or a landscape.

### 1.3.2 Empirical research: Butterflies and Agriculture

In Europe, the last few decades has seen an alarming decline in the abundance of many species of butterfly (Pollard and Yates 1993, Warren 1993a, Pullin 1995, van Swaay and Warren 1999, Thomas *et al.* 2004) and factors such as urbanisation, road construction and the habitat loss associated with intensive agriculture are considered to be largely responsible (Thomas 1991, New *et al.* 1995, Pullin 1996, Maes and van Dyck 2001). Habitat fragmentation, chemical inputs and landscape homogeneity directly or indirectly create ecosystems inhospitable to butterflies (van Swaay and Warren 1999, Summerville and Crist 2001, Steffan-Dewenter and Tscharntke 2002, Tscharntke *et al.* 2002b, Benton *et al.* 2003, Rundlof and Smith 2006, Ockinger and Smith 2007). Declines of a similar nature have also been described for Japan (Sibatani, 1990) and North America (Pyle 1976, Swengel and Swengel 1999).

While the principal components of arable, pastoral and other types of farmland are unsuitable for the majority of butterflies (Thomas 1984), the discussion above concerning the importance of agriculture to biodiversity does apply to this group. Many farmland features associated with the traditional land uses are potentially vital for species survival (Dover 1994, 1996, Pywell *et al.* 2004, Schmitt *et al.* 2008). Hedgerows (reviewed by Dover and Sparks 2000), field margins (Dover 1994, Feber *et al.* 1996, MacDonald *et al.* 2000, Feber *et al.* 2007), green lanes (Croxtton *et al.* 2005), grass banks (Sotherton *et al.* 1989, Dover 1991), riparian features (Nelson and Anderson 1994, Nelson and Wydoski 2008), open areas of woodland (Greatorex-Davies *et al.* 1993, Dover 1999, Dover *et al.* 2000), grassland (Steffan-Dewenter and Tscharntke 2000), field nodes (Fry 1991) and other fallow or remnant areas of native vegetation (Shepherd and Debinski 2005, Schmitt *et al.* 2008, Franzen and Nilsson 2008) have all been shown to be important to individual species or butterfly species assemblages. Habitat management practices which conserve these features usually result in increased butterfly species richness or abundance or both.

In addition, organic farms tend to host greater numbers of butterflies than do conventionally managed farms (Rundlof and Smith 2006, Feber *et al.* 2007) and features of traditional agricultural management that maintain successional stages of vegetation such as grazing, mowing, burning and coppicing (Thomas *et al.* 1986, Munguira and Martin 1993) or rotational management (Sparks *et al.* 1994) are also important. The butterfly species associated with these habitats utilise many of the arable plant species, including ‘weeds’, as host plants or as adult nectar sources (Dover 1990, Tudor *et al.* 2004). Farmland features can also benefit butterflies in a number of other ways, however. For example, although hedgerows may impede the movement of some species (Dover and Fry 2001), these structures have been



shown to offer shelter, provide nursery conditions for host plants and perennial nectar sources and can act as conduits for movement between habitats (Dover 1990, 1991, Dover *et al.* 1992, Haddad 1999). In addition, shrubs in marginal areas can be important roosting locations (Dennis and Sparks 2006, Hardy and Dennis 2007), bare ground and other structures are important basking sites for the generation of body warmth (Gilbert and Singer 1975, van Dyck and Matthysen 1998) and different species may require high habitat heterogeneity for mate location (Shreeve 1992). In summary, as butterflies require many different structures and resources in addition to host plants and adult food to survive and persist (Dennis 2004), high butterfly species richness, abundance and diversity is closely linked to landscape heterogeneity (Bergman 2004, Dennis and Sparks 2006, Ockinger and Smith 2006).

Recent studies by Dennis *et al.* (2003) and Dennis and Sparks (2006) have led to the discussion that the definition of ‘habitat’ should be reassessed for butterflies. The mere presence of larval and adult food is not enough for the majority of species, often due to exacting requirements which can differ widely between species. Instead, features of the so-called matrix, areas between apparent habitat patches, might be utilised by adults at different times of the day and season (Hardy and Dennis 2007). Such findings highlight the need to understand individual species habitat requirements and their relation to the resources available on individual farms and across landscapes if changes to management are to benefit a number of species.

### **1.3.3 Empirical research: Butterflies in New Zealand**

Although New Zealand does not benefit from a long history of entomological interest, as Britain does (Pullin 1996), and thus lacks detailed historical data on butterfly population dynamics, much of lowland New Zealand is likely to have experienced a decline in its butterfly fauna due to the loss of native host plants (Gibbs 1980). Lowland New Zealand has come under similar or more severe agricultural pressures to counterpart regions in the northern hemisphere (McGlone 1989), so it is reasonable to expect that wildlife numbers have reacted in a comparable way. The fact that there are no endangered butterflies in New Zealand (Patrick and Dugdale 2000) may be by virtue of the dearth of long-term population monitoring records.

Surprisingly little is known about native butterfly population dynamics in New Zealand (Gibbs 1980, Patrick and Dugdale 2000, Barron *et al.* 2003). Besides the early work by the first European entomologists to reach New Zealand, work has been restricted to regional studies reported in national publications. Flux (1968) made flight period, sex ratio and abundance observations of three copper species (Lycaenidae) near Wellington and Craw

(1975) conducted a mark and release study on the same three species in Otago, with notes on food plants and flight behaviour. Gibbs (1980) completed the most comprehensive work on the New Zealand butterfly fauna to date in an attempt to bring published information in line with that available in Australia and Britain. However, in terms of ecological studies, little work has been conducted. Only pest control studies of the adventive cabbage white (*Pieris rapae* L. (Pieridae)) have engaged the international literature to any extent (Cameron and Walker 2002, Pearce *et al.* 2006, Creaser *et al.* 2008), and a recent suite of studies evaluated the non-target effects of an introduced biocontrol agent on the New Zealand red admiral (*Bassaris gonerilla* F. (Nymphalidae)) (Barron *et al.* 2003, 2004, Barron 2007).

While many of the plants known to act as hosts for some butterfly species are being re-introduced by the Greening Waipara project, they have not been included to target butterflies *per se*. However, evidence of the improvement of the conservation of butterflies could assist in the marketing of wine products to the consumer and of the project to government policy-makers and funders. The presence of butterfly species would also add an aesthetic dimension of appeal to eco-tourists, providing a visible sign that the process of wine growing in this region is less environmentally harmful than are more conventional agriculture practices.

For these reasons, the Greening Waipara project provides a unique opportunity to simultaneously study the population dynamics of native New Zealand butterflies in highly modified landscapes and to quantify the ‘stacked ecosystem service’ effect of habitat management on butterfly populations.

#### **1.4 CURRENT RESEARCH**

Against the backdrop of the Greening Waipara project, this thesis investigates the importance of enhanced ecosystem services to butterflies in a viticultural setting with the general aim of establishing if and how habitat management can provide non-target benefits to overall biodiversity. Due to the lack of knowledge surrounding much of the native and endemic butterfly fauna, it is impractical to incorporate autecological studies of all species present in a single thesis. It is therefore necessary and appropriate to attempt general survey techniques on a farm scale, and then focus more detailed ecological studies on the most common species and those of the most immediate conservation concern. For the purposes of this thesis, the term ‘native’ refers to species that are thought to occur naturally in New Zealand since prior to European settlement, and ‘endemic’ refers to those only occurring in New Zealand.

With the above remit in mind, Chapter 2 of this thesis details the results of survey work on six participating properties in Waipara to identify the species present and their use of

habitat features on vineyards. By incorporating plantings conducted as part of the Greening Waipara project, an evaluation of their effectiveness is also included. Regression techniques have been employed to identify biotic and abiotic features of the environment of most importance to butterfly abundance and diversity, an aspect of butterfly ecology not yet attempted in New Zealand or in vineyards. From this analysis, it became apparent that the most common species in these ecosystems are the endemic southern blue, *Zizina oxleyi* Felder & Felder (Lycaenidae), and the endemic common copper, *Lycaena salustius* Fabricius (Lycaenidae), which were subsequently chosen for more detailed work.

Since Gibbs (1980) suggested that *Z. oxleyi* may be hybridising with the introduced and closely related common blue, *Z. labradus* Godart, the issue has been stated as of conservation concern (Patrick and Dugdale 2000, Yago *et al.* 2008) without any corroborating evidence. Gibbs (1980) initially based hybrid identification on male genitalia dissection and suggested that the hybrid zone incorporated the Waipara valley. DNA analysis can be an important determinant of hybridisation (Sonnenberg *et al.* 2007) and although Yago *et al.* (2008) concluded from a phylogenetic study of all species in the genus that the above two species were unlikely to interbreed, an extensive molecular survey of New Zealand *Zizina* butterflies has never been conducted. Chapter 3 details the results of *Zizina* spp. collection from around New Zealand and the subsequent comparison of wing coloration, genitalia morphology and the sequences of a mitochondrial gene. The aim of this study was to establish a more accurate distribution of the two *Zizina* species in New Zealand, clarify the existence of hybrids and assess the implications of this on the conservation status of the endemic species, *Z. oxleyi*.

Numerous studies in the northern hemisphere have highlighted the importance of nectar sources to adult butterfly fitness (Murphy 1983, Boggs 2003, Mevi-Schutz and Erhardt 2003, 2005), and in butterfly abundance and species richness measures (Munguira and Thomas 1992, Murphy 1983, Shepherd and Debinski 2005) particularly in agro-ecosystems (Dover 1996, Clausen *et al.* 2001, Pywell *et al.* 2004, Ouin *et al.* 2004). However, there are mixed results concerning the effect of nectar abundance on butterfly population growth with positive (Feber *et al.* 1996, Forrester *et al.* 2005) and neutral effects (Waltz *et al.* 2004, Matter *et al.* 2009) reported. While there are no known negative effects of flower abundance on butterfly abundance, nectar sources in New Zealand agricultural landscapes tend to be adventive ‘weed’ species in the absence of native species. Nectar sources such as yarrow (*Achillea millefolium* L. (Asteraceae)) and Californian thistle (*Cirsium arvense* L. (Asteraceae)) may be important sources of nectar for New Zealand butterflies and may even be a limiting resource to butterfly populations on farmland (Dover and Sparks 2000), but they are unlikely to be encouraged by farmers due to their weed status. The work in Chapter 4 uses laboratory and

field techniques to establish nectar preferences of adults of *L. salustius*, with the aim of finding a suite of native nectar sources suitable to use in butterfly conservation efforts and which may be acceptable to landowners.

Chapter 5, the final experimental chapter, investigates a similarly important area of butterfly conservation. Larvae of *L. salustius* are thought to feed on any of the New Zealand *Muehlenbeckia* spp. Meisn. (Polygonaceae) or native dock (*Rumex flexuosus* Spreng (Polygonaceae)). However, some food resources can be more important than others for oligophagous herbivores (Kursar *et al.* 2006, Forister *et al.* 2009) which can impact conservation efforts. While one food species, *Muehlenbeckia astonii* Petri, is used in Greening Waipara plantings, there is no guarantee that this species is ‘preferred’ by ovipositing females or feeding larvae. Chapter 5 is a study into the oviposition and feeding preferences of *L. salustius*. Studies of this kind have been conducted in the northern hemisphere recently in order to investigate host range evolution and expansion. Results have shown positive (Singer *et al.* 1988, Bossart 2003), negative (Bossart and Schrieber 1995, Berdegue *et al.* 1998) and neutral (Valladeres and Lawton 1991, Fox 1993) correlations between female oviposition preference and larval performance. A positive correlation between choice and performance would help elucidate ideal plants to be used for conservation, while a negative correlation suggests additional factors at work in host plant choice.

The final chapter of the thesis draws the conclusions of the experimental chapters together and discusses their contribution to the understanding of New Zealand butterfly ecology and the role of ecosystem service enhancement in their conservation, both in terms of the Greening Waipara project and possible future enhancements.

## **Chapter 2 The importance of viticultural landscape features and ecosystem service enhancement for native butterflies in New Zealand vineyards**

### **2.1 ABSTRACT**

Butterfly populations have declined alarmingly in Europe and the USA and the fragmentation of habitats in intensively managed farming landscapes is often considered to be partly responsible. Although relatively little is known about the ecology of the New Zealand butterfly fauna, agricultural landscapes in lowland New Zealand are managed in a similar way to those in Europe and ecosystem services in these landscapes are generally at a low level. In the northern hemisphere, the problem is tackled through agri-environment schemes, but such farmer compensation is not available in New Zealand. Instead, landowner and research lead initiatives are currently the only potential solutions. One such project in Canterbury, the Greening Waipara project, seeks to return native plants to viticultural landscapes, and while research has sought to quantify economic benefits, there has been no work to establish if the plantings are improving or are likely to improve non-target invertebrate biodiversity, for example non-biocontrol agents. In the first study of its kind in New Zealand, ‘Pollard walk’ butterfly surveys are applied to vineyards to gain insight into the population dynamics of native butterflies, and linear regression techniques are applied to the data to identify the most important habitat features to butterfly distribution. While the native planting areas were not important for butterflies, remnant patches of native vegetation in unproductive areas were vital for sedentary species, not least due to the presence of host plants and nectar sources. These results are discussed in relation to the conservation of native species and recommendations are made for enhancing habitats on New Zealand vineyards and farmland in general.

### **2.2 INTRODUCTION**

In some countries such as the USA, the UK and Japan, the decline of butterfly populations have been inexorably linked with changes in land use and the fragmentation of habitats in intensively managed farming landscapes (Pyle 1976, Erhardt 1985, Sibatani 1990, Kleijn and Snoeijs 1997, Steffan-Dewenter and Tscharntke 2000, Thomas *et al.* 2001). However, the

study of butterflies and agriculture has demonstrated the importance to butterflies of farmland features such as field margins (Dover 1994, Feber *et al.* 2007), hedgerows (Dover and Sparks 2000) and remnant areas of native vegetation (Shepherd and Debinski 2005, Schmitt *et al.* 2008, Franzen and Nilsson 2008). In addition, to complement the monitoring data of programs such as the UK Butterfly Monitoring Scheme, recent farm-scale studies have sought to assess the influence of other biotic and abiotic factors influencing butterfly abundance and distribution in different landscapes (Dover 1996, Clausen *et al.* 2001, Pywell *et al.* 2004). An understanding of individual species' requirements and the identification of influential features of the landscape is vital to butterfly conservation on farmland, particularly for some of the rarer and specialist species (Dennis 2004, Pywell *et al.* 2004). Much of this research can also inform the improvement of agri-environment schemes (AES) (Kleijn and Sutherland 2003, Kuussaari *et al.* 2007, Franzen and Nilsson 2008), in widespread attempts to encourage farmers to enhance ecosystem service provision in agricultural landscapes, including the aesthetic ecosystem service of butterfly conservation.

In New Zealand, farmer incentives of this kind do not exist and the study of butterflies is considerably undeveloped (Gibbs 1980). Although there are no endangered species (Patrick and Dugdale 2000), this may be because population records are insufficient to detect downward trends in abundance. However, the lowland agricultural areas of New Zealand may be even simpler than those in Europe (Tschardt *et al.* 2007), with less than 1% of native vegetation remaining (C. Meurk, pers. com.), so associated invertebrate species are likely to have suffered a similar fate to their European counterparts. The use of land for viticulture is one of the most intensive forms of agriculture in terms of monocultures, chemical application and the fragmentation resulting from field re-alignment in the conversion from arable or pastoral farming (Schmitt *et al.* 2008). While ecosystem services in such extreme monocultures are at a low level (Fiedler *et al.* 2008), there are opportunities for enhancement and to halt any unrecorded decline in native butterfly populations.

In place of assistance in the form of AES in New Zealand, a government funded project led by Lincoln University and Landcare Research in the viticultural region of Waipara, North Canterbury is an example of a grower and research led initiative to address the paucity of ecosystem services in these environments. The Greening Waipara project (<http://bioprotection.org.nz/greening-waipara>) is encouraging and assisting participating vineyard owners to replant once commonplace native plant species in and around the properties. The emphasis is on providing 'stacked ecosystem services' (Fiedler *et al.* 2008), such as weed and disease suppression through planting low growing plants beneath vines, natural enemy population enhancement via flowering plants and cultural services from

educational biodiversity trails close to wineries and other native plantings outside vine-blocks. While research surrounding the different plantings has focussed on tangible economic benefits (Scarratt *et al.* 2004, Berndt *et al.* 2006, Tompkins *et al.* 2009), there has been no work to evaluate the impact to general biodiversity not specifically targeted by the plantings. The Greening Waipara project therefore provides an ideal opportunity to study both the butterfly fauna of New Zealand vineyards and their habitat requirements, and the extent to which ecosystem service enhancement is improving or has potential to improve the biodiversity of the viticultural landscape through ‘stacked ecosystem services’.

This study explores the factors that influence butterfly distribution within New Zealand vineyards. It consists of the first known survey of butterfly species on farmland of any type in New Zealand and the first attempt at associating farmscale features with the New Zealand butterfly fauna using multiple linear regression. As butterflies are considered to be a useful group of organisms in evaluating habitat quality (Erhardt 1985, Erhardt and Thomas 1991, Panzer *et al.* 1995, Oostermeijer and van Swaay 1998; Pywell *et al.* 2004, Poyry *et al.* 2005), the work also includes an assessment of the habitat management measures in improving overall biodiversity in vineyards.

## 2.3 METHODS

### 2.3.1 Study sites

All measurements were carried out on and around six vineyards in the Waipara Valley, North Canterbury (Appendix 1). The term “vineyards” in this study is a catch-all term to include all parts of a property the predominant product of which is wine grapes. Therefore, despite being largely monocultural areas, the vineyard sites included a wide range of habitats including pasture, wide field margins for turning machinery, tracks, river beds and Greening Waipara planting areas. In addition, each site was chosen due to the presence of adjacent areas of remnant native vegetation (hereafter termed ‘remnants’), either on the property or on the neighbouring land. These remnants typically consisted of stands of matagouri (*Discaria toumatou* Raoul (Rhamnaceae)), *Muehlenbeckia* spp. (Polygonaceae), NZ bindweed (*Calystegia tuguriorum* G. Forst. (Convolvulaceae)), as well as European Gorse (*Ulex europaeus* L. (Fabaceae)) and Broom (*Cytisus scoparius* L. (Fabaceae)) and a range of exotic herbs and grass species. They were also considered marginal and relatively unproductive land due to thin soils or impractical topography, although many of the remnants were grazed occasionally by livestock. The vineyards were spread across the valley so that no study site

was closer than 1.5km to its nearest neighbour (Appendix 1). The sites all underwent typical vineyard management operations, although there were differences between mowing and spraying regimes for example, which have not been accounted for.

### **2.3.2 Butterfly recording**

Butterflies were counted along fixed transect routes following the standard methodology used by the UK Butterfly Monitoring Scheme (UKBMS) described by Pollard *et al.* (1986) and Pollard and Yates (1993). Transects were established at each site so that they passed through the different vegetation types represented on or adjacent to the properties. Homogenous sections of each transect were identified on the basis of topography and vegetation type. Transects had between 9 and 14 sections, with a total of 66 across the six sites. GPS co-ordinates of the start and end of each section are included in Appendix 1.

Transects were walked every two weeks between October 2008 and April 2009 covering the flight periods of all common butterfly species, with a total of 13 visits per transect. The route was walked at a steady pace counting all butterflies encountered up to 2.5m either side and in front of the observer. Where possible, the butterflies were recorded to species and sex. Where the species was uncertain, the most common species was assumed. In the case of the blue butterfly species, the southern blue (*Z. oxleyi*) and the common blue (*Z. labradus*) are difficult to distinguish in the field. However, because of the results of molecular and morphological work in this thesis (Chapter 3), all blues were classed as *Z. oxleyi*. To ensure consistency in data collection, transect counts were completed only after 10:00 and before 16:00 when butterflies are most active. In addition, counts were performed only when the air temperature exceeded 13° C in sunny weather and 17° C in cloudy weather, and when the wind speed did not exceed 5 on the Beaufort scale (Pollard and Yates 1977).

### **2.3.3 Environmental variables**

Biotic and abiotic habitat parameters were recorded in January 2009 for each transect section and are listed in Table 2.1. The choice of variables followed those of Pywell *et al.* (2004), Clausen *et al.* (2001) and Dover (1996) where applicable. The variables were recorded for the 5m belt of land in which butterflies were observed. Also, the vegetation composition of each section was estimated using quadrats. Each transect section over 100m was divided into 10 subsections in which a circular quadrat of 1.5m diameter was placed randomly. Where sections were less than 100m in length, subsections were allocated every 10m. Within each quadrat, the percentage cover of each plant species was estimated visually. For simplicity in data analysis, these observations were subsequently grouped by family (except for grasses



which were grouped together), and additionally into native and non-native species. The abundance of nectar sources was estimated using the five point DAFOR scale (*sensu* Clausen *et al.* 2001) (5 = D – dominant, 4 = A – abundant, 3 = F – frequent, 2 = O – occasional, 1 = R – rare) and this was recorded during most of the butterfly transect data collection sessions. The number of each species in flower was visually estimated in blocks of 20 inflorescences. A particular flower was considered dominant if more than 10 blocks were counted in a section, abundant if 5-10 blocks were counted, frequent if 2-5 were counted, occasional if 1-2 were counted, and rare if counts did not constitute a block of 20. Nectar source abundance categories were summed for each section for an overall flower abundance score, and the total species richness across the summer was also calculated.

### 2.3.4 Statistical Analysis

All butterfly counts of individual species and of total butterflies were summed over the summer for each transect section in order to account for possible temporal correlation between fortnightly counts. In order to normalise the residuals, the number of butterfly species (species richness) was transformed by first dividing observations by the natural log of the length of the section, and then taking the natural log of these standardised values. This is because species richness is logarithmically proportional to the length of each section. This pattern is less likely to hold for total butterfly abundance, so these values were transformed using  $\log(n/\text{length})$ . Total abundance of *Z. oxleyi* was transformed in a similar way, except that the formula was  $\log(n+1/\text{length})$  to account for the presence of zeros. It was not possible to transform data of other species due to the large number of zero counts, so data for these species were not analysed separately.

The first analysis consisted of a separate linear regression model for the three response variables (species richness, total butterfly abundance, abundance of *Z. oxleyi*) to habitat type. This was favoured ahead of an ANOVA analysis due to the unbalanced nature of the data. In these regression analyses the factor variable ‘site’ was forced as primary term to accommodate differences between sites (i.e. ‘site’ was included in regression models regardless of significance) (*sensu* Pywell *et al.* 2004).

Secondly, environmental variable linear regression models were parametised by performing a separate multiple regression model for each response variable, with the explanatory variables in Table 2.1 (except habitat type). Backwards stepwise selection was used to eliminate variables that were not significant at the  $p = 0.01$  level. The remaining variables were then considered those that best explained variation in the response variables (Zuur *et al.* 2009). A primary variable was also forced for ‘site’ for this analysis.

**Table 2.1:** Environmental variables recorded for each transect section.

Variable	Description and scale
Habitat type	Remnant, vine rows, river, planting, pasture, margin, track
Insolation	Estimated from aspect 0 = S, 2 = SE or SW, 4 = W or E, 6 = NE or NW, 8 = N
Shelter	Estimated on 0-8 scale following Dover (1996); number of directions from which section is sheltered from wind
Slope	Presence of slope: 0 = no slope, 1 = slight slope, 2 = steep slope.
Shade	Degree of shade over section: 0 = no shade, 1 = under 50% shaded, 2 = over 50% shaded
Grazing	0 = ungrazed, 1 = grazed by rabbits only, 2 = grazed by rabbits and occasionally by stock (sheep or cattle), 3 = heavily grazed
Mowing	0 = no mowing, 1 = occasional mowing, 2 = frequent mowing
Grass height	Mean height of grass (cm) (N =20 per section)
Vegetation height	Mean height of ground (non woody) vegetation (cm) (N=20 per section)
Native Cover	% cover of native plant species estimated for length of section
Tree Cover	% cover of trees estimated for length of section
Shrub cover	% cover of all shrub species for length of section,
Herb cover	% cover of all herb species for length of section
Grass cover	% cover of grasses for length of section
Bare Ground cover	% cover of bare ground for length of section
Nectar	Summed nectar source abundance score per 100m of transect section
Nectar richness	Maximum number of flower species per section over summer

The use of the 0.01 cut-off criteria as opposed to the traditional 0.05 level was due to the fact that regression models may suffer from multi-collinearity between explanatory variables and the data may violate the assumption of independence due to the proximity of sections and sites (Dover 1996). Additionally, prior to analysis, the transect sections with zero scores for all butterflies were removed because they could not be transformed as above. This reduced the number of sections to 61.

Thirdly, a separate multiple regression model for each response variable was performed using individual flower species as explanatory variables. Backwards stepwise selection was used in the same way as above, until only significant terms remained (the flower species that were significant in explaining the variation in response variables). All analyses were performed using R 2.9.2 (R Core Development Team 2009).

## **2.4 RESULTS**

### **2.4.1 Butterfly assemblage in Waipara vineyards**

A total of 2988 butterfly individuals were recorded on 13 visits to the six vineyards. The butterfly fauna in these habitats was limited to a maximum of 5 species on any one site at any one recording session, a maximum of 7 species over the summer on any one site, and a total of 8 species over the summer (Table 2.2). The majority of individuals belonged to the Lycaenidae (87%); 74% were *Z. oxleyi* which was prevalent in most places due to the ubiquity of its host plants, members of the Fabaceae. The New Zealand lycaenids, like those in other countries, are relatively sedentary species, and were almost always observed close to host plants. The other four species recorded are all vagile and as the low number of observations suggest, were most often seen on the wing passing through the sites. With the exception of the pestiferous cabbage white (*P. rapae*), it cannot be stated with any confidence whether any of these vagile species were breeding on the study sites as none of their host plants were encountered. The site with the highest mean species number was Waipara West ( $2.1 \pm 0.4$ ) and the lowest was Fancrest ( $0.9 \pm 0.4$ ). The highest mean abundance of butterflies per 100m was observed at Greystone ( $36.5 \pm 13.1$ ) and the lowest was at Dickson vineyard ( $11.2 \pm 3.4$ ).

**Table 2.2:** Mean butterfly abundance and species richness per 100m of transect recorded at each of the 6 study sites (n = number of sections after removal of sections where no butterflies were found).

Species	Riverside sites			Hillside sites				
	Dickson n = 10	Dunstaffnage n = 9	Waipara West n = 13	Fancrest n = 9	Greystone n = 9	Mountford n = 11	Total mean n = 61	
Lycaenidae								
<i>Zizina oxleyi</i>	Southern blue	6.8 ± 2.5	4.8 ± 1.4	9.5 ± 3.1	27.0 ± 6.4	30.5 ± 12.8	18.9 ± 6.8	15.3 ± 2.7
<i>Lycaena salustius</i>	Common copper	2.8 ± 1.9	2.1 ± 1.5	3.3 ± 2.6	0.7 ± 0.7	0.8 ± 0.7	2.2 ± 1.5	2.1 ± 0.7
<i>Lycaena feredeyi</i>	Glade copper	0.1 ± 0.05	0.1 ± 0.06	0.1 ± 0.1	0.0	0.0	0.1 ± 0.1	0.05 ± 0.03
<i>Lycaena boldenarum</i>	Boulder copper	0.0	3.5 ± 2.7	1.1 ± 0.7	0.0	0.0	0.0	0.8 ± 0.5
Nymphalidae								
<i>Bassaris gonerilla</i>	Red admiral	0.0	0.0	0.0	0.1 ± 0.07	0.0	0.1 ± 0.04	0.02 ± 0.01
<i>Bassaris itea</i>	Yellow admiral	0.1 ± 0.1	0.0	0.1 ± 0.04	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.08	0.09 ± 0.04
<i>Danaus plexippus</i>	Monarch	0.0	0.0	0.1 ± 0.06	0.0	0.1 ± 0.05	0.0	0.06 ± 0.04
Pieridae								
<i>Pieris rapae</i>	Cabbage white	1.4 ± 0.9	2.7 ± 0.9	3.4 ± 1.0	0.8 ± 0.7	5.1 ± 1.0	2.8 ± 1.0	2.75 ± 0.4
	Total butterflies	11.2 ± 3.4	13.2 ± 3.6	17.4 ± 4.5	28.8 ± 6.3	36.5 ± 13.1	24.0 ± 6.6	3.5 ± 2.8
	Species richness	1.7 ± 0.3	1.3 ± 0.4	2.1 ± 0.4	0.9 ± 0.4	1.2 ± 0.3	1.4 ± 0.5	1.5 ± 0.2

Host plant species were encountered for all four of the lycaenids and for *P. rapae*. For *Z. oxleyi*, white clover (*Trifolium repens* L. (Fabaceae)) was the most abundant host plant species. *Muehlenbeckia complexa* A. Cunn (Polygonaceae) was the most abundant host for both the common copper (*L. salustius*) and glade copper (*Lycaena feredayi* Bates (Lycaenidae)), although *M. australis* G. Forst (Polygonaceae) and *M. astonii* were present in smaller numbers; *M. astonii* was a frequently used species in the Greening Waipara plantings. *Muehlenbeckia axillaris* Hook f. (Polygonaceae), the host plant of the boulder copper (*Lycaena boldenarum* White (Lycaenidae)) was found either abundantly in river beds or was irregularly seen in some of the remnants. Host plants for *P. rapae* included hedge mustard (*Sisymbrium officinale* L. (Brassicaceae)) and brassica crops in nearby arable farmland.

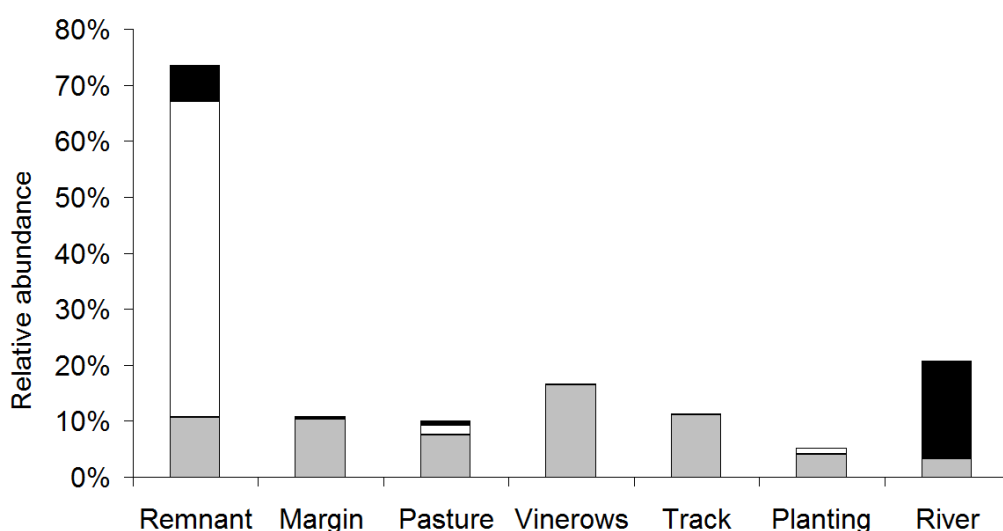
The most common nectar sources known to be used by butterflies, and observed being used by NZ butterflies could all be categorised as agricultural weeds. Species common at most sites were: dandelion (*Taraxacum officinale* Weber (Asteraceae), mean total abundance score per section  $7.8 \pm 0.7$ ), *T. repens* ( $4.8 \pm 0.8$ ), California thistle (*C. arvensis*,  $2.9 \pm 0.5$ ), and common yarrow (*A. millefolium*),  $1.9 \pm 0.6$ ). However, the most abundant flower species on certain sites that were unused by butterflies were Viper's bugloss (*Echium vulgare* L. (Boraginaceae),  $4.9 \pm 0.8$ ), large flowered mallow (*Malva alcea* L. (Malvaceae),  $4.3 \pm 0.8$ ), small flowered mallow (*Malva parviflora* L. (Malvaceae),  $2.3 \pm 0.5$ ) and dog rose (*Rosa canina* L. (Rosaceae),  $1.1 \pm 0.3$ ). Native flowers consisted only of *C. tuguriorum* ( $0.2 \pm 0.1$ ) which was not used, and *Muehlenbeckia* spp. (*M. axillaris*:  $0.4 \pm 0.3$ , *M. complexa*:  $1.4 \pm 0.5$ ) which were used occasionally (butterfly nectar feeding data analysed in Chapter 4).

#### 2.4.2 Lycaenid life history observations

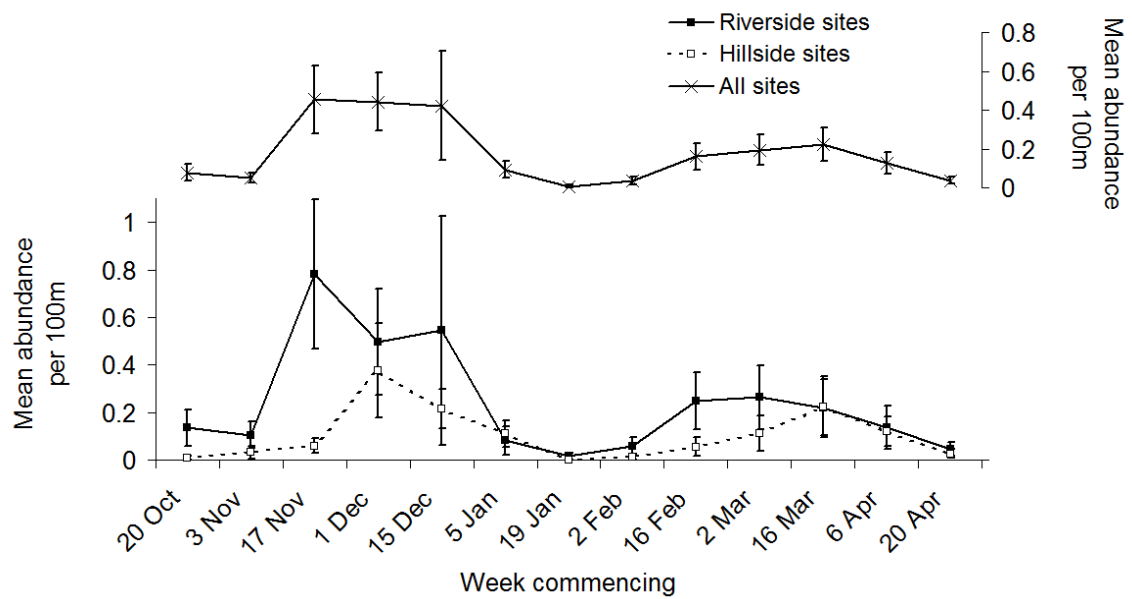
While the more vagile species were not recorded in great numbers, three of the lycaenid species were present at sufficient densities to allow analysis of population dynamics. Two of the species in this group show clear fidelity to certain section types (Fig. 2.1). *Lycaena salustius* was found almost exclusively in the remnants where its larval host plants were abundant. Similarly, *L. boldenarum* was restricted to the section types where its larval host was present. In contrast, *Z. oxleyi* was relatively well represented in all habitat types. This distribution of species also illustrates the relative importance of the different habitat types. In particular, while the remnants were most important for butterflies, the plantings were least important.

Fig. 2.2 shows the mean abundance of *L. salustius* at each sampling date across all sites, and also separates these data between the three sites located close to the Waipara river (riverside sites) and the three situated on the foothills of Mt Cass (hillside sites). There are two clear generation peaks during the flight season. Furthermore, more adults were observed on the riverside sites where the remnants were on north facing river terraces, than on the hillside sites where the remnants were in south facing gullies. In addition, the populations reached their peak earlier at the riverside sites.

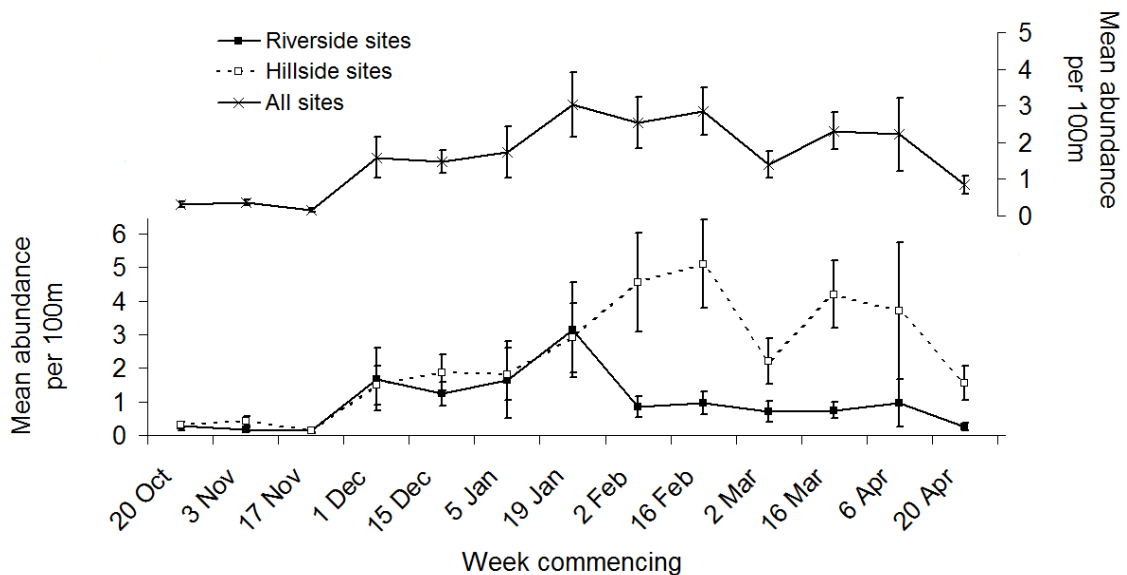
Generation peaks for *Z. oxleyi* were more difficult to discern (Fig. 2.3). Instead it appears that the population gradually builds to a sustained peak towards the end of the summer. There were more individuals on average on hillside sites than at riverside sites. *Lycaena boldenarum* was found only on riverside sites and clear generation peaks are similarly difficult to discern (Fig. 2.4).



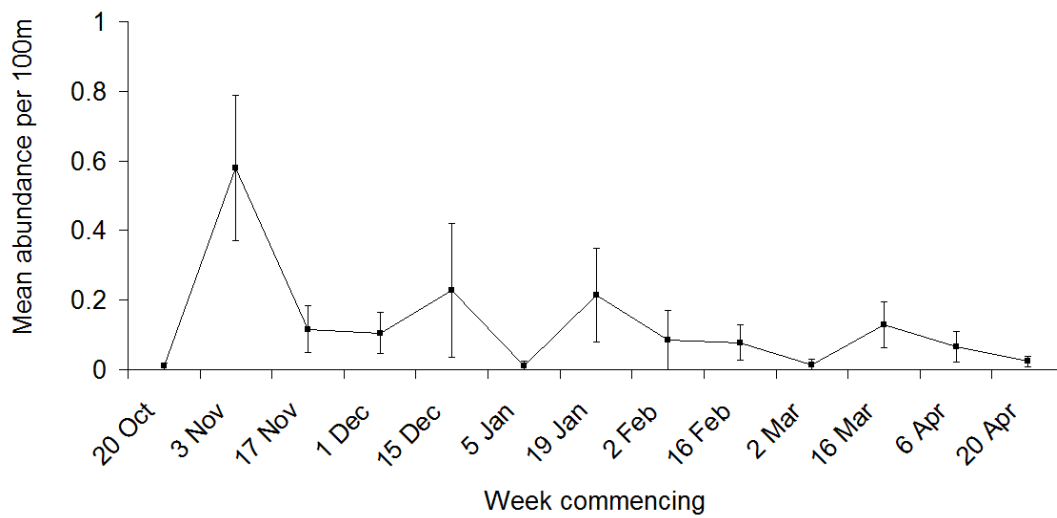
**Figure 2.1:** Mean relative abundance of species on different transect section types. Relative abundance per section type was calculated for each species on each vineyard as follows: Number of individuals on section type/total number of individuals on vineyard. Mean relative abundance was then calculated from these vineyard figures (n = 6). Black bars: *L. boldenarum*; white bars: *L. salustius*; grey bars: *Z. oxleyi*.



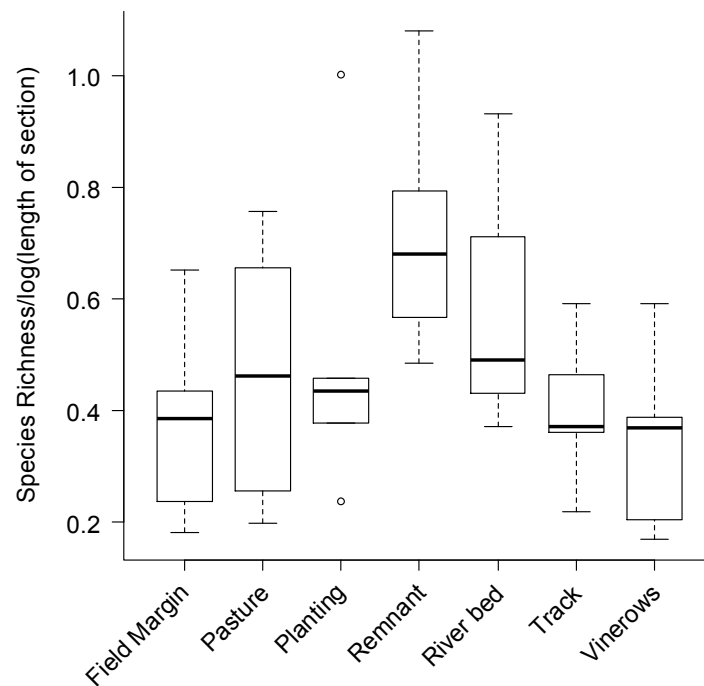
**Figure 2.2:** Mean abundance of *L. salustius* per 100m of transect section for each sampling date. Top graph shows data for all sites, bottom graph shows data separated by site location. With  $\pm 1$  standard error bars



**Figure 2.3:** Mean abundance of *Z. oxleyi* per 100m of transect section for each sampling date. Top graph shows data for all sites, bottom graph shows data separated by site location. With  $\pm 1$  standard error bars



**Figure 2.4:** Mean abundance of *L. boldenarum* per 100m of transect section for each sampling date for all sites (recorded only at riverside sites). With  $\pm 1$  standard error bars



**Figure 2.5:** Boxplot of Species richness per log-length of section (m) plotted by section type. The solid line in each box indicates the median, and the lower and upper edges of the box are the first and third quartiles. The whisker of the box reaches to the largest or smallest value which is not an outlier. Outliers are indicated by the open circles.



### 2.4.3 Linear regression analysis: Habitat type

The first linear regression analysis of the response variables modelled by site and habitat type returned a significant effect of habitat type for species richness ( $F = 4.686$ ,  $df = 6, 54$ ,  $p < 0.001$ ,  $R^2 = 0.40$ ), but not total abundance or abundance of *Z. oxleyi*. Subsequent examination of the effects of the different habitat types on species richness (Fig. 2.5) showed that the remnants were the most important.

### 2.4.4 Multiple regression analysis: Environmental factors

In the multiple regression analysis of environmental factors (Table 2.3) the only significant explanatory variable for species richness was percentage cover of native plant species. The total abundance of butterfly individuals was explained by percentage of native plant cover, abundance of nectar, the percentage cover of legumes and, of secondary importance, a negative relationship with grass height. The abundance of *Z. oxleyi* was explained by nectar abundance, percentage cover of legumes and grass height to a lesser extent.

**Table 2.3:** Significant environmental variables in the linear regression models of species richness, total butterfly abundance and abundance of *Z. oxleyi*, after backwards stepwise selection

Response variable	F value	R <sup>2</sup>	Native cover	Nectar	Fabaceae cover	Grass height
Species richness	16.43	0.31	***	ns	ns	ns
Total abundance	6.05	0.52	***	***	*	* (–)
<i>Zizina oxleyi</i>	7.48	0.54	ns	***	**	** (–)

(–) – negative correlation

\*\*\* -  $p < 0.001$

\*\* -  $p < 0.01$

\* -  $p < 0.05$

ns - not significant

#### 2.4.5 Multiple regression analysis: nectar abundance.

The linear regression of the response variables modelled by the abundance of individual flowering species had less explanatory power than the models of environmental variables in terms of R squared values (Table 2.4). The most important flowers explaining species richness were *C. arvense* and *T. repens*. *Cirsium arvense* was also important for abundance of individuals, together with *T. officinale*, while only *T. repens* was significant for *Z. oxleyi* abundance.

**Table 2.4:** Results of linear multiple regression analysis of nectar sources with species richness, total individuals and *Z. oxleyi* individuals after backwards stepwise selection.

Response variable	F value	R <sup>2</sup>	P value	<i>Cirsium arvense</i>	<i>Trifolium repens</i>	<i>Taraxacum officinale</i>
Species richness	11.03	0.276	<0.001	***	***	ns
Total individuals	21.15	0.422	<0.001	***	ns	***
<i>Zizina oxleyi</i>	15.3	0.206	<0.001	ns	***	ns

\*\*\* - p<0.001

ns - not significant

## 2.5 DISCUSSION

### 2.5.1 Butterfly abundance and population dynamics

The number of butterfly species and individuals recorded in Waipara vineyards was low compared to similar studies in Europe (Clausen *et al.* 2001, Pywell *et al.* 2004, Schmitt *et al.* 2008) and the USA (Nelson and Wydoski 2008) and this is likely to be due in part to the nature of the New Zealand butterfly fauna. New Zealand accommodates an extremely small number of butterfly species compared with most countries in the northern hemisphere. The reasons for this are unknown, although may be linked to the relative isolation of the archipelago and the changes that took place during the Gondwana separation (Gibbs 1980).

The species recorded in this study were either mobile species (*P. rapae*, *Bassaritis itea* F. (Nymphalidae), *B. gonerilla*, *Danaus plexippus* L. (Nymphalidae)) or relatively immobile species

closely associated with host plants that were present on the study sites (*Z. oxleyi*, *L. salustius*, *L. feredeyi*, *L. boldenarum*). Of the mobile species, only the introduced pest species *P. rapae* occurred in large numbers. This species is able to utilise a number of members of the Brassicaceae as host plants and feeds on the nectar of numerous perennial ‘weed’ species (Gibbs 1980). The remaining three mobile species encountered, *B. itea*, *B. gonerilla* and *D. plexippus*, also use non-native nectar sources, but could not be said to be supported by the vineyards due to extremely low numbers and an absence of host plants.

The immobile species consisted of four native species of the Lycaenidae. Only the southern blue, *Z. oxleyi*, the most abundant butterfly overall by far (74%), occurred in all habitat types. The strong presence of *Z. oxleyi* can be explained by the ubiquity of the species’ host plants, members of the Fabaceae, and this is supported by the importance of Fabaceae cover in both total abundance and *Z. oxleyi* abundance regression models. The three other lycaenids were restricted to the remnants and river beds that contained their *Muehlenbeckia* spp. host plants. For example, *L. salustius* and *L. feredeyi*, were restricted almost entirely to the remnants of native vegetation on or adjacent to vineyard properties. When *L. salustius* was recorded in other habitat types, it was observed nectaring suggesting that it may search for food sources away from the host plant patches. This is supported by Gibbs (1980) who observed the butterfly nectaring up to 250m from the nearest larval host plant. *Lycaena boldenarum* was recorded only in association with *M. axillaris*, the sole host plant of this species, which was most often found on the gravelly river banks adjacent to Dickson, Dunstaffnage and Waipara West vineyards, although some records of both plant and butterfly came from the remnants of the same sites.

Observations of the population dynamics of *L. salustius* in New Zealand have not been conducted since the work of Craw (1975), and have not been completed at all for *Z. oxleyi*. However, such information from a wide range of locations is important in establishing species’ conservation status (Pollard and Yates 1977, Gibbs 1980). In Waipara, the riverside sites held more *L. salustius* than did hillside sites, and this may be due to the orientation of the main habitat type within each site. Remnants on riverside sites were all north facing steep river terraces, receiving heat and sunshine for more of the day than the remnants of the hillside sites, which were generally south facing. This may explain the earlier peaks of the riverside populations for both generations, although both insolation and slope were not significant factors in species richness and total abundance regression models. The data are comparable to those of Craw (1975) who conducted a mark-release-recapture study of *L. salustius* in Central Otago. In that study,

similar generation peaks to those shown in this study were observed, but they occurred in January and March, as opposed to November and February in Waipara, suggesting that climate is an important factor in the regional phenology of *L. salustius*. This has been shown for other species of butterfly (Warren 1987a, Nylin and Svard 1991, Munguira and Martin 1993).

*Zizina oxleyi* is certainly also affected by climate. For this species, the hillside sites held greater numbers than did riverside sites. It is thought that *Z. oxleyi* was able to take advantage of the greater range of north facing slopes on hillside sites as its host plant was ubiquitous. From the data, it was not possible to determine clear generation peaks of this species because the populations seem to build up to larger numbers towards late summer. This pattern may be affected by mowing. While the temporal aspect of mowing was not incorporated into regression models or population dynamics work, fewer *Z. oxleyi* adults were observed up to two weeks after the mowing of field and track margins, vineyards and pasture fields. The timing of mowing has been shown to affect butterfly populations (Warren 1993b) and different mowing practices on the six vineyards may have affected the natural occurrence of population peaks.

### **2.5.2 Habitat associations of butterflies in Waipara vineyards**

Few butterflies were associated with the plantings of the Greening Waipara project. *Zizina oxleyi* was the most abundant in this habitat type, but this was likely to be due to the presence of legumes or flowering species in the vicinity. While some *M. astonii* plants were included in most of the plantings, these sites were isolated from native patches, with apparently insufficient nectar resources to attract *L. salustius*. While the more mobile species are not constrained by larval host plants in their distribution on farmland (Pywell *et al.* 2004), a general lack of suitable nectar sources and host plants in plantings and elsewhere is likely to explain at least in part the paucity of these species on vineyards.

Significantly more species were recorded in the remnants than any other habitat type. The remnants were the main location for native shrubs, which was an important factor in both species richness and abundance models. The presence of the *Muehlenbeckia* spp. and associated *Lycaena* spp. are likely to have inflated both summary response variables (species richness and total butterfly abundance) for such a small fauna.

The importance of the remnants is unsurprising aside from the presence of native host plants. Remote from much of the disturbance of intensive agricultural operations such as machinery use and chemical application, which are detrimental to butterflies (Marini *et al.* 2008,

Franzen and Nilsson 2008), fallow, marginal or unproductive lands have been found to be important refuges of specialist butterflies in urban areas (New 2007), European vineyards (Schmitt *et al.* 2008) and other types of farmland (Clausen *et al.* 2001, Sumerville *et al.* 2005, Ockinger *et al.* 2006, Kuussaari *et al.* 2007). As a diverse structural component of the ecosystem, they provide host plants, nectar for adult feeding and shelter in otherwise open farmland (Dover 1996, 1997, Dover *et al.* 2000, Pywell *et al.* 2004), corridors to facilitate movement between patches of habitat (Haddad 1999) and other non-consumable resources for roosting, perching, basking and mate location (Dover and Fry 2001, Dennis, 2004, Hardy and Dennis 2007).

While grazing was not an important factor in regressions, the secondary importance of grass height in the abundance model may suggest that it is beneficial as reported in previous work. Although overgrazing can be detrimental and some grass feeding butterfly species prefer the microclimates of tall grass for oviposition (Poyry *et al.* 2004, Franzen and Nilsson 2008), a lower sward height increases the temperature at ground level (Thomas 1983) which is beneficial to butterflies. Similarly, moderate grazing maintains the successional stages required by many butterfly species (Erhardt 1985), prevents the dominance of grasses (Krahulec *et al.* 2001) and thus encourages flowering-plant diversity (Croxtton *et al.* 2005). Moderate and well timed mowing can also be important (Warren 1993b). Mowing was not conducted in remnants, and the presence of grass height in both the total abundance and *Z. oxleyi* abundance models may suggest that it is beneficial. However, while *Z. oxleyi* seems to prefer a low sward on this evidence, extensive mowing is likely to be detrimental to host plants and nectar sources, and therefore to other butterfly species. More detailed research is required to explore the effects of grazing and mowing on the New Zealand butterfly fauna before conclusions can be drawn on optimal management regimes.

The abundance of nectar was also important in this work in explaining variation in total butterfly abundance and the abundance of *Z. oxleyi*. In agricultural landscapes, where perennial flowering plants are removed directly or indirectly through the use of artificial fertilisation (Boatman 1992), nectar sources can be the limiting factor for many butterfly species. Nectar is important for adult butterflies for both longevity and fecundity (Watt *et al.* 1974, Murphy *et al.* 1983, Alm *et al.* 1990), and has been correlated with butterfly abundance and species richness in a number of similar studies (Munguira and Thomas 1992, Dover 1996, Clausen *et al.* 2001, Pywell *et al.* 2004, Nelson and Wydoski 2008). In this study, the multiple regression analysis of the response variables and individual flower abundance showed that *C. arvense*, *Trifolium repens*

and *Taraxacum officinale* were important species. In a similar study in Denmark, Clausen *et al.* (2001) also found that the abundance of nectar sources such as *C. arvense* and *T. repens* were significant in explaining variation in butterfly abundance, and associated much of the nectar sources usable by butterfly species with strips of uncultivated land at field margins.

While the findings of the regression analyses can be interpreted with apparently simple explanations, butterfly habitat provision is complex and the results should be considered with the potential drawbacks of the methodology. Firstly, while the ‘Pollard walk’ technique is a recognised butterfly survey method, results of which have compared favourably to those of mark-release-recapture methods (Pollard 1977, Thomas 1983, Warren *et al.* 1984), the system may suffer from bias due to the greater apparency of some species over others. Dennis *et al.* (2006) found that UKBMS butterfly abundance indices were positively correlated with basking behaviour and activity height, which may have caused an underestimation in nymphalid species in the present study. The weather may similarly affect recorded butterfly densities, as cooler cloudy weather may result in *L. salustius* perching with wings closed for example, making it difficult to detect against vegetation. Different weather conditions may also cause butterflies to alter their distribution away from the transect (Dennis *et al.* 2006). Despite this, Dennis *et al.* (2006) suggested that bias is less likely to affect surveys where the fauna is smaller (e.g., New Zealand), and the repetitive nature and standardisation of the counts is likely to have minimised these effects here.

The limitations of linear regression using count data include the possibility of lost information during the pooling of data across the summer and through data transformation (Zuur *et al.* 2008). The data may also suffer from multi-collinearity and a lack of independence (Kivinen *et al.* 2006). Also, the use of multiple explanatory variables can result in the identification of significant values for terms by chance or due to correlation between variables (Dover 1996, Clausen *et al.* 2001). Every effort has been made to minimise collinearity in the present study and the strict 0.01 significance level has been used in stepwise selection to account for these possibilities.

### **2.5.3 Conservation implications and the importance of enhanced ecosystem services**

It is evident that the remnant sites are important pockets of habitat for butterflies in Waipara vineyards and for the less mobile species, these are isolated patches. For the more mobile species, they may provide important sources of nectar that are absent from other habitat types (e.g., *C.*

*arvense*), although host plants for these vagile species were not found. Thus, remnants have much potential to form the focus of future conservation efforts on farmland. In contrast, the plantings were the least important habitat type and this is likely to be because of their young age, low numbers of host plants and isolation from larger patches of native vegetation. While butterflies were not the target of these plantings, such areas could be developed with some form of connection to the larger remnants and more focussed planting of host plants and nectar sources.

Findings by numerous other workers have emphasised that providing habitats for a number of butterfly species is not simply about improving larval and adult food supply, but should be concerned with creating a mosaic of habitat types which may be used for different aspects of the adult behaviour and stages of the life cycle (Clausen *et al.* 2001, Pywell *et al.* 2004). The environmental factors that were not significant in the regression models such as shelter, insolation and grazing, are variables of importance in other studies, and their influence on butterfly populations in this study may be more subtle at spatial scales other than at that scale measured. Individual species requirements for different structures may also differ through time (Hardy and Dennis 2007) and in different weather conditions (Wikstrom *et al.* 2009). Such aspects have not been measured here, but this work supports the general message of butterfly conservation that the more diverse the conservation areas in terms of vegetation (e.g., host plants, nectar sources, roosting sites) and topography (e.g., for basking and shelter), the better (Poyry *et al.* 2004, Shorka *et al.* 2007, Franzen and Nilsson 2008).

This emphasis can also be extended to the whole farm, or the so-called matrix (the area between habitat patches as defined largely by host plants (Dennis and Hardy 2007)), which may make conservation more successful at greater scales (Maes *et al.* 2006). For example, mobile species are likely to take advantage of ‘weed’ nectar sources in and around the crop/vines (Dover 1996) and more of these patches can be created by restricting herbicide application and by the active planting of strips of flowers such as buckwheat (*Fagopyrum esculentum* Moench (Polygonaceae)) and phacelia (*Phacelia tanacetifolia* Benth. (Hydrophyllaceae)), which have been shown to support beneficial invertebrate species (Berndt *et al.* 2006, Robinson 2008). The planting of species of climbing plants such as *M. australis* and *M. complexa* along the many fence lines on a vineyard property, may also provide the corridor and habitat effects found for hedgerows in Europe (Dover 1997). However, in vineyards the challenge remains in convincing landowners to commit to such measures. In Europe this challenge is addressed by subsidising farmers to diversify through agri-environment schemes. These schemes have had variable success

(Kleijn *et al.* 2006), and are more likely to have positive outcomes when the surrounding landscape adopts similar practices (Rundlof *et al.* 2008). Such financial compensation is not available to New Zealand vineyard owners and managers, and landowners are unlikely to be able to incorporate many diversification ideas into working practice due to the semi-permanent nature of the vine row and the dedication of resources to the production of wine only. Instead, incentives in the form of evidence of the economic benefits of habitat management techniques are required. For example, research has shown that the application of mulches beneath vines can reduce the incidence of fungal infection below the economic threshold, removing the need for chemical sprays (Jacometti *et al.* 2007, 2010).

Although the Greening Waipara project was not specifically aimed at butterfly conservation, much work is required before butterflies could be said to benefit. However, in a country such as New Zealand where the provision of ecosystem services is not paid for, this extra work may be unlikely to occur in the near future. Therefore, subsequent research should be focussed on linking the conservation of biodiversity indicators such as butterflies with beneficial outcomes such as the enhancement of associated beneficial biodiversity and the aesthetic effects on tourism and marketing.



## **Chapter 3 Morphological variation and molecular divergence in the genus *Zizina* (Lepidoptera: Lycaenidae) in New Zealand: hybridisation or recent speciation?**

### **3.1 ABSTRACT**

In taxonomy, closely related taxa are often separated by distinct differences in morphological characters. However, the situation becomes complex when individuals with intermediate characters between two species occur, and when genetic markers identify species boundaries that do not match those of morphological markers. Intermediate individuals may represent natural variation within, or hybridisation between the two species and the study of such species complexes can assist in advancing the understanding of morphological divergence and speciation. The butterfly genus *Zizina* is poorly studied in New Zealand, despite a historically difficult taxonomy due to the presence of morphological intermediates between the two species, the endemic *Z. oxleyi* and the introduced *Z. labradus* in a putative hybrid zone on the east coast of the South Island. The aims of this study were to address this uncertainty through morphological and molecular analyses. Specimens were collected from a range of locations in New Zealand, as well as from Australia, and measurements were made of male genitalia and ventral wing coloration. The mitochondrial cytochrome oxidase subunit I (COI) gene was also sequenced for a selection of individuals. Morphological analysis showed that the two species could be separated in morphological space, although there was some overlap, and the contact zone appeared to be around Kaikoura on the east coast of the South Island. Furthermore, specimens from the putative hybrid zone could be classified as *Z. oxleyi* using morphological characters individually, but not when these were used in a principal component analysis. Molecular analysis showed that there was a mean sequence divergence of 2.1% between two clades, but suggested that the contact zone between them was in the north west of the South Island. It is thought that this incongruence between morphological and molecular markers is indicative of a hybridisation which is more extensive than previously thought. However, the possibility that recent speciation has occurred or is occurring is not ruled out.

### 3.2 INTRODUCTION

Invertebrate morphological characters such as genitalia and wing coloration typically vary within a biological species, but that variation is often confined to a discrete morphological range with little or no overlap with other species (Coyne and Orr 2004). Such clusters of traits are important under various species concepts to delimit species boundaries. For example, the maintenance of a discrete range of genitalia infers a mechanism of reproductive isolation (Eberhard 1985), also known as the isolation species concept and central to the Biological Species Concept (Mayr 1942). Similarly, the recognition species concept implies that species maintain a discrete range of morphological characters such as wing markings to ensure a conspecific mate recognition system and thus prevent hybridisation (Fordyce *et al.* 2002). However, the presence of individuals with characters intermediate between two closely related species causes problems in the identification of taxa and may compromise the species concept used, especially when genetic markers identify alternative species boundaries (Nice and Shapiro 1999, Sperling 2003, Oliver and Shapiro 2007). This can further lead to false conclusions about allopatric speciation (Mallet 1995, Isaac *et al.* 2004), particularly for island populations where differentiation at the specific level may be subjective (Mallet 1995, Dapporto *et al.* 2007). The ‘intermediates’ may be either hybrids between two species or reflect natural variation within a single species. If the natural variation appears as clusters in morphological space this may also represent evidence of recent or ongoing speciation. Reconciling these hypotheses can assist in the understanding of morphological divergence (Nice and Shapiro 1999) and in identifying areas of conservation priority (Isaac *et al.* 2004, Dapporto *et al.* 2007). Furthermore species complexes exhibiting ambiguous delimitation provide excellent opportunities for studies of speciation (Sperling 2003).

Despite the presence of large numbers of intermediates based on wing coloration and male genitalia in sympatric areas of *Zizina labradus* and *Zizina oxleyi* in New Zealand (Gibbs 1980), an extensive study of the relationship between the two species has not been conducted. Such work is perhaps overdue however, particularly given recent speculation that the introduced *Z. labradus* may have reduced the range of the endemic *Z. oxleyi* (Yago *et al.* 2008). In the present study, the hypotheses of hybridisation and intraspecific variation are investigated using morphological and molecular analyses, with the further aim of elucidating the evolutionary history of the two species in New Zealand.

The lycaenid butterfly genus *Zizina* Chapman is widely distributed with members occurring from tropical to temperate zones of the Palearctic, Oriental, Australian and Afrotropical regions (Parsons 1999). The genus has also suffered from a historically difficult taxonomy. Different authors have regarded the group as having between one and four species due to variability in previously diagnostic male genitalia, the incongruence of male genitalia and wing coloration and unclear geographical borders (see Yago *et al.* 2008 for a synopsis). Until recently, the most widely accepted classification of four species was that of Bridges (1988), but molecular work by Yago *et al.* (2008) has reduced this number to three. However, the classification of the genus in New Zealand is still not satisfactory, with Gibbs' (1980) suggestion of hybridisation not being addressed fully by Yago *et al.* (2008) and continuing to add to the problems of classification. For this reason and for simplicity, the classification used in this study is that of Bridges (1988). This does not represent a taxonomic revision however. In addition, the distribution of the species of the genus in New Zealand described by Gibbs (1980) is assumed.

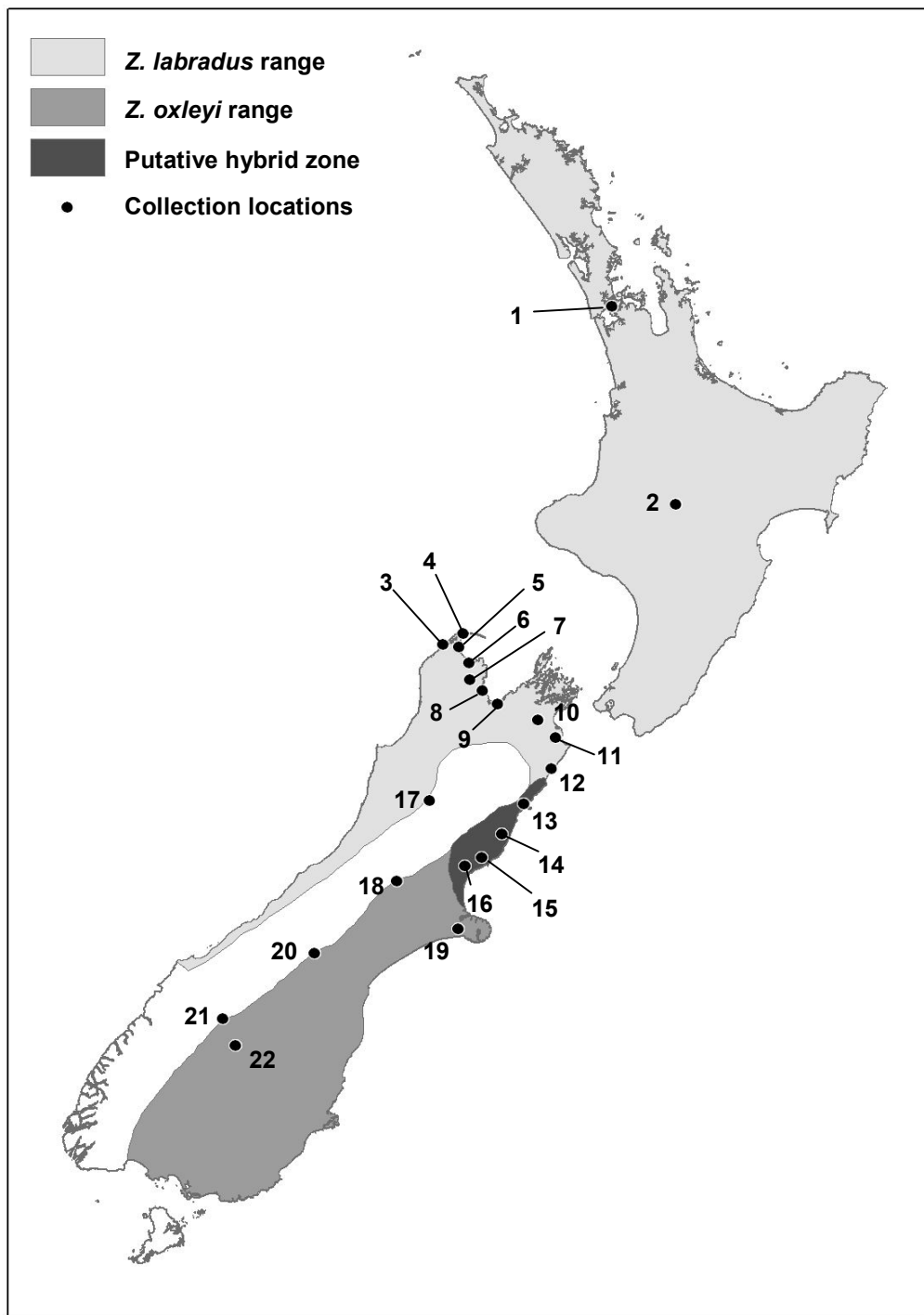
Only two species of *Zizina* occur in New Zealand. *Zizina labradus* (common blue), characterised by faint grey spots on the ventral surface of the hind wings, is found throughout Australia, Melanesia and Polynesia (Parsons 1999) and in New Zealand on the North Island, north and west of the South Island and on the Chatham Islands (Gibbs 1980; Fig. 3.1). *Zizina oxleyi* (southern blue), distinguishable from *Z. labradus* by dark brown markings on the ventral surface of the hind wings, is endemic to New Zealand and occurs in the south and east of the South Island (Fig. 3.1). The two species are virtually indistinguishable in behaviour, phenology and habitat preferences, both utilising members of the Fabaceae as host plants, although it is thought that *Z. oxleyi* originally fed on native *Carmichaelia* R.Br. (Fabaceae) species or native pea *Montigena novae-zelandiae* Hook. f. (Fabaceae) before European settlement and the range of exotic legume species that came with it (Gibbs 1980). The origin of the two species in New Zealand is unknown, although *Z. oxleyi* is thought to have diverged from a common ancestor either following the Gondwana separation or a more recent chance event in which windblown individuals arrived from Australia (Gibbs 1980). The introduction of *Z. labradus* either anthropogenically or via a similar chance event, may have checked the endemic species' progress through the country after the establishment of exotic European grasslands, due to superior performance of the former (Gibbs 1980). A lack of genetic distance between Australian and New Zealand specimens of *Z. labradus* suggests that a recent introduction is more likely to be the explanation rather than divergence after the Gondwana separation (Yago *et al.* 2008).

The most recent distribution maps (Fig. 3.1) imply that the ranges of these two species overlap between the Lower Awatere valley in Marlborough and Christchurch, Canterbury and that within this sympatric zone the two species interbreed freely (Gibbs 1980). This conclusion is based partly on the discovery of a number of specimens in the sympatric zone that represent intermediates between the two species in terms of male genitalia and ventral wing coloration. Gibbs (1980) found that in two regions of the sympatric zone, intermediates made up around 25% of the individuals captured, and has successfully crossed the two species in captivity (G.W. Gibbs, pers. comm.). Despite this, in an otherwise wide ranging molecular study of the *Zizina* genus, Yago *et al.* (2008) used a single mitochondrial gene (ND5) and seven specimens from one location in the sympatric zone to conclude that introgression of mtDNA via hybridisation had not occurred and that successive copulation between the two species was unlikely. However, this molecular work may not have been suitable for diagnosing the existence of hybrids. Furthermore it was not conducted on specimens from the supposed allopatric range of *Z. oxleyi*, so the degree of genetic and morphological variation remains uncertain. Such information is important in a conservation context, not least because it is vital to understand the genetic basis of organisms for which conservation is planned and their associated threats (Oliver and Shapiro 2007), but because it raises questions about the potential continuance of competitive exclusion of *Z. oxleyi* by *Z. labradus*. From an evolutionary context, the hypotheses concerning the origin of the intermediates remain untested and may provide important information on the evolutionary history of these two species. This study aims to clarify the *Zizina* taxonomy in New Zealand through a combination of morphological and molecular analyses. In doing so, it is anticipated that the theory of hybridisation will be tested and gaps in the knowledge of the *Zizina* genus in New Zealand outlined above will be addressed.

### **3.3 METHODS**

#### **3.3.1 Sampling**

A total of 163 adult males were used in the analysis of genitalia morphology. Most of the specimens were collected from a wide range of sites in New Zealand in March 2009 and April 2010. However these collections were supplemented with specimens from Sydney, Australia and from a collection provided by G.W. Gibbs dating from March 2002. The collection sites in New Zealand are shown in Fig. 3.1 and are listed in Table 3.1.



**Figure 3.1:** Map showing current distribution of *Z. labradus*, *Z. oxleyi* and the putative hybrid zone redrawn from Gibbs (1980). Numbers refer to the locations of sampling and are listed in Table 3.1.

**Table 3.1:** The details of specimens collected for morphological and molecular analyses with map reference numbers (Fig. 3.1), location and number of specimens used in analyses. Distribution determination is based on the ranges of the two species from Gibbs (1980), shown in Fig. 3.1. In the putative hybrid zone locations, the term “both” refers to the possibility that either species or intermediates between the two may be found.

Map reference number (Fig. 3.1)	Location	Number of specimens – morphology analysis	Number of specimens – molecular analysis	Distribution determination
1	Auckland	5	1	<i>Z. labradus</i>
2	Lake Rotoaira	10	1	<i>Z. labradus</i>
3	Paturau River	4	3	<i>Z. labradus</i>
4	Puponga	2	1	<i>Z. labradus</i>
5	Collingwood	4	1	<i>Z. labradus</i>
6	Takaka	7	1	<i>Z. labradus</i>
7	Upper Takaka	9	1	<i>Z. labradus</i>
8	Motueka	8	1	<i>Z. labradus</i>
9	Nelson	9	4	<i>Z. labradus</i>
10	Renwick	6	1	<i>Z. labradus</i>
11	Seddon	8	1	<i>Z. labradus</i>
12	Kekerengu	1	1	<i>Z. labradus</i>
13	Kaikoura	5	1	both
14	Parnassus	9	1	both
15	Motunau	7	1	both
16	Waipara	45	10	both
17	Marble Hill	4	0	<i>Z. labradus</i>
18	Castle Hill	4	1	<i>Z. oxleyi</i>
19	Lake Ellesmere	2	2	<i>Z. oxleyi</i>
20	Lake Tekapo	1	1	<i>Z. oxleyi</i>
21	Wanaka	7	5	<i>Z. oxleyi</i>
22	Lowburn	2	1	<i>Z. oxleyi</i>
23*	Chatham Islands	1	2	<i>Z. labradus</i>
24*	Sydney, Australia	3	7	<i>Z. labradus</i>
	Total	163	48	

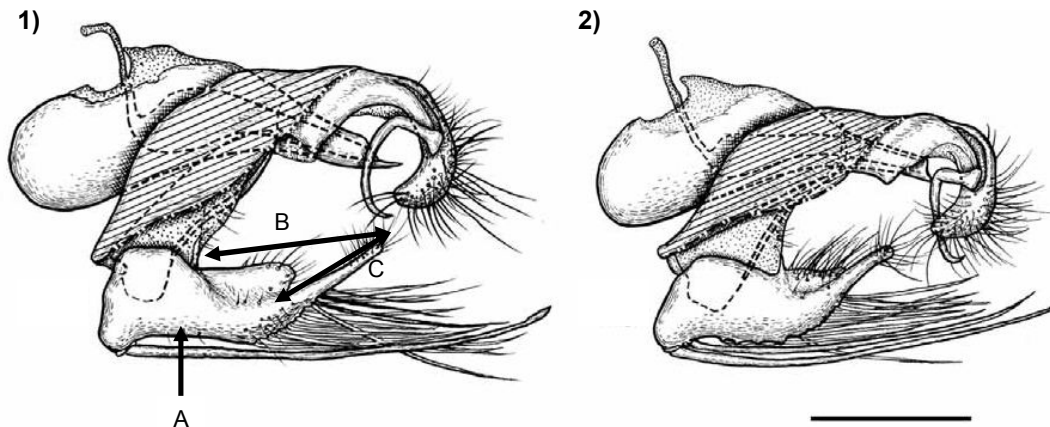
\* locations not shown on Fig. 3.1

### 3.3.2 Genitalia morphology

The abdomen of each male specimen was removed before DNA analysis, and cut in half using sterilised equipment on each occasion to prevent DNA contamination. The apical section was placed in 5% KOH solution overnight to macerate the abdomen tissue, and the remaining section was stored in 96% ethanol in a freezer. Following maceration of the apical section, the genitalia could be pulled apart from the tissue with fine forceps and examined under a binocular microscope at x63 magnification. Firstly, using the classification of Yago *et al.* (2008), the valve itself and the distal margin of the process were measured (Fig. 3.2) using the microscope's reticle. Measurements from the reticle were then transformed to mm for data analysis. Secondly, genitalia were scored on the setal pattern on the valves because a further distinction between the two species is the presence of two long setae on each genital valve of *Z. oxleyi*, and a single seta on each valve of *Z. labradus*. Gibbs (1980) found that intermediate individuals were detectable due to a combination of these features (a single seta on one genital valve and two on the other) or to the position of a second seta halfway between the posterior seta and the smaller distal setae (shown by the letter A in Fig 3.2). This latter arrangement is referred to henceforth as the 'intermediate position' and may be present on either or both valves. A scoring system for the genital setae arrangement was developed by Gibbs (1980) ranging from 0% resemblance to *Z. oxleyi* to 100% resemblance to *Z. oxleyi*. This system is used in this study (Table 3.2).

**Table 3.2:** The scoring system used for the genital valve setal arrangement, developed by G.W. Gibbs (pers. comm.).

Score	Description
0	A single seta on both genital valves ( <i>Z. labradus</i> )
25	One seta on one valve, two on the other with one seta in the intermediate position.
50	One seta on one valve and two on the other, or Two seta on either side, with both second setae in intermediate positions
75	Two setae on one valve and two on the other valve but with the second seta in the intermediate position
100	Two setae on both genital valves ( <i>Z. oxleyi</i> )

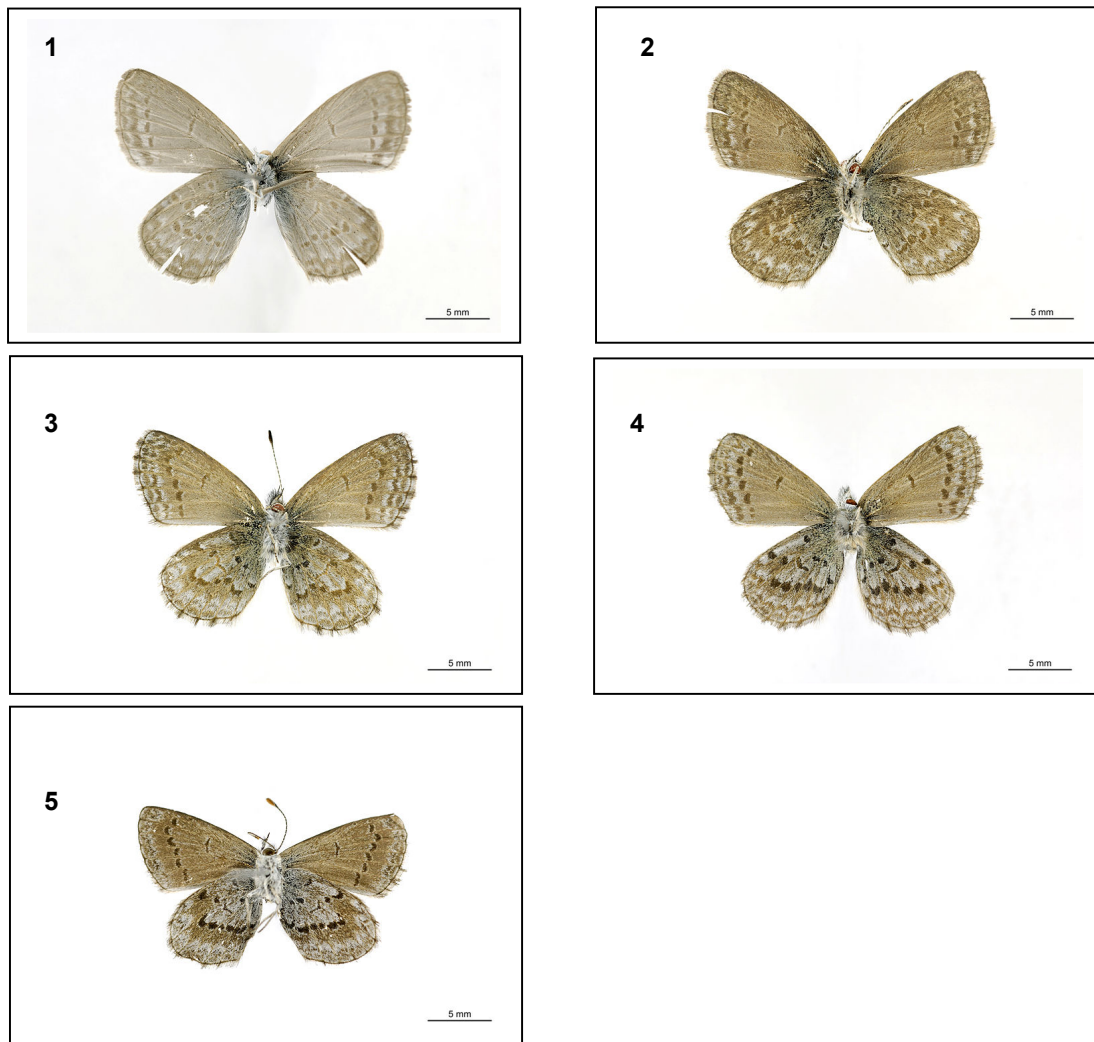


**Figure 3.2:** Male genitalia of 1) *Zizina labradus* and 2) *Zizina oxleyi*, reproduced from Yago *et al.* (2008). *Z. labradus* is differentiated from *Z. oxleyi* by a stouter valve distal process (C), and by the presence of a single posterior valve seta, compared to two of *Z. oxleyi*. The intermediate position of the second seta in putative hybrids is indicated by A. Measurements were also taken of the valve (B and C). Scale bar = 0.5mm.

### 3.3.3 Wing morphology

Following removal of the abdomen, each specimen was scored on ventral hind wing spot coloration using a five point index: 1 = markings barely distinguishable from other parts of the underside of the wings, 2 = light grey markings, 3 = light brown markings, 4 = dark brown markings and 5 = nearly black markings. Examples of each of the 5 scores are shown in Fig. 3.3. Although the index scoring system is relatively arbitrary compared to more sophisticated geometric techniques, time and resources were insufficient to apply such a system. In order to limit bias in the scoring system, however, specimens were scored twice and ‘blind’ on both occasions so that the scorer was not aware of the origin of each specimen to ensure independent scoring.





**Figure 3.3:** Typical ventral hind wing coloration of individual *Zizina* butterflies studied, showing the variation in dark spots. The numbers of the photographs refer to their index score. Locations: 1) Sydney, Australia, 2) Paturau River, NW South Island, 3) Seddon, NE South Island, 4) Lake Ellesmere, Central South Island, 5) Wanaka, S. South Island.

### 3.3.4 Data analysis

For the purposes of data analysis, specimens were allocated to three groups based on the distribution determination in Table 3.1: 1) those specimens collected within the *Z. labradus* range as far as the putative hybrid zone, 2) those specimens collected within the *Z. oxleyi* range as far as the putative hybrid zone, and 3) those specimens collected within the putative hybrid zone.

Morphological data were not normally distributed so the non-parametric Kruskal-Wallis test was used to test for differences in mean values between the three groups of specimens. Variation in all

morphological variables was assessed using principal component analysis. Differences in morphology were determined using MANOVA on the principal component scores of each male individual. These scores were then used to plot the location of each male in morphological space to identify differences between the three groups. All analyses were performed using R 2.9.2 (R Core Development Team, 2009).

### 3.3.5 Phylogenetic analysis

Total genomic DNA was extracted from the remaining tissues of the abdomen of a small selection of specimens using the Qiagen DNeasy Tissue kit (Qiagen Inc.) following the manufacturer's instructions. Negative controls were included with each set of extractions. The mitochondrial gene cytochrome oxidase subunit I (COI) was amplified and sequenced using the primers HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' and LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' (Folmer *et al.* 1994). The polymerase chain reactions (PCR) were conducted in 25µL final volume reactions containing 0.2µL *itaq* polymerase (5µ/µL), 2.5µL of x 10 buffer, 0.2 mM of each dntp, 1.0µL of each primer from a 10µM stock (all from Invitrogen), 2.5µL of DNA extraction and 15.3µL of water. A PCR MasterCycler (Eppendorf) was used with the following cycling conditions: 94°C denaturation for 1 min followed by 30 cycles of 94°C for 30s, annealing at 45°C for 30s and extension at 72°C for 90s, with a final extension of 72°C for 10 min. Negative controls were included with each set of PCRs. PCR products were examined by running 5µL of PCR product by gel electrophoresis at 80V for 40 min on a 1.5% agarose gel made with SYBRSafe (Invitrogen) and visualising bands with the program GenSnap (Syngene, Synoptics Ltd). Clearly visible bands were considered to be positive PCR products. Subsequent sequencing reactions contained 0.5µL of BigDye Terminator (version 3.1) and 2µL of x5 sequencing buffer (both from ABI Prism Cycle Sequencing Kit), 0.8µL of primer (from a 10µM stock), 0.5µL of PCR product and 6.2 µL of water. The sequencing PCR was at 96°C for 1 min, followed by 25 cycles of 96°C for 10s, 50°C for 5s and 60°C for 4 min. The resulting sequence PCR products were processed in an ABI Prism 3130xl Genetic Analyser with a 16 capillary 50cm array installed and using Performance Optimized Polymer 7 (POP7). A total of 604bp of COI was sequenced for each sample. Sequences were aligned by eye using the program MEGA 4.1 (Temura *et al.* 2007) and identical sequences were treated as a single operational unit (OUT). In total 30 sequences, including an outgroup species *Lampides boeticus* L. (Lycaenidae) (long-tailed blue) taken from GenBank (Accession No: EU919326.1),

were used to create a Neighbour joining tree using Maximum Composite Likelihood in MEGA 4.1 (Tamura *et al.* 2007).

### 3.4 RESULTS

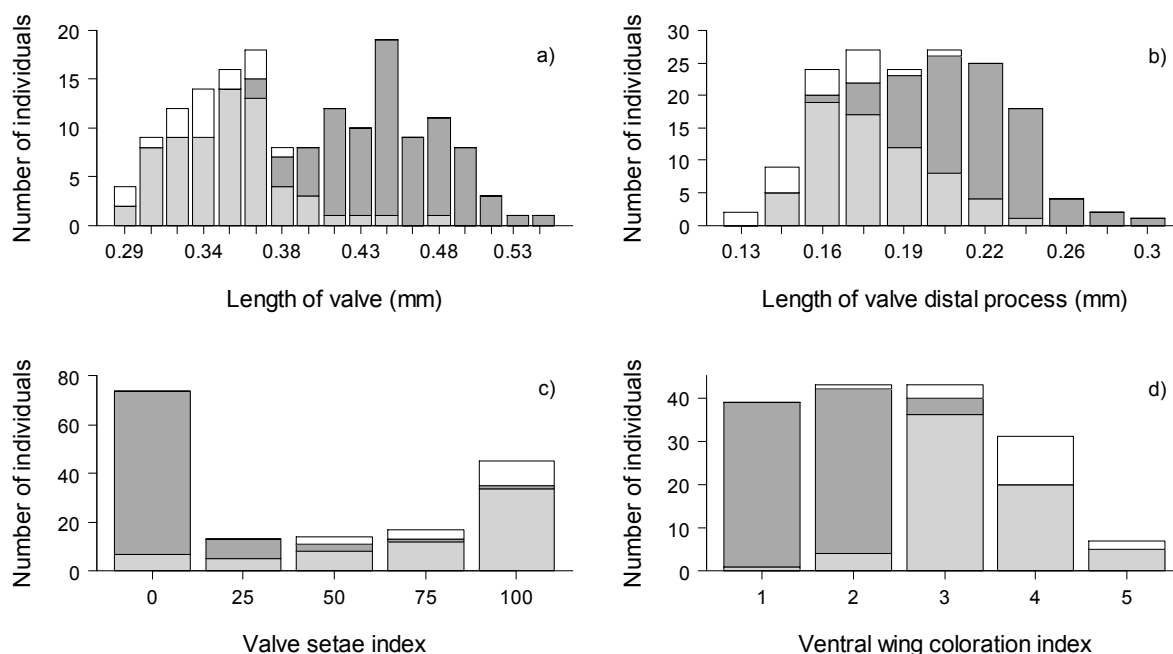
#### 3.4.1 Genitalia morphology

The valve lengths of individual specimens exhibited a bimodal distribution with some overlap with the majority of putative hybrid specimens showing similar measurements to specimens from the *Z. oxleyi* range (Fig. 3.4a). There was a significant difference between mean valve lengths of the three groups (Kruskal-Wallis chi squared=108.9,  $P<0.0001$ ), and multiple comparisons showed that there were significant differences between the mean valve length of specimens from the ranges of *Z. oxleyi* and *Z. labradus* ( $P<0.0001$ ) and *Z. labradus* and putative hybrids ( $P<0.0001$ ) but not between mean valve lengths of specimens from the *Z. oxleyi* range and the putative hybrid zone. Comparison of specimens from the allopatric zones only (Lake Ellesmere and south for *Z. oxleyi*; North Island, Chatham Islands and Australia for *Z. labradus*) indicated that the separation point between the two species is around 0.38mm: 95% of all *Z. oxleyi* range specimens and 90% of putative hybrids had valve lengths equal to or below this value, and 92% of all *Z. labradus* range specimens had valve lengths above this value.

Measurements of the distal process showed a similar pattern but there was a greater degree of overlap (Fig. 3.4b). The means for the three groups of specimens were significantly different (Kruskal-Wallis chi squared = 79.5,  $P<0.0001$ ), and multiple comparisons showed that the differences were between specimens from the ranges of *Z. oxleyi* and *Z. labradus*, and *Z. labradus* and putative hybrids only ( $P<0.0001$ ). The value of separation between allopatric specimens appeared to be around 0.19mm: 87% of *Z. oxleyi* range specimens and 80% of putative hybrids had measurements equal to or below this value, and 79% of *Z. labradus* range specimens had measurements above it.

The valve setae index appeared less useful for identifying species. While 80% of *Z. labradus* range individuals had only a single seta on each valve, 20% showed intermediate indices. Of the *Z. oxleyi* range individuals, only 59% exhibited typical valve setae arrangements. Of the putative hybrids, 55% exhibited typical *Z. oxleyi* patterns and 9% exhibited *Z. labradus* patterns (Fig. 3.4c). Despite this overlap, there was a significant difference between the mean

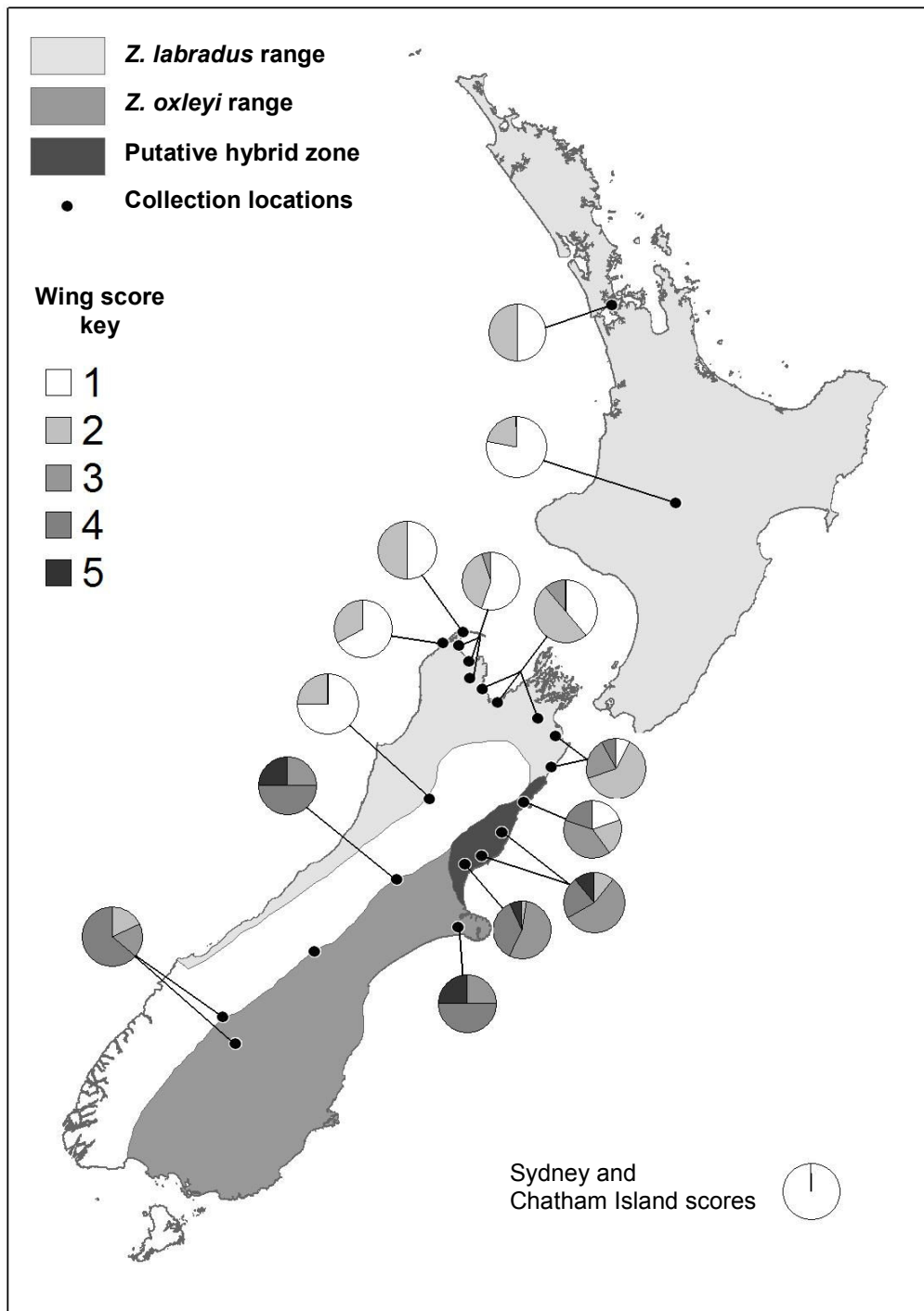
scores of the groups (Kruskal-Wallis chi squared = 103.3,  $P < 0.0001$ ), and multiple comparison differences showed the same pattern as above at the  $P < 0.0001$  level.



**Figure 3.4:** Distributions of the three genitalia variables and wing coloration score used for morphological differentiation of the two *Zizina* species: a) Valve length measurements, b) valve distal process measurements, c) valve setae indices and d) ventral wing coloration index. White bars: specimens from *Z. oxleyi* range, Light grey bars: putative hybrids, Dark grey bars: specimens from *Z. labradus* range.

### 3.4.2 Wing coloration

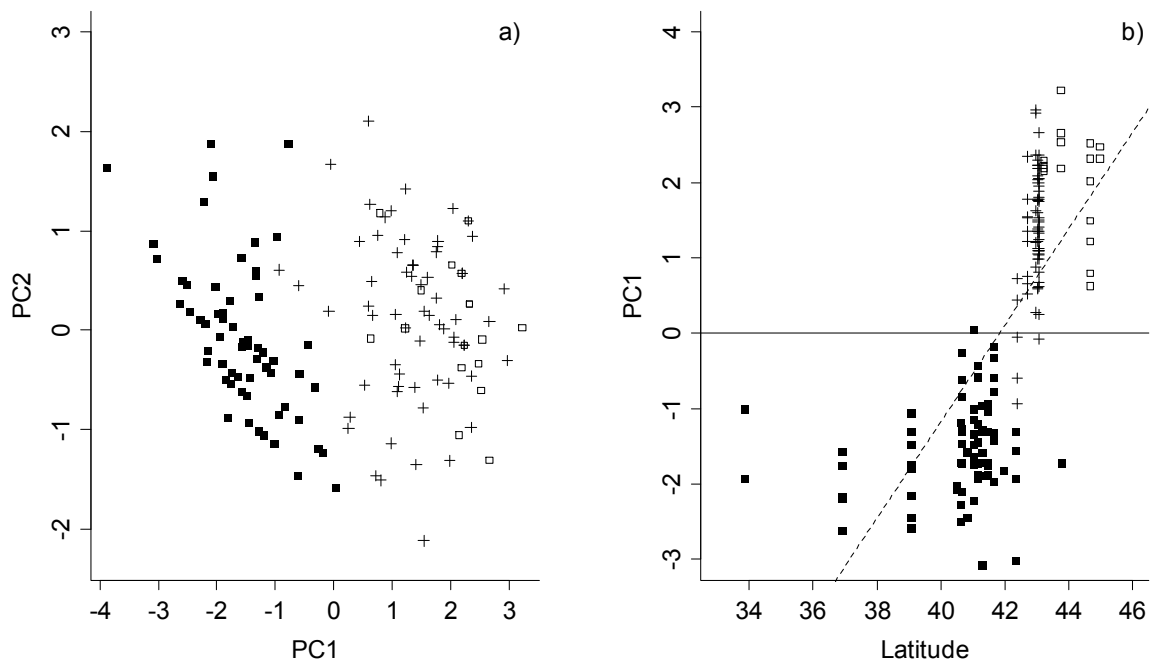
While 92% of *Z. labradus* range specimens were scored as pattern 1 or 2, the *Z. oxleyi* range individuals were more difficult to identify with certainty with 24% being identified as exhibiting an “intermediate” or *Z. labradus* wing pattern (Fig. 3.4d). Of the putative hybrids, 38% had *Z. oxleyi* wing coloration, and 55% had “intermediate” colouring. There was a significant difference between the mean scores of the groups (Kruskal Wallis chi squared = 111.6,  $P < 0.0001$ ), and multiple comparison showed that differences in wing score were significant between specimens from the ranges of *Z. labradus* and *Z. oxleyi*, and *Z. labradus* and putative hybrids ( $P < 0.0001$ ), but not between those from the range of *Z. oxleyi* and the putative hybrid zone. Fig. 3.5 further shows the broad overlap in wing coloration. Here the variation seems to show latitudinal clinal variation with a greater proportion of lighter wing coloration further north.



**Figure 3.5:** Distribution of ventral wing coloration scores for all specimens analysed. Pie charts show proportions of wing scores at each location or group of locations. Specimens were grouped with closest location if there were less than 5 individuals. The location with no pie chart is the Lake Tekapo site. Sampled individuals from this site were too old to determine a score.

### 3.4.3 Principal component analysis

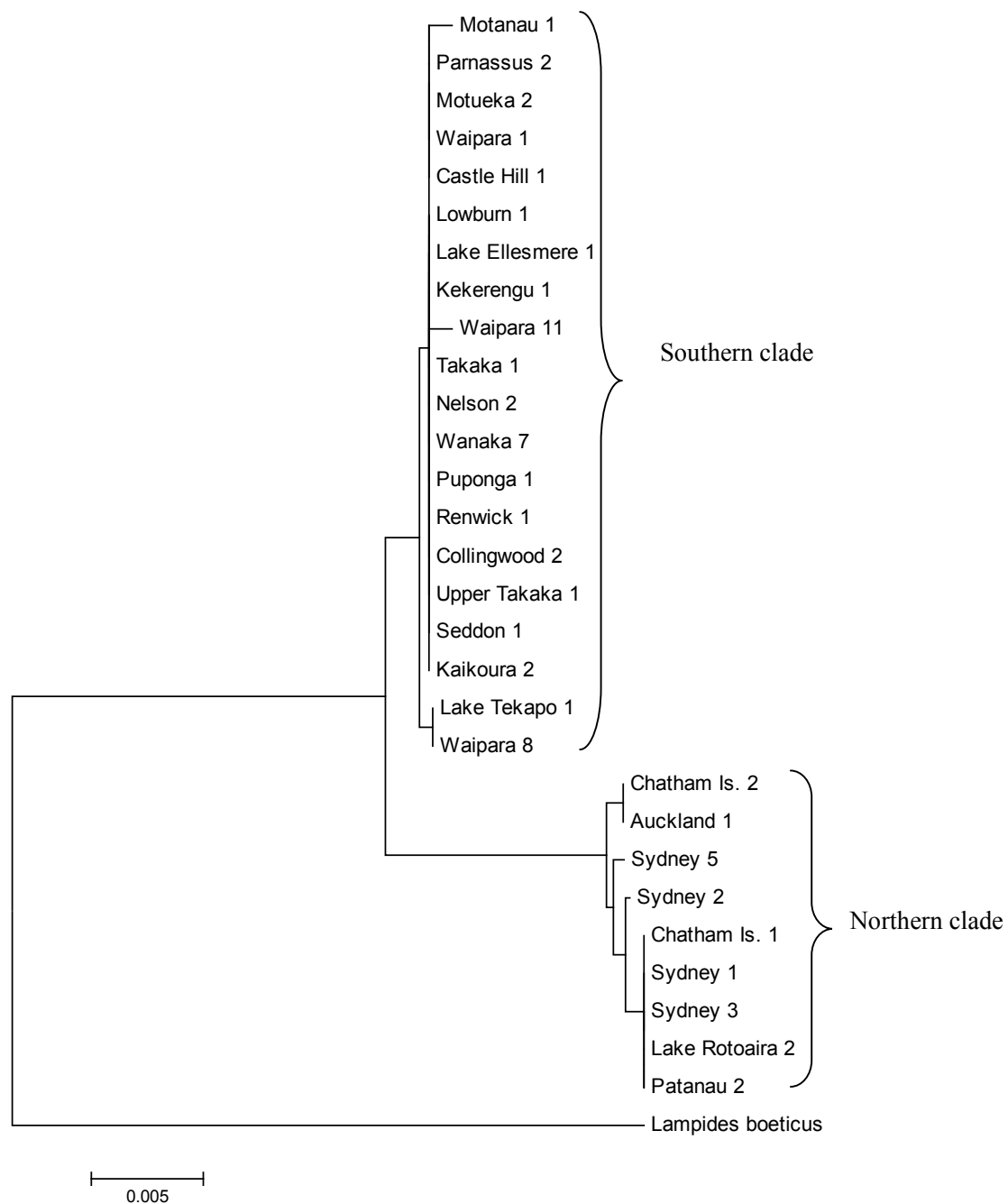
The first principal component (PC1) accounted for 73% of the observed variation in male morphology and the second (PC2) accounted for a further 15%. PC1 was negatively loaded by the size measurements of valves (eigenvector coefficients: valve length: -0.53; distal process: -0.49) but positively loaded by the indices for setae and wing coloration (eigenvector coefficients: setae score: 0.47; wing index: 0.51). As *Z. oxleyi* is supposedly distinguishable from *Z. labradus* by shorter valve measurements and higher scores in the setae and wing indices, PC1 appears to represent the main distinguishing component between the two species. PC2 was positively loaded by all four variables, most strongly by the valve distal process measurements (0.64) and the setae index (0.66). The MANOVA analysis revealed that only PC1 differed significantly between the three groups ( $F_{2,160} = 391.47$ ,  $P < 0.0001$ ). However, in contrast to the individual character analysis, Tukey HSD multiple comparisons showed that there were significant differences between all groups ( $P < 0.0001$ , except for differences between *Z. oxleyi* and putative hybrids:  $P < 0.01$ ). The morphological separation of the groups is shown in Fig. 3.6a. There are two clear clusters with specimens from the *Z. oxleyi* range and the putative hybrid zone grouped together, although with some overlap with the individuals from the *Z. labradus* range. In addition to the PCA plot, PC1 scores were plotted against the latitude at which each individual was collected (Fig. 3.6b). There was a strong positive correlation between PC1 scores and latitude (Spearman's rank correlation coefficient = 0.81,  $P < 0.0001$ ). There was also almost complete clustering of specimens in groups above and below the PC1 = 0 line. All specimens from the *Z. oxleyi* range lie above this line, and all specimens from the *Z. labradus* range lie below it. Only four putative hybrids lie below the line: three are from Kaikoura (out of 5 specimens from Kaikoura), and one is from Waipara. This may suggest that the contact zone, or the point where the ranges of the two species meet is in Kaikoura.



**Figure 3.6:** a) Principal component scores from morphological analysis for individuals from the ranges *Z. labradus*, *Z. oxleyi* and the putative hybrid zone, b) the correlation between PC1 scores from morphological analysis and latitude is indicated by the dashed line which is the line of best fit. The horizontal line at PC1 = 0 demonstrates the strength of the clustering. Individuals are plotted on both plots according to ranges as delimited by Gibbs (1980): solid squares = *Z. labradus*, crosses = putative hybrids, open squares = *Z. oxleyi*.

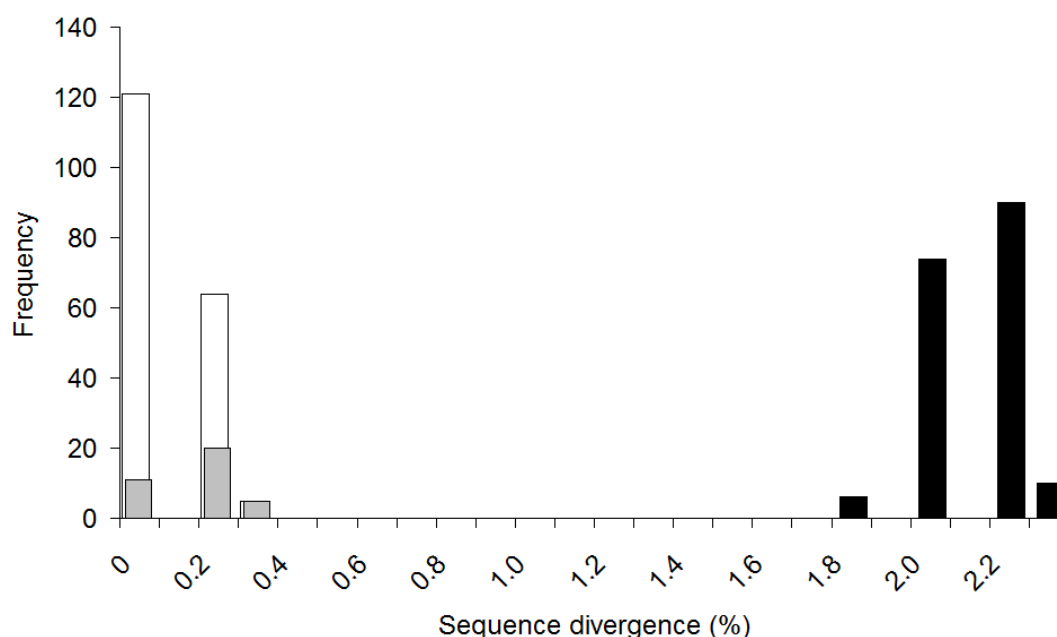
### 3.4.4 Molecular data

Individuals from all populations except Marble Hill were sequenced successfully resulting in 604bp regions of the mitochondrial cytochrome oxidase subunit I (COI) gene. Two distinct clades are indicated by the tree topology (Fig. 5.7), although neither forms a monophyletic group. Interestingly, the northern clade is restricted on the South Island to Paturau River (point 3 on the map in Fig. 3.1), as well as the North Island, the Chatham Islands and Australia. The southern clade appears to span the entire east side of the South Island and the north coast of the South Island, and therefore incorporates individuals from all three distribution groups. Mean sequence divergence between the clades, regardless of haplotype is 2.1%, but intra-clade sequence divergence was extremely low (Fig. 5.8). However, the separation between intra-clade variation and inter-clade variation suggests that the two clades form distinct genetic entities (Fig. 5.8).



**Figure 3.7:** Neighbour-joining tree of mtDNA cytochrome oxidase subunit I haplotypes. The optimal tree with the sum of branch length = 0.062 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. Phylogenetic analyses were conducted in MEGA 4.1 (Tamura *et al.* 2007). *Lampides boeticus* is the outgroup species.





**Figure 3.8:** Divergence of COI sequences of the two clades resulting from DNA analysis of specimens from the *Zizina* genus using p-distance. The distribution of interclade divergence is shown by black bars. The distribution of intraclade divergence is shown by white bars (southern clade) and grey bars (northern clade).

### 3.5 DISCUSSION

#### 3.5.1 The distribution of the *Zizina* species

The distribution of the *Zizina* species in New Zealand has not been studied in detail to date and identification in the field has largely relied on ventral wing coloration (Gibbs 1980). However, this study has shown that this sole exterior diagnostic character is particularly variable and that morphological and geographical identification is incongruent with molecular data.

Genitalia morphological traits showed clear distinctions between specimens from the ranges of *Z. oxleyi* and *Z. labradus*. However, specimens from the putative hybrid zone were not significantly different from those from the *Z. oxleyi* range using individual morphological characters, suggesting that they belong to the same species. When specimens are analysed in this way, it appears that there is no ambiguity and that the ranges of the two species meet in Kaikoura but do not overlap. However, in support of Gibbs' (1980) findings, morphologically ambiguous individuals were found in all areas, with overlap in genitalia measurements, valve setae number

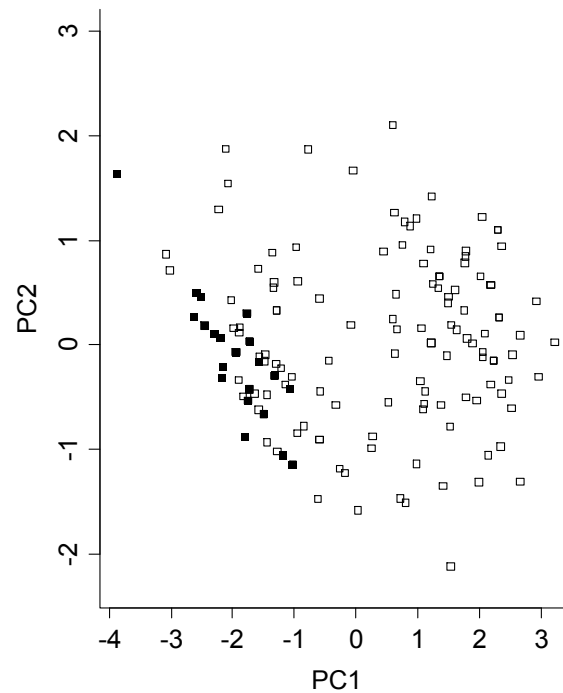
and position and wing coloration. Individually, all three groups were identifiable from morphological characters in all three ranges, particularly when using wing coloration, the only distinguishable exterior character used. Similarly, while the PCA plot appears to support the conclusions that there are only two clearly delimited species, multiple comparisons showed that specimens from the putative hybrid zone were significantly different from both allopatric ranges. This may be due to the wide range of overlap between all three groups.

The COI sequences described a different distribution. Here, specimens from the putative hybrid zone were genetically similar to those from the range of *Z. oxleyi*. In addition, specimens from part of the *Z. labradus* range in the north of the South Island were also included in this southern clade. Specimens from only one location on the South Island, Paturau River, were included in the northern clade. Although all specimens were not analysed genetically, a broad range of intermediates were analysed and showed extremely low intra-clade genetic variation. There was 2.1% divergence between the two clades which is on the borderline of species delimitation (Sperling 2003), but within the range observed for fully distinct species in various butterfly families (Aubert *et al.* 1997, Martin *et al.* 2000), including the Lycaenidae (Kandul *et al.* 2004, Als *et al.* 2004). Using a phylogenetic species definition, this may indicate that *Z. oxleyi* is more widespread than originally thought, and that *Z. labradus* only occurs on the North Island, the Chatham Islands and in the north west of the South Island, although other regions of the west coast of the South Island were not surveyed. Further diagnostic phylogenetic work is required to corroborate this finding, and sampling from a greater range of locations would help to identify the contact zone in more detail.

The incongruence of the data is demonstrated in Fig. 3.9. Here, species delimitation is inferred from the molecular analysis by grouping together the specimens from the two clades and assuming that all specimens not analysed have the same sequences as those from the same location. This broad overlap of the clades in morphological space further shows the ambiguous nature of the two *Zizina* species, indicating that geographical, molecular and morphological separation of the two species is uncertain.

The situation concerning the potential hybrid zone is similar to that found for other species complexes in the Lepidoptera. For example, *Papilio rutulus* and *P. eurymedon* are distinct species in North America under most species definitions but do not vary in mtDNA sequences (Sperling 1993), and *Lycaeides idas* and *L. melissa* lack phylogenetic structure despite differences in genitalia morphology, wing coloration and habitat use (Nice and Shapiro 1999,

Nice *et al.* 2002). However, the *Zizina* species in this study show an alternative pattern in another part of the country. In the north west of the South Island, the opposite situation occurs, where genetic differentiation is apparent, but morphological differentiation is not. This situation is rarer in the literature. It would therefore provide an opportunity to research an unusual species relationship and additional phylogenetic data and data on ecological and behavioural traits across all ranges are required to fully resolve this issue.



**Figure 3.9:** Principal component scores from morphological analysis for individuals from the ranges of *Z. labradus*, *Z. oxleyi* and the putative hybrid zone, grouped by molecular clade: solid squares = northern clade, open squares = southern clade.

Despite this complexity, attempts may be made to update the current theory on the evolutionary origin of the two species in New Zealand. The lack of congruence between morphological traits and molecular markers may be evidence of either recent or ancient gene flow through hybridisation (Arnold 1997). The data presented here are consistent with this hypothesis and suggest that introgression of *Z. oxleyi* mitochondrial DNA is occurring or has occurred in the putative hybrid zone of Gibbs (1980). However, the distribution of southern clade mtDNA suggests that this introgression is more widespread reaching as far as Puponga, but not yet as far Paturau River. This would explain the overlapping morphological characters throughout

the South Island and the conservation of the northern clade type DNA in Paturau River populations.

Hybridisation is not rare in butterflies (Descimon *et al.* 1989, Verovnik and Glogovcan 2007) and any slight overlap in morphology is likely to allow this to occur. Reproductive isolation appears incomplete between the two species studied here and individuals with wing coloration resembling *Z. labradus* can be found in *Z. oxleyi* allopatric zones due to the loss of dark markings with age (Gibbs 1980). Although courting rituals between the two species may differ and other prezygotic isolating mechanisms are not known, lycaenids in particular are known to demonstrate mate choice based on wing coloration (Bernard and Remington 1991), so the overlap of wing markings makes hybridisation a possibility.

In breeding experiments, the two butterflies have been shown to interbreed (G.W. Gibbs, pers. comm.) although the viability of offspring is not known. Haldane's rule (c.f. Orr 1997) states that the heterogametic sex, the female in Lepidoptera, should be more negatively affected by hybridisation. These effects have been observed in a number of butterfly species (Sperling 1993, Jiggins *et al.* 2001, Presgraves 2002), and are thought to imply that maternally inherited genes such as COI only rarely penetrate species boundaries (Sperling 1993, Nice and Shapiro 1999), a case shown for swallowtail butterflies for example (Sperling 1993, Aubert *et al.* 1997). Viability of hybrids is not always reduced however (Aubert *et al.* 1997, Porter *et al.* 1997). In this study most individuals in the north of the South Island are morphologically similar to *Z. labradus* but are genetically identical to those from the *Z. oxleyi* range. This implies the mating of a *Z. oxleyi* female and a *Z. labradus* male (Aubert *et al.* 1997, Verovnik and Glogovcan 2007). If this is the predominant direction of hybridisation, hybrid females with the maternally inherited mtDNA of *Z. oxleyi* which are able to mate with both parent species would lead to a proliferation of intermediate individuals with *Z. oxleyi* COI sequences. If this is the case, this proliferation has reached as far as Paturau River, where populations of *Z. labradus* remain intact. Although *Z. oxleyi* are not capable of strong flight (Gibbs 1980), their food plants are contiguous throughout the country, enabling dispersal along road-sides for example. Sedentary species have been shown to disperse relatively long distances, such as Fender's blue (*Icaricia icarioides fenderi* Macy (Lycaenidae)) which may disperse up to 2km between habitat patches (Schultz 1998). Nevertheless, the possible dispersal of hybrid individuals with *Z. oxleyi* mtDNA as far as Paturau River would suggest that if hybridisation is occurring, it is in favour of the endemic *Z. oxleyi*, rather than *Z. labradus* as was previously feared (Barlow and Goldson 2002, Yago *et al.* 2008).

The clinal variation of morphological traits also could be tentatively suggested as evidence of a hybrid zone. Clinal variation is a common form of geographic variation in nature (Endler 1977) and may occur due to factors, acting singly or in combination, such as gene flow, random drift or hybridisation between two isolated populations (Arnold 1997). Steep clines can be associated with hybrid zones and the evolution of isolating mechanisms (Endler 1977), but this should not be assumed unless evidence of variability in fitness, morphology or other factors is available (Mayr 1963). Variation with latitude may also simply be an adaptation to ecological or climatic conditions (e.g., Nylin and Svard 1991).

The hybridisation hypothesis is partially suited to current evolutionary theory of *Z. oxleyi* origin. Prior to European settlement, there were no records of *Z. labradus*, and *Z. oxleyi* is thought to have been the common species on both islands (Gibbs 1980), suggesting that divergence occurred as a result of the geographical separation. The later arrival of *Z. labradus* therefore suggests secondary contact between the two forms. However, as this study has shown, it is difficult to determine the species on the basis of morphology alone, so it is possible that early naturalists were mistaken about the distribution of *Z. oxleyi*, taking wing coloration as natural variation of a single species. Furthermore, if proliferation of hybrids with *Z. oxleyi* mtDNA has occurred northwards from around Kaikoura recently, this implies that the range of *Z. oxleyi* prior to hybridisation was south of this region as per the distribution of Gibbs (1980). However, if *Z. oxleyi* was actually the common species on both islands prior to European settlement, range retraction must have occurred in the past. This begs the question of why hybridisation did not occur immediately following secondary contact on the arrival of *Z. labradus* after European settlement. Without historical data and more extensive phylogenetic work, it is difficult to accurately retrace the evolutionary origin of the two species.

Incongruence between morphological and molecular distributions may also be indicative of recent speciation (Nice and Shapiro 1999, Oliver and Shapiro 2007). Lycaenid butterflies are particularly considered to be capable of rapid speciation (Nice & Shapiro 1999; Aagaard *et al.* 2002, Eastwood & Hughes 2003, Oliver and Shapiro 2007). Overlaps in morphological space between individuals from the two ranges on the east coast of the South Island in this study may point to recent speciation if there has been insufficient time for morphological reproductive isolation to evolve completely (Oliver and Shapiro 2007). Levels of morphological reproductive isolation have been correlated with Nei's *D* and thus time since divergence for *Drosophila* sp. (Coyne and Orr 1989). Furthermore, introgression may be expected to continue between recently

diverged species close to the sympatric zone resulting in intermediate individuals, and the lack of differentiation between COI sequences may be due to differing rates of morphological and genetic evolution which have been shown to be uncoupled in nature for butterflies (Martin *et al.* 2003). However, while it is not possible to rule this hypothesis out completely, it does not explain the population at Paturau River with distinct COI sequences from the rest of the South Island, or those on the North Island. These populations may be the result of isolated and more recent invasion events of *Z. labradus* from Australia, but this subsequently suggests that individuals resembling *Z. labradus* but with *Z. oxleyi* mtDNA have undergone convergent evolution. There is insufficient data to establish whether this is the case. This hypothesis also does not explain the apparent displacement of *Z. oxleyi* from the North Island.

### 3.5.2 Conclusion

Overall, the relationship between the two species requires more study in all areas for adequate testing of these hypotheses. For example, modern geometric morphometric methods provide a greater appraisal of size and shape differences in both male and female genitalia and allow a greater distinction to be made between closely related species (Marcus *et al.* 1996, Lestrel 1997, Mutanen 2005). This is especially important as the genitalia markers studied here may not be the most functionally important for genital coupling and therefore not subjected to strong evolutionary forces (e.g., Goulson 1993). Furthermore, mtDNA is not suitable for determining the presence of hybrids because it is maternally inherited and therefore not subject to recombination. The analysis of nuclear markers or microsatellite loci is required to make more sound conclusions. These more comprehensive techniques should be applied to this interesting case study and over a broader range of sites, and where possible using museum specimens to test whether genetic differentiation in the putative hybrid zone has changed over time. Overall, the ranges and evolutionary origins of the two species in New Zealand remain uncertain, but offer an ideal opportunity to study speciation and/or hybridisation in depth. Nevertheless, this study has added support to the suggestion that the two *Zizina* species in New Zealand are hybridising. The new data suggest that if this is occurring, it is likely to be in favour of the endemic *Z. oxleyi*, rather than the introduced *Z. labradus* as was previously feared (Barlow and Goldson 2002, Yago *et al.* 2008). Furthermore, it would appear that the blue butterfly species encountered in vineyard surveys (Chapter 2) is *Z. oxleyi*, based on both morphological and mtDNA analysis, although the further work described above would corroborate this.

## **Chapter 4 The role of nectars of New Zealand endemic dicotyledons in the fitness of adult *Lycaena salustius* (Lepidoptera: Lycaenidae).**

### **4.1 ABSTRACT**

One of the most important factors influencing the stability of butterfly populations is adult nutrition, yet beyond general observations, information on preferred nectar sources in farmland settings is lacking, particularly in New Zealand. The importance of sugars and amino acids in the adult diet is clear, but the amino acid requirements of different butterfly species differ, so the richest sources of amino acids are not necessarily the best for all species. Flower preference may be affected by time of day or season, weather, flower structure and butterfly behaviour and morphology. However, studies have not yet linked butterfly flower choices in the field to the existing laboratory work on the role of particular nectar compounds in adult fitness. Although nectar sources are available for *Lycaena salustius* on New Zealand vineyards, it is unclear whether these are optimal species or are those used opportunistically in the absence of true preferences and thus possibly preventing maximal reproduction. This study aims to quantify the use of nectar by *L. salustius* adults in three ways: 1) field observations of nectar use in current habitat patches of New Zealand vineyards, 2) a field-choice experiment involving species currently available and those species expected to be preferred, and 3) a greenhouse no-choice bioassay investigating fitness enhancement by different flower species. This latter experiment is thought to be the first of its kind as applied to butterflies. *Lycaena salustius* was found to be an opportunistic nectar feeder, relying largely on exotic flower species. However, when provided with additional choices, males spent significantly longer on flowers of *Hebe youngii* and *Fagopyrum esculentum* than on species already present in the field. In the laboratory, *Hebe salicifolia* and *F. esculentum* flowers also significantly enhanced the fitness of females over *Achillea millefolium* and the water control. The results are discussed with reference to the implications for population dynamics and improvements to Greening Waipara plantings are suggested.

## 4.2 INTRODUCTION

Species conservation management is primarily concerned with maintaining the critical resources for the target organism (New 2007). Understanding these requirements in detail is a major initial step in enhancing the persistence of butterfly populations (Thomas 1991, New *et al.* 1995, Dennis and Hardy 2007, Dennis and Sparks 2006). Although many non-consumable resources are required for butterflies to persist in time and space (Dennis *et al.* 2003, Dennis and Hardy 2007, Hardy and Dennis 2007), the nutrition of larval and adult stages is essential. Host plant preferences and the importance of host plant quality to larval growth are well studied (Thomas 1983, 1984, Bourn and Thomas 1993, Thomas *et al.* 2001, Fowles and Smith 2005), but information on optimal nectar sources for adults, particularly in farmland settings where nectar is often lacking, is less readily available (Tudor *et al.* 2004).

In general, nectarivorous butterfly species rely on the sugars and water in nectar for optimal longevity and reproduction (Baker and Baker 1975, Boggs 1997, Fischer and Fiedler 2001). Glucose and amino acids obtained by adults may be used in varying ratios with nitrogenous resources stored from larval feeding for egg production, maintenance, and defence (Boggs 1987, Boggs 2003). Under optimal feeding conditions, larval-derived amino acids are usually the major contributor to egg production, because oogenesis begins before adult emergence in the pupal stage (Boggs 1986). However, recent studies have shown that when larval feeding is completed on poor quality host plants, i.e. plants of low nutrient value or low water content, the shortfall in larval-derived amino acids is substitutable by amino acids from nectar (Mevi-Schutz and Erhardt 2003, 2005, Jervis and Boggs 2005). Even if larval feeding is not limited, adults emerge with at most 20% of their optimal egg capacity (Jervis and Boggs 2005). Feeding on nectar with a high source of amino acids is therefore potentially important in overcoming egg shortfall, a key mortality factor in some species (Hayes 1981, Courteney and Duggan 1983).

Despite the clear importance of high amino acids in the adult diet, nectar preferences within and between plant species are complex. The quality and quantity of nectar from flowers may differ between species and over time (Corbet 1978, Shreeve 1992). While there is evidence that perennial sources are more energetically valuable than annual plant species (Corbet 1995), butterfly species vary widely in their flower species preferences based on nectar concentration (Watt *et al.* 1974), flower colour (Faegri and van der Pijl 1979), flower and butterfly morphology



(Porter 1992, Corbet 2000), butterfly behaviour (May 1992) and as discussed above, larval food quality (Mevi-Schutz and Erhardt 2003).

In the case of farmland butterflies, this complexity has been addressed through a ‘shot gun’ approach, despite there being some positive results. Wild flower mixes have been shown to improve populations of butterflies, bumble bees and other invertebrates spatially and temporally (Feber *et al.* 1996), and conservation headlands which preserve nectar in the form of perennial ‘weed’ species in selectively sprayed field borders of cereal fields have been especially successful in increasing farmland species abundance (Dover *et al.* 1990, Dover 1996, 1997, Feber *et al.* 1996). Questions are rarely asked about whether species used by adults in the field act as useful sources of carbohydrates and amino acids or are simply a common source of low quality fluid in the absence of true optimal nectar sources, a consequence of agricultural intensification and resulting monoculture (Sandhu *et al.* 2008).

In New Zealand, the ongoing Greening Waipara project seeks to demonstrate less environmentally damaging land management, particularly in vineyards, and the enhancement of native butterfly populations may be a key indicator of its success. However, while perennial ‘weed’ species are an important source of nectar to farmland butterflies (Dover and Sparks 2000), the encouragement of agricultural pest plant species is unlikely to gain support in New Zealand. In this study, an attempt was made to find a native flowering plant species or group of species which may be beneficial to butterflies and be acceptable as an addition to Greening Waipara plantings.

The flower preferences of a species of endemic New Zealand butterfly, *Lycaena salustius* were assessed in three ways. Initial survey work established which resources were utilised through the summer of 2008/2009 by adults in vineyards in the Waipara region. Subsequently, an outdoor choice experiment was devised using some of these preferences and additional native flowers to test towards which flowers adults were attracted. Preferences taken from these two studies were then applied to a caged fitness experiment which seems to be the first of its kind for butterflies. The effect of different prepared concentrations of amino acids and sugars on butterfly longevity and fecundity in cages is well studied (Watt *et al.* 1974, Murphy 1983, Hill and Pierce 1989, Rusterholz and Erhardt 1997, Fischer and Fielder 2001, Mevi-Schutz and Erhardt 2003, 2005), but this method of fitness assessment has not been used to test the benefits of actual flower species. This type of flower ‘screening’ has been used frequently in conservation biological

control research involving insect parasitoids (Baggen and Gurr 1998, Wackers 2004, Lavandero *et al.* 2006, Wade and Wratten 2006).

Due to the low level of native plant species present in vineyards, particularly flowering species with which native butterflies may have coevolved, it is expected that *L. salustius* will opportunistically utilise the nectar of exotic perennial species, but prefer and receive most benefit from suitable native nectar sources when available. Subsequently identifying the species of most benefit to *L. salustius* will help to inform conservation efforts as part of native plantings which form part of the Greening Waipara project, and help to determine whether the butterfly is well adapted to farmland habitats, or is only surviving in substandard refuges.

## **4.3 METHODS**

### **4.3.1 Study species**

The common copper, *L. salustius*, is endemic to New Zealand and is not an endangered species (Patrick and Dugdale 2000). It is a relatively sedentary species with a wing span of 24-33mm found in most areas of the country with two broods during early and late summer depending on climate. Often associated with shrubland and coastal environments, its larval host plants consist of members of the *Muehlenbeckia* genus, particularly *M. complexa* and *M. australis* (Gibbs 1980). Apart from the early records (summarised by Gibbs 1980), and observations of population dynamics by Flux (1968) and Craw (1975), there have been no published studies on the ecology of *L. salustius*. This species has been chosen for these experiments because it was prevalent in survey studies (Chapter 2, this thesis) and was conducive to lab rearing, readily mating and ovipositing in caged environments.

### **4.3.2 Field observations**

Field observations of nectar use by adult *L. salustius* were taken during transect survey work in the summer of 2008/09 using the 'Pollard walk' (Pollard and Yates 1993) method of the UK Butterfly Monitoring Scheme (this thesis, Chapter 2). On each of six vineyards in Waipara, a fixed transect route was established passing through different habitat types on the vineyards. The transects were split into sections based on vegetation type and topography so that observations could be analysed by habitat type. Transects were walked 13 times during the summer, separated by 2-3 weeks. For the nectar use observations, a record was made each time an adult *L. salustius*

was observed feeding as to its sex and flower species used. However, as some flowers are more abundant than others, the flower abundance scoring system from Chapter 2 was also used here to calculate a rank of flower species preference of adult butterflies. On most of the transect visits throughout the summer, the abundance of flowers of each species or group of species was scored for each section of transect passing through a different habitat type using the DAFOR estimating method (*sensu* Clausen *et al.* 2001) (5 = Dominant, 4 = Abundant, 3 = Frequent, 2 = Occasional, 1 = Rare). The number of each species in flower was visually estimated in blocks of 20 inflorescences. A particular flower was considered dominant if more than 10 blocks were counted, abundant if 5-10 blocks were counted, frequent if 2-5 were counted, occasional if 1-2 were counted, and rare if counts did not constitute a block of 20. Nectar source abundance categories were summed for each section for an overall nectar abundance score. These scores were summed over the summer and a mean per section was calculated. The number of observations of butterfly feeding was then divided by this score to give a rank of preference for the flower species (Table 4.1).

#### 4.3.3 Field experiment

In the summer of 2009/10, a 15m x 8m plot was marked out at the interface of the remnant of native vegetation (the main *L. salustius* habitat in Chapter 2) and the adjacent field margin at Waipara West vineyard. A rabbit-proof fence was built around the perimeter of the plot. A total of 32 plants, 4 each of 8 species were then planted within the plot in a randomised block design, with each plant placed approximately 1.5m from the edge of the plot and its nearest neighbours. The eight plant species consisted of: *Hebe youngii* C. Teschner (Scrophulariaceae) (shrubby veronica), *Trifolium repens* (white clover), *Fagopyrum esculentum* (buckwheat), *Muehlenbeckia complexa* (pohuehue), *Phacelia tanacetifolia* (phacelia), *Achillea millefolium* (common yarrow), *Chrysanthemum maximum* Ram. (Asteraceae) (Shasta daisy) and *Leptinella minor* Hook.f. (Asteraceae) (Banks Peninsula button daisy). The plants were selected either because they were attractive to *L. salustius* adults in the previous field season (*T. repens*, *M. complexa*, *A. millefolium*), because they were native species absent from Waipara but potentially attractive to butterflies (*H. youngii*, *L. minor*; M. Gillespie, pers. obs.) or because their presence would allow the testing of plant species prescribed to vineyards for conservation biological control (*F. esculentum*, *P. tanacetifolia*; Berndt *et al.* 2006). Finally, *C. maximum* was chosen as a last-minute replacement for *Cirsium arvense* (Californian thistle), which failed to flower at the time

of proposed data collection. Seeds of *C. maximum*, *F. esculentum* (c.v. Katowase) and *P. tanacetifolia* (c.v. Balo) were obtained commercially from Kings Seeds, Katikati, Bay of Plenty, NZ (*C. maximum*), Midland Seeds, Ashburton, Canterbury, NZ (*F. esculentum*) and Kiwi Seeds, Blenheim, NZ (*P. tanacetifolia*). These seeds were sown in potting mix at the nursery at Lincoln University. All other plants were grown from Canterbury seed stock in potting mix either in the nursery at Lincoln University (*T. repens*, *A. millefolium*), or by Hurunui Nurseries (*H. youngii*, *L. minor*, *M. complexa*). Plants were transplanted in September 2009 to allow them to overcome root shock and reach flowering stages in coincidence with peak butterfly populations. Plants were watered weekly as required.

Unfortunately, only four species of plant were in flower at the time of data collection: *H. youngii*, *P. tanacetifolia*, *T. repens*, and *A. millefolium*. The four remaining species were therefore replaced by potted plants of those species in flower. Pots were sunk into the ground so that the top of the pot was level with the ground and the soil covered the pot's rim. On 4 December 2009, the grass around the plants was mown at 08.30h, to ensure a uniform sward and to remove other flowering species in and around the plot. From 09.30h to 12.00h, the plot was observed continuously. The weather on this day was cloudless and still with a mean morning air temperature of 25°C. When a *L. salustius* adult entered the plot, a stopwatch was started and the butterfly was followed until it began feeding on a plant species in the experiment. The duration of feeding activity, as assessed through binoculars, was recorded for each individual flowering plant until the butterfly left the plot, at which point it was caught with a sweep net and kept in a small, dark container so that it would play no further part in the experiment. All butterflies were subsequently released at the end of the day. The experiment was repeated two weeks later on 18 December. It is assumed that the adults involved in the second experiment were independent from the first as this period is longer than the known field life span of adult *L. salustius* (10.4 days, Craw 1975). In total, 36 butterflies were observed.

#### **4.3.4 Greenhouse bioassay**

The effect of different nectar sources on adult female fitness as estimated by fecundity and longevity were investigated in a no-choice bioassay. Eight cages measuring 0.8m x 0.8m x 0.8m and consisting of a metal frame and nylon mesh netting were erected and placed on a table in a greenhouse at the Lincoln University nursery. A length of black mesh was placed on the western half of the roof of each cage to provide periodic shade and prevent overheating. In each of the

cages, the oviposition substrate of a small potted plant of *M. australis* was placed in a corner. A small plastic vial filled with water and a dental wick protruding through a hole in the yellow lid was also placed in the middle of each cage on top of an upturned plant pot.

The eight cages were then randomly assigned to one of four treatments: *F. esculentum*, *Hebe salicifolia* G. Forst. (Scrophulariaceae), *A. millefolium* and a water control. In the cases of *F. esculentum* and *A. millefolium*, potted flowering plants were grown in the nursery at Lincoln University from seeds obtained as above. As the *Hebe* species used in the field choice experiment was not flowering at the time of this experiment, an alternative was used. However, *H. salicifolia* is too large for the small cages, so a flowering shoot was cut from a *H. salicifolia* bush in the nursery grounds, and placed in a plastic vial filled with water and held in place by a dental wick protruding through a hole in the lid. The flower was replaced daily and the vial was placed on an upturned flower pot in the cage. This form of flower provision in cage experiments has been shown to act as satisfactory substitute for intact inflorescences (Wade and Wratten 2007). For the water control, no flower was placed in the cage but water was available from the plastic vial and dental wick arrangement described above. All nectar treatments were placed in the unshaded corner opposite the oviposition treatment.

In addition to these plants, a potted *F. esculentum* plant with flowers removed was placed in each of the cages. This was due to the fact that *L. salustius* females were observed laying a large number of eggs on *F. esculentum* in a pilot study. The addition of a non-flowering specimen to each cage was to balance the effect of the plant on oviposition.

A newly eclosed, unfed and unmated female of laboratory reared *L. salustius* was randomly assigned to each cage. Females were reared from eggs laid in the laboratory by wild-caught females from near Lake Ellesmere, Canterbury. Wild males caught from this location were used to mate the females as required. The cages were revisited daily to check for survival, count the number of eggs on each plant, and to water or replace plants where necessary. The males were removed from the cages when the females had begun to lay eggs. On the rare occasions that this did not occur after two days, a new wild-caught male was introduced. Plants holding eggs were placed in the insectary at the nursery and checked regularly for egg hatching. When the females of all eight cages had died, they were subsequently dissected and the eggs remaining in the abdomen were counted under a x10 binocular microscope. The experiment was started in January 2010 and was repeated 5 times in total, although with only 4 cages on the last run ( $n = 9$ ), with the final run ending in the second week of March 2010.

#### 4.3.5 Statistical analysis

The data from the field experiment were analysed using the Friedman test, a non-parametric analogue of a two-way ANOVA used when there is a single observation for each factor combination. ‘Flower species’ was the grouping variable and ‘Row’ was the blocking variable. The response variables were frequency of flower visits to each individual flowering plant, and total time (s) of feeding activity at each individual flowering plant. Only two females were recorded feeding in the plot, so the sexes were not treated separately.

For the greenhouse bioassay, survival analysis was used to compare the effect of treatment on the life span (days) of butterflies. The Kaplan-Meier estimate of the survival function was calculated and Cox’s Proportional Hazard Model (Afifi and Clark 1990) was used to compare the survival curves. Mean total eggs laid, potential fecundity, reproductive period and eggs laid per day were analysed using ANOVA after ensuring normality and homogeneity of variance, with cage number and experiment run added as covariates. Pairwise comparisons were tested using Tukey’s HSD. All analyses were performed in R version 2.9.2 (R Core Development Team, 2009).

### 4.4 RESULTS

#### 4.4.1 Field observations

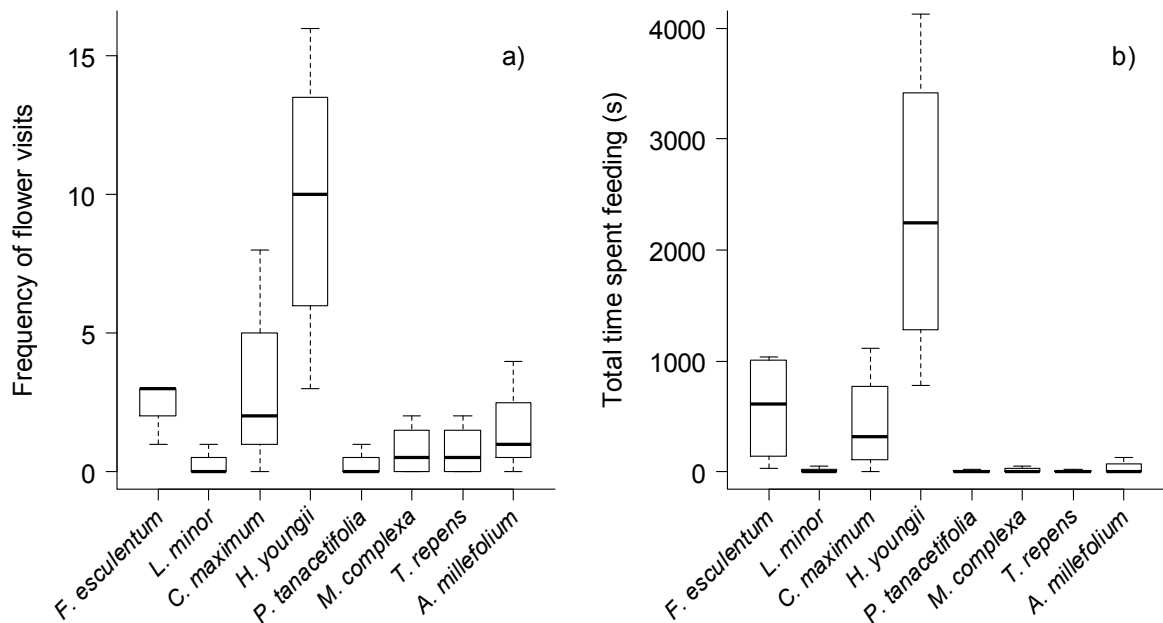
Feeding records from the 2008/09 summer season are summarised in Table 4.1. The most frequented species was *C. arvensis*, with *A. millefolium*, *Geranium* spp. L. (Geraniaceae) and *M. axillaris* also visited a large number of times. However when feeding observations were divided by the abundance of flowers, the *Geranium* spp. were the most visited species, followed by *M. axillaris*, *Chenopodium* sp. L. (Amaranthaceae), *C. arvensis* and *A. millefolium*. In total, *L. salustius* was observed visiting 16 different species. Due to the low number of females recorded, data for males and females were summed.

**Table 4.1:** The 16 flower species on which *L. salustius* was observed feeding throughout the summer survey of 2008/09, listed in descending order of observation frequency. Plant abundance scores are taken from Chapter 2 (see methods) and the flower rank is based on the number of feeding observations/plant abundance score. Thus, the third most visited flower is the top ranking flower because of the large number of visits despite its relative scarcity.

Species		No of feeding observations	Plant abundance score	Flower rank
Californian thistle	<i>Cirsium arvense</i>	120	2.91	4
Common yarrow	<i>Achillea millefolium</i>	59	1.89	5
Cranesbill spp.	<i>Geranium</i> spp. (not <i>G. dissectum</i> L.)	55	0.36	1
Sprawling wire-vine	<i>Muehlenbeckia axillaris</i>	48	0.44	2
Goosefoot sp.	<i>Chenopodium</i> sp.	28	0.39	3
White clover	<i>Trifolium repens</i>	24	4.83	11
Black medick	<i>Medicago lupulina</i> L. (Fabaceae)	24	1.30	6
Pohuehue	<i>Muehlenbeckia complexa</i>	20	1.44	9
Hedge mustard	<i>Sisymbrium officinale</i>	13	0.73	7
Yellow Asteraceae	Asteraceae spp.	12	7.77	14
Viper's bugloss	<i>Echium vulgare</i>	8	4.89	13
Lucerne	<i>Medicago sativa</i> L. (Fabaceae)	4	0.97	12
Large flowered mallow	<i>Malva alcea</i>	4	4.32	15
Vetch	<i>Vicia sativa</i> L. (Fabaceae)	2	0.12	8
Cutleaved cranesbill	<i>Geranium dissectum</i>	2	0.39	10
Small flowered mallow	<i>Malva parviflora</i>	1	2.30	16

#### 4.4.2 Field experiment

The explanatory variable ‘Flower species’ was significant for both the frequency of visits (Friedman Chi-squared = 16.92, df = 7,  $P = 0.018$ ) and the total time spent feeding (Friedman Chi-squared = 17.59, df = 7,  $P = 0.014$ ). Performing the same analysis with the explanatory variables reversed showed that the effect of row was not significant (Frequency: Friedman Chi-squared = 1.7344, df = 3,  $P = 0.6293$ ; Time: Friedman Chi-squared = 1.0714, df = 3,  $P = 0.784$ ). Plots of the effects of flower species (Fig. 4.1) show that *H. youngii* was the most visited flower species, followed by *F. esculentum*, *C. maximum* and *A. millefolium*. All species were visited at least once, but not all individual plants were visited.



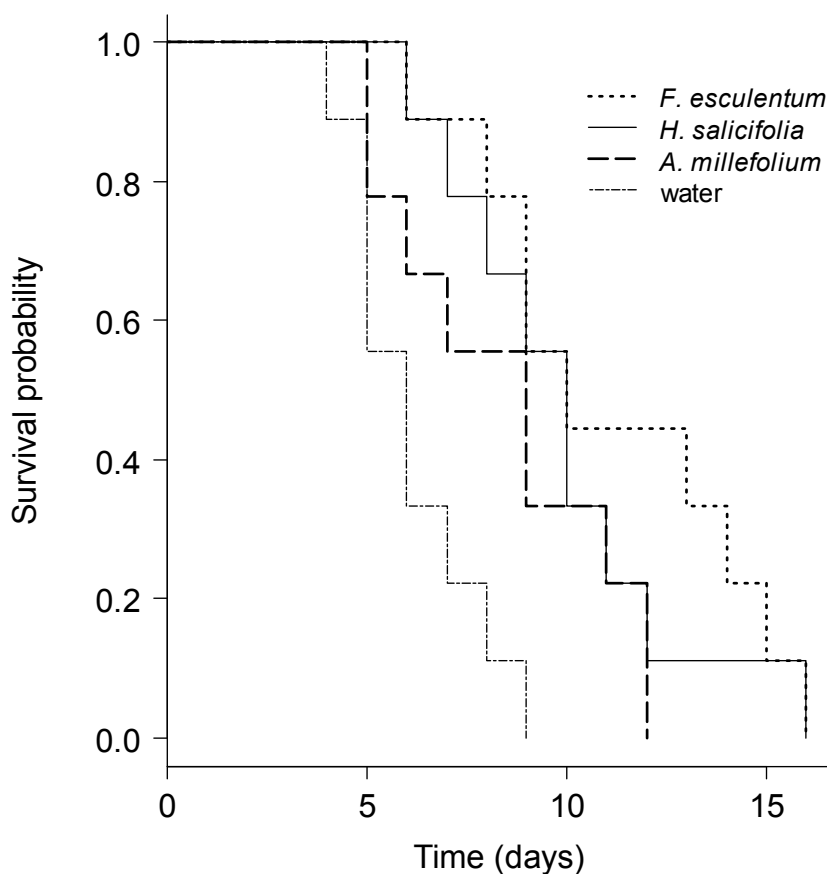
**Figure 4.1:** Field choice experiment results showing a) the frequency of feeding visits of adult *L. salustius* to different flowering species and b) the total time spent by adult *L. salustius* feeding from the flowering species. The solid line in each box indicates the median, and the lower and upper edges of the box are the first and third quartiles. The whisker of the box reaches to the largest or smallest value which is not an outlier.

#### 4.4.3 Adult fitness

In all fitness analyses the covariates ‘run’ and ‘cage’ were not significant and were removed as factors. Survival curves were significantly different between treatments (log-rank:  $P < 0.001$ , Wald test:  $P < 0.01$ ) (Fig. 4.2). Bonferroni-corrected paired comparisons using Cox’s proportional hazard model showed that *F. esculentum* ( $P < 0.01$ ) and *H. salicifolia* ( $P < 0.01$ ) significantly



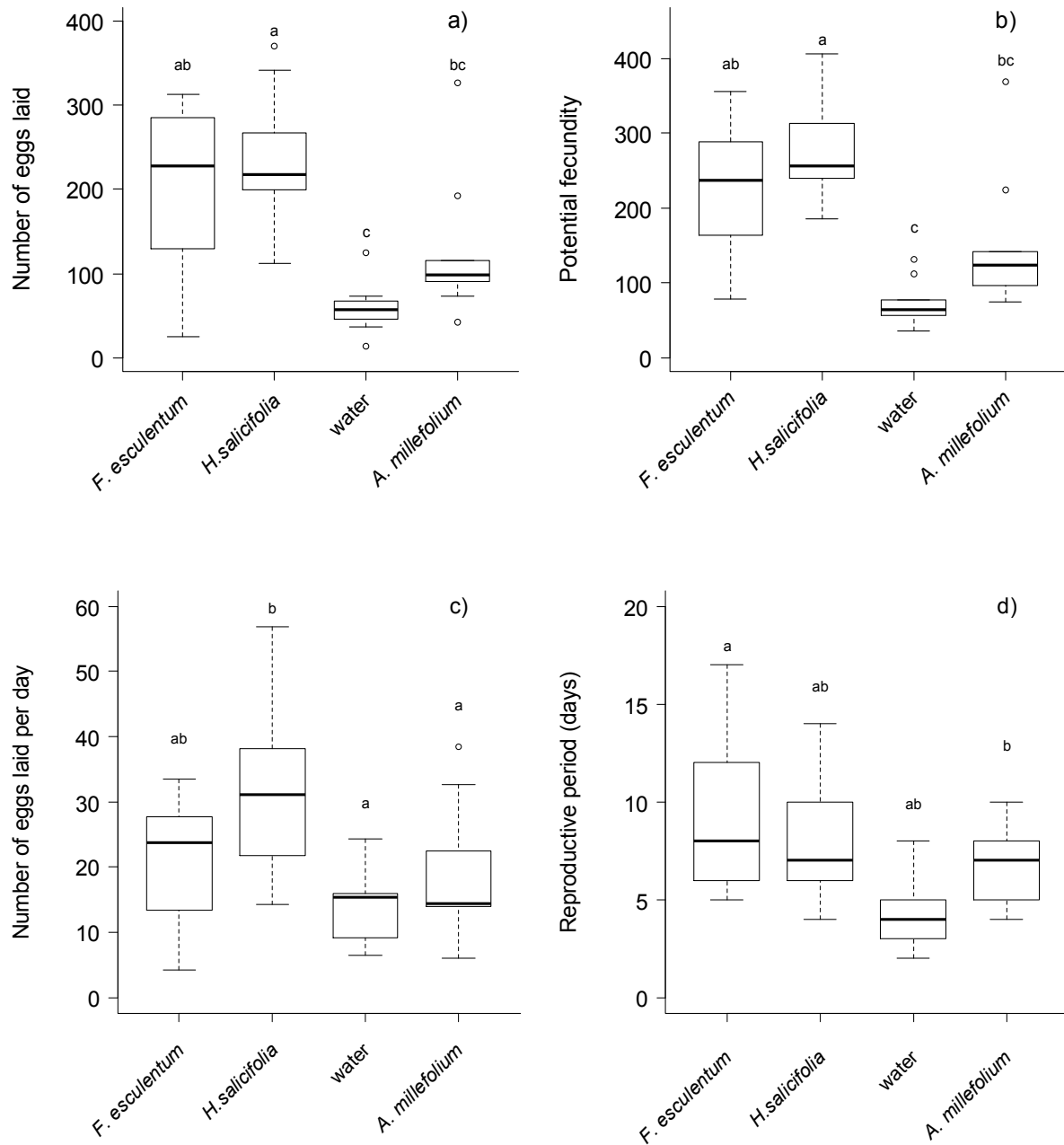
enhanced longevity of *L. salustius* females compared with water. There were no significant differences in longevity between the three flower species, but the longevity of females fed *A. millefolium* was not significantly different from those fed only water ( $P = 0.10$ ). Mean longevity and standard errors are shown in Table 4.2.



**Figure 4.2:** Kaplan-Meier estimates of the survivorship function of female *L. salustius* when fed different flower species or water.

**Table 4.2:** Mean life span in days, and standard error, of *L. salustius* females given *F. esculentum*, *H. salicifolia* and *A. millefolium* flowers and water. ( $n = 9$ ).

	<i>F. esculentum</i>	<i>H. salicifolia</i>	<i>A. millefolium</i>	Water
Mean	11.11	9.89	8.44	6.11
Standard error	1.16	0.99	0.94	0.54



**Figure 4.3:** a) Fecundity, b) potential fecundity (number of eggs laid + unlaied eggs after death), c) number of eggs laid per day and d) reproductive period of lab reared female *L. salustius* adults on different nectar sources and water. The solid line in each box indicates the median, and the lower and upper edges of the box are the first and third quartiles. The open circles refer to outliers. The whisker of the box reaches to the largest or smallest value which is not an outlier. Treatments with the same lower case letter did not differ significantly with Tukey's HSD ( $P < 0.05$ ).

There was a significant effect of treatment on all measures of fecundity: eggs laid ( $F=8.13$ ,  $df = 3$ ,  $P<0.001$ ), potential fecundity ( $F=12.07$ ,  $df = 3$ ,  $P<0.0001$ ), reproductive period ( $F = 4.93$ ,  $df = 3$ ,  $P<0.01$ ) and eggs laid per day ( $F = 5.35$ ,  $df = 3$ ,  $P<0.01$ ) (Fig. 4.3). The number of eggs laid by females fed *H. salicifolia* did not differ from those fed *F. esculentum*, but both laid significantly more eggs than those fed only water ( $P<0.001$  and  $P<0.01$  respectively). In addition, *H. salicifolia* was slightly superior to *A. millefolium* in enhancing the egg laying capacity of females ( $P<0.05$ ). The number of eggs laid by females feeding on *A. millefolium* did not differ from those fed only water ( $P=0.35$ ).

Potential fecundity (number of eggs laid + eggs in abdomen after death) showed the same patterns: females fed *H. salicifolia* had significantly higher potential fecundity than those fed water ( $P<0.0001$ ) and *A. millefolium* ( $P<0.01$ ). Adults fed *F. esculentum* also had a higher potential fecundity than the control insects ( $P<0.01$ ). For eggs laid per day, females fed *H. salicifolia* also differed significantly from those provided with water ( $P<0.01$ ) and *A. millefolium* ( $P<0.05$ ). Only females fed *F. esculentum* had a longer reproductive period than those fed water ( $P<0.01$ ).

## 4.5 DISCUSSION

### 4.5.1 Nectar availability and choice in Waipara vineyards

*Lycaena salustius* adults used a range of 16 flower species although only half of these were used 20 or more times. Only two species were native, *M. axillaris* and *M. complexa*, ranked 2 and 9 respectively, with the remainder consisting of common farmland perennial ‘weed’ species as expected. The list in Table 4.1 is not an exhaustive account of the butterfly’s potential resources: Craw (1975) observed *L. salustius* feeding from native jasmine (*Parsonsia heterophylla* A. Cunn. (Apocynaceae)), white climbing rata (*Metrosideros perforata* JR & G. Forst. (Myrtaceae)) and the introduced watercress (*Nasturtium officinale* R. Br. (Cruciferae); formerly *Rorippa nasturtium-aquaticum*) in Central Otago. Flux (1968) found that *A. millefolium* and *Rubus fruticosus* L. (Rosaceae) (blackberry) attracted most adults in Wellington.

Many butterflies are known to be opportunistic nectar feeders (Gilbert and Singer 1975), opting for those species that are abundant and accessible (Shreeve 1992). For example the meadow brown (*Maniola jurtina* L. (Nymphalidae)) has been observed in the UK preferentially feeding on different flower species at different sites (Brakefield 1982, Bourn and Thomas 1993).

The choice of flower by a butterfly species may depend on a number of factors. Wing loading and the clustering of flowers have been shown to determine foraging profitability (Corbet 2000), and other factors such as the quality and quantity of nectar at a given time, weather, flower colour, proboscis length and adult behaviour may also be important (Corbet 1978, Faegri and van der Pijl 1979, Shreeve 1992, Porter 1992, May 1992). These varying factors mean that flower choice can vary in time within a butterfly population and species rarely make use of all nectar sources or focus feeding on a single source.

Tudor *et al.* (2004) found that nectar ‘generalists’ were correlated more with generalism in host plant use and biotope. Thus the more mobile species are exposed to a greater range of opportunities for nectar feeding than the less mobile ‘specialists’ (Tudor *et al.* 2004). *Lycaena salustius* may fall between the extremes of ‘specialist’ and ‘generalist’: relatively sedentary adults use a small number of flower species and host plants, but they also disperse to a range of habitats outside the vineyard remnants and display the opportunistic behaviour of generalists such as *M. jurtina*, preferring different species in different habitats (Flux 1968, Craw 1975).

This potential for opportunism was reflected in the findings of the field choice experiment. When apparently more optimal plants (*H. youngii*, *F. esculentum*, *C. maximum*) were brought to the habitat of *L. salustius*, they were used in preference to useable species already present nearby (*M. complexa*, *A. millefolium*, *T. repens*). This in turn suggests that if butterflies always choose the most profitable source of nectar, optimal resources are not currently present in this particular habitat.

These findings rely on a number of assumptions regarding the nature of the field choice experiment. The records of feeding in the plot are a ‘snapshot’ of a butterfly individual’s feeding history. It is assumed that individuals arriving in the plot are identical in nutritional needs and have been attracted to the plot due to a lack of preferred food elsewhere. In reality, butterflies may have used species in the plot during transit from one end of the habitat to the other, for a fraction of their lives without actually being drawn to feed preferentially on *H. youngii*, for example. A true, but impractical, assessment of preference would record feeding for the life of a number of butterfly individuals and include other aspects of nutrition such as larval feeding. Furthermore, despite the capture of each *L. salustius* individual following its vacating the plot (in an attempt to ensure independence), butterfly choices may have been influenced by edge effects, interactions with other nectar feeding invertebrates and the interactions of other invertebrates with the plant species. Finally, butterflies may have shown a preference for *H. youngii* due to

elements of adaption and learning known to occur during flower feeding (Gilbert and Singer 1975, Wiklund 1977, Goulson *et al.* 1997). The *H. youngii* plants, as well as those of *M. complexa*, *T. repens* and *P. tanacetifolia*, were in flower for several days before the start of data collection. In contrast, the potted species were introduced on the day of the study. Adult butterflies may therefore have had the opportunity to find, use and become accustomed to the presence of *H. youngii* (Gilbert and Singer 1975) which may have resulted in a preference for this species over the potted flowers. Any further study of this kind should use either all potted flowers of a similar age, or all planted individuals. Despite this, potted *F. esculentum* and *C. maximum* were utilised, and the preference of *H. youngii* over planted white clover and *M. complexa*, as well as ‘weed’ species directly outside the plot, suggests that this plant is optimal at least over these species.

*Hebe* species have been suggested to be favoured by native butterflies (G. W. Gibbs, pers. comm.) and a number of butterfly species have been seen feeding voraciously on *Hebe* sp. nectar in garden settings (M. Gillespie, pers. obs.) but this ‘attraction’ has never been quantified. *Fagopyrum esculentum*, also used in the trial plot, has been shown to be a useful nectar source for attracting beneficial insects such as the parasitoids and predators of agricultural invertebrate pests (Landis *et al.* 2000, Berndt and Wratten 2005, Berndt *et al.* 2006). Despite the drawbacks of the methodology, the clear attraction of these species highlights the need to consider the possibility that optimal flower species may not be present, abundant or accessible in a habitat, despite observed patterns of preferential feeding on potentially substandard species.

#### **4.5.2 Adult fitness**

The longevity and fitness estimates of the greenhouse bioassay showed that only *F. esculentum* and *H. salicifolia* enhanced female *L. salustius* fitness compared with water. While there was a trend for *A. millefolium* to increase female *L. salustius* fitness above that provided by water, it is not an optimal plant by this evidence, supporting the suggestion that *A. millefolium* may be used opportunistically in the field in the absence of superior resources. Further work would be required to assess if this is the case for all species in Table 4.1, but these findings provide evidence for the preference of another *Hebe* species and *F. esculentum* in the field choice experiment. Although the cages used here may have altered butterfly behaviour, the life span results are comparable with those of field studies. Flux (1968) reported from mark-release-recapture work that *L.*

*salustius* adults lived for 8 days, and Craw (1975) put the figure at 10.4 days, with a maximum of 14 days. These figures compare favourably with all three of the tested flower species (Table 4.2).

There are no other studies that compare the fitness effects on butterflies from feeding on different flower species in cages, but there has been much work on the effects of different concentrations of artificial nectars. Fischer and Fiedler (2001) found that another lycaenid, *Lycaena hippothoe* L. in Germany showed increased longevity by 3-5 times and increased fecundity by up to 7 times when fed sucrose compared to feeding only on water. Hill and Pierce (1989) found sugar to double the longevity and egg production of *Jalmenus evagoras* Donovan (Lycaenidae) in Australia. Explanations of the effects observed in the present study can be inferred from these earlier studies. Due to the low fitness of *L. salustius* adults fed water, the production of eggs in *L. salustius* is evidently limited by a lack of carbohydrate and amino acid intake (Fischer and Fiedler 2001), and larval stores of these compounds were not enough to ensure optimal life span and high levels of egg production.

Despite the fact that the *Hebe* species used in the field choice experiment was different to that used here, and that the field experiment observations consisted mainly of males, the results may help to explain the preference for the *H. youngii* in the field experiment as the morphology of the flowers is similar. Males and females of butterflies are known to use different resources due to different requirements (Shreeve 1992), but both also feed preferentially from sources with high levels of amino acids (Baker and Baker 1973, 1975, Watt *et al.* 1974, Mevi-Schutz and Erhardt 2003). These nitrogenous compounds, also carried over from larval feeding, are important to females for egg maturation (Murphy *et al.* 1983), particularly when larval feeding is poor (Mevi-Schutz and Erhardt 2003, 2005), and are important to males for longevity, sperm production and the possible transmission of important nutrients during mating (Porter 1992). Although the nectar composition is not known for the *Hebe* species used in this study, *Hebe stricta* Benth. produces large quantities of nectar (0.46 ul per day per flower for male flowers and 0.10 ul for female flowers) (Delph and Lively 1992). *Fagopyrum esculentum* is similarly a known source of high nectar concentrations (0.17 ul per flower, Cawroy *et al.* 2008). Amino acid concentrations are not known for these two species, but *A. millefolium* does have a high concentration of amino acids (Baker and Baker 1973, Rathman *et al.* 1990). Flowers visited by dung- and carrion-feeding flies are known to be the richest in amino acids (Baker and Baker 1973) and *A. millefolium* is particularly attractive to these fly species (Rathman 1990). Plants primarily pollinated by butterflies are also known to be slightly less rich in amino acid sources

than these fly-pollinated plants however (Baker and Baker 1973). The amino acid concentrations of both *H. salicifolia* and *F. esculentum* may therefore be more suited to *L. salustius* and butterflies in general, than *A. millefolium* although further study would help ascertain this. Similarly, further work comparing the apparently optimal flower species with species already present in butterfly habitats which have amino acid levels more suited to butterflies would help to support these findings.

#### 4.5.3 Implications for conservation

The implications of these findings for *L. salustius* and other species in the field are clear from these three measures of flower use. Male *L. salustius* preferred *H. youngii* in the field over many of the species that they were observed using in a previous field season (Chapter 2; although the most favoured plant species were absent). Female *L. salustius* in cages gained most fitness benefit from a related species, *H. salicifolia*, closely followed by *F. esculentum*, a plant recommended to growers for the biocontrol of insect pests (Berndt *et al.* 2006, Robinson *et al.* 2008). A longer life span and a larger egg load resulting from feeding on these species in the field may contribute to the conservation and enhancement of this species by enhancing reproductive output considerably. If *L. salustius* is not restricted by nectar provision *per se* in the field as butterflies may be in other agricultural landscapes (Dover 1997), they may be restricted by a lack of optimal nectar sources and this may also extend to other butterfly species.

The inclusion of the *Hebe* species used in this study in future plantings are therefore likely to improve populations of butterfly species in the local area, although further experimentation is required to determine whether fitness improvements demonstrated in caged environments are exhibited in nature. Many species of butterfly have comparably high potential reproduction, but actual oviposition in the field is much lower because overall fecundity will depend on the density and availability of suitable oviposition sites (Warren 1992). The positioning of *Hebe* spp. in plantings may also be an important factor. For example, work in this thesis (Chapter 2) and by Flux (1968) and Craw (1975) has attested to the sedentary nature of *L. salustius*. It is uncertain how far adults of this species will regularly travel to reach preferred nectar sources over local substandard species. Feeding from more common but less rewarding species, e.g., Asteraceae species, growing in the native remnant habitats, may be more efficient over the life span of the adult (Shreeve 1992). As work elsewhere has shown, butterflies, particularly relatively immobile species are more likely to reach other patches of native species, e.g., Greening Waipara plantings

including biodiversity trails, if patches are connected by linear features which accommodate resources such as nectar sources and host plants (Dover 1997, Haddad 1999, Dover *et al.* 2000, Shepherd and Debinski 2005). The incorporation of shrubby *Hebe* spp. into such connecting features is therefore likely to provide both nectar sources and dispersal corridors. In addition *Hebe* spp. may provide an additional service of enhancing natural enemy populations, although further work is required to confirm this.

Finally, the usefulness of flowering species with high nectar production will depend on a number of other factors such as the time available to use them as determined by climate, the density of flowers available and their location (Fischer and Fielder 2001). Use of these resources by other species of butterflies will also depend on the extent of competition with other invertebrates (Porter 1992, Boggs 2003) and the ability of other butterfly species to use *Hebe* spp.

It is recommended therefore that perennial nectar sources in or around vineyards be encouraged and supplemented by a range of *Hebe* spp. providing flowers throughout the summer, in patches close to known habitat. Mobile species may visit any planted *Hebe* species, but immobile butterflies are likely to be attracted to those close to vineyards for example, if a sheltered corridor of host plants and other flowers forms a link to them.

#### **4.5.4 Recommended further study**

In addition to the above recommendations, there are a number of ways that a study of this type can be improved upon. Only male *L. salustius* were included in the field choice experiment and only females were included in the greenhouse experiment. Studies have shown that the sexes of butterflies may differ in their choice of nectar source for different reasons (e.g., Shreeve 1992). Similarly nectar requirements may differ for different species over time, and nectar provision by flower species may also differ over time (Watt *et al.* 1974). There are also a large number of different *Hebe* species which are commercially available and flower at different times of the year, which may be beneficial to different butterflies to varying degrees. Work incorporating all of these features, as well as factors such as the sugar and amino acid content of preferred plant species and the effects of recommended plants to both beneficial and pestiferous species would return valuable information. This information would contribute to a better understanding of adult butterfly nutrition in New Zealand and elsewhere and would subsequently assist in optimising future conservation efforts of native butterflies, general biodiversity and thus potentially, ecosystem function.



## **Chapter 5    Adult oviposition preferences and larval performance of *Lycaena salustius* (Lepidoptera, Lycaenidae) on four native hosts and an exotic novel host**

### **5.1 ABSTRACT**

Optimal theory in evolutionary ecology predicts that female phytophagous insects should oviposit on the host-plant species that impart maximum fitness to offspring, but this is not always the case. The relationship between adult oviposition preference and larval growth and survivorship can take many forms and for decades studies have sought to understand the role the relationship plays in host range evolution, host specificity and host shifts. In particular, the details of the preference/performance correlation can provide evidence concerning the incorporation of potential introduced novel hosts into the host range. In this study, the oviposition preferences and larval performance of the common copper, *Lycaena salustius* are evaluated on five species: two native species (*Muehlenbeckia complexa* and *M. australis*) and two endemic species (*M. astonii*, and *Rumex flexuosus*) chosen because they are the perceived ancestral host plants of *L. salustius* and the importance hierarchy of which is unknown; and a potential novel host, *Fagopyrum esculentum*, a plant promoted to farmers for its attraction of, and benefit to natural enemies of agricultural pests. Although *L. salustius* is unlikely to have encountered the novel host before, the butterfly was observed ovipositing large numbers of eggs on *F. esculentum* in pilot studies. Choice oviposition assays were conducted in field cages and in the greenhouse alongside laboratory no-choice larval performance experiments. In the absence of *F. esculentum*, adults were relatively non-discriminatory, although they laid significantly more eggs on *M. astonii*, a rare host in the field. When *F. esculentum* was present, adults laid a large number of eggs on this novel host. In performance assays, components of fitness were not correlated with each other, leading to conflicting results. The different results are discussed in relation to host-association evolution theory and conservation implications.

## 5.2 INTRODUCTION

The relationship between the host-plant preferences of ovipositing polyphagous insect herbivores and the growth and survival of their offspring on those plants has been a central concern of theories of host association evolution (Jaenike 1978, Thompson 1988, Mayhew 2001). Host selection for many Lepidoptera is made by the adult female, but the consequences of the decision are borne by the offspring, particularly if they are relatively sedentary with limited opportunities to change hosts. Distinct preference hierarchies have been shown for Lepidoptera in a number of studies (Nylin and Janz 1993, Thompson 1998, Forister 2004), but preferences are not always positively correlated with larval performance measures such as growth and survival. The relationship between these factors plays an important role in host specificity, host-range evolution and influences how insects ‘shift’ onto new hosts (Tabashnik 1983, Futuyma and Peterson 1985, Mayhew 1997, 2001, Mercader and Scriber 2007).

Optimisation theory of host-use suggests that female phytophagous insects should choose those plants that impart the greatest fitness benefits on their offspring (Jaenike 1978), and while this does occur (Via 1986, Singer *et al.* 1988, Craig *et al.* 1989), many studies have found no correlation (Valladeres and Lawton 1991, Fox 1993, Singer *et al.* 1994, Friberg and Wiklund 2009), or even a negative relationship (Courteney 1982, Berdegue *et al.* 1998, Bossart 2003, Keeler and Chew 2008). Some species will lay eggs even on food plants that are toxic to their offspring (Berenbaum 1981, Chew and Robbins 1984, Moreteau *et al.* 1994, Bossart and Scriber 1995). The lack of congruence between adult oviposition preference (hereafter termed ‘preference’) and offspring survival, growth and mass (hereafter termed ‘performance’) has been explained in the literature by the potential influences of differing ecological conditions and selection pressures in nature (Thompson 1988). For example, adults may select inferior plants because they constitute enemy free space compared to higher quality species (Murphy 2004), or choices may represent trade-offs due to the rarity of plants at the top of the preference hierarchy (Futuyma and Moreno 1988).

Alternatively, preference towards an inferior but novel host may occur because the organism has not had sufficient time to adapt to the new plant (Thompson 1988). When a butterfly encounters a potential new host a number of reactions may be observed. The new host may not be incorporated into the host range because it is not recognised by adults or does not allow larvae to survive. In this case, natural selection acts to decrease the oviposition behaviour

on the novel host (Karowe 1990). If larvae can survive on the latter, the new plant may be incorporated into the host range either due to physiological pre-adaptations without the need for evolutionary change, or through adaptation of larval fitness (Thomas *et al.* 1987). Subsequently, if the new host is preferred and enhances performance over the ancestral host (the host plant with which the butterfly co-evolved), the result is a ‘host shift’ on to the new plant, excluding the original plant from the diet. If the performance is enhanced equal to that on the ancestral host, the incorporation of the new food plant into the diet represents a ‘host-range expansion’ (Tabashnik 1983).

Identifying the nature of the ‘preference/performance’ relationship (Thompson 1988) for a particular species is therefore particularly useful in understanding the processes involved when butterflies incorporate new host plants into their range, sometimes in favour of original hosts (Tabashnik 1983, Thomas *et al.* 1987, Bossart *et al.* 2003, Murphy 2007, Keeler and Chew 2008). In addition, this information is important in a conservation context because the identification of optimal requirements will inform managers of which resources to promote and conserve (New 2007). In this study, the preference/performance problem is investigated for an endemic New Zealand butterfly species, *Lycaena salustius*, with the aim of identifying overall resource hierarchies and evaluating the likelihood of the host range expanding to incorporate a novel host, *Fagopyrum esculentum* (buckwheat). *Lycaena salustius* is known to feed on *Muehlenbeckia complexa*, *M. axillaris* and *M. australis* in the field. In addition, the two other species of *Muehlenbeckia* (*M. astonii*, *M. ephedroides* Hook f.) have been fed successfully to larvae in the laboratory and there are records of eggs being laid on *Rumex acetosa* L. (Polygonaceae) and the New Zealand endemic *R. flexuosus* (Gibbs 1980). In pilot laboratory experiments, female *L. salustius* were also observed ovipositing on *F. esculentum*, and larvae are able to survive to the pupal stage on this plant in no-choice experiments. The plant is promoted to vineyards for its attractiveness to natural enemies of agricultural pests, and as the nectar is also attractive to *L. salustius* adults, populations of *L. salustius* on vineyards may have access to *F. esculentum* in the future.

An investigation into the preferences and performance of *L. salustius* on ancestral species (taken to be the native and endemic species in this study) compared to a potential novel host, will provide important information on the host selection of this species and on the consequences of introducing a plant species which is perceived as beneficial. This study will also lay the foundations for future research in host shifts or host-range expansion in the field.

## 5.3 METHODS

### 5.3.1 Study species

Occurring throughout the country, *L. salustius* is largely sedentary although can on occasion be found some distance from the host plants of the *Muehlenbeckia* genus. The species is active between November and April, although variations in flight patterns due to local conditions are common (Craw 1975). There is usually a large first brood in January derived from overwintering larvae, followed by a smaller second brood in March-April in sites near Wellington (Flux, 1968, Craw 1975), while in Waipara the broods occur in November and February (Chapter 2, this study).

*Lycaena salustius* butterflies were reared in captivity at Lincoln University using 12 initial females caught in Waipara, Canterbury in October 2009. The butterflies were placed individually in nylon mesh cages measuring 0.8m<sup>3</sup>, which were then placed in an insectary. The butterflies were maintained in cages with a larval food plant, and a pot of Shasta daisy (*Chrysanthemum maximum*) as a nectar source. The host plant was changed regularly, so that eggs were laid on five different species: *M. complexa*, *M. australis*, *M. astonii*, *Rumex flexuosus* and *Fagopyrum esculentum*. *Muehlenbeckia complexa* and *M. astonii* potted plants were supplied from Hurunui Natives Plant Nursery, Cheviot, (grown from Canterbury seed stock) and were kept at a Lincoln University nursery shade house until required. The *M. australis* plants were grown from cuttings taken from the gardens of Landcare Research in Lincoln. The *R. flexuosus* plants were grown from seed obtained from B&T World Seeds (France, <http://b-and-t-world-seeds.com/>) and *F. esculentum* (c.v. Katowase) was grown from seeds obtained from Midland Seeds, Ashburton, Canterbury, New Zealand. A previous pilot study showed that adults would not oviposit on *R. acetosa*, ruling it out of the study.

### 5.3.2 Larval development

Plants on which eggs were laid were left in the insectary until hatching. Hatching was checked every 2-3 days, so each larva was at most 3 days old when transferred carefully using a '00' size paint brush to a cut shoot of the same plant species. This cutting was placed in a 5ml Eppendorf tube with a hole cut in the lid. The tube was filled with tap water and the shoot held in place by part of a dental wick. This ensured the leaves remained turgid for as long as possible. The tube and shoot arrangement was then placed in an 8cm Petri dish, with moistened filter paper on the

base. The Petri dish was labelled appropriately and stored in an incubator on a 16:8h L:D cycle, at 22°C and 55% humidity during the light phase and 15°C and 55% humidity during the dark phase. 30 Petri dishes were set up for each plant species.

Every day, the filter paper of each dish was moistened, food of the appropriate species was replenished if required and the larvae were checked. New shoots of each plant species were taken from a number of different plants, and leaves were selected to be young and of the best quality based on a visual examination of colour and turgidity. When the pupation stage was reached, each pupa was weighed using an analytical balance (Sartorius LE225D, sensitivity: 0.0001g). Larvae were also weighed initially one day after the first moult. These data were then used to calculate relative growth rate (RGR, in milligrams per day), which quantifies mass gained per day based on the exponential growth model (Lederhouse *et al.* 1992) as given by the formula:

$$\text{RGR} = [\ln(W_P) - \ln(W_I)]/D,$$

where  $W_P$  is weight of the pupa,  $W_I$  is initial weight, and  $D$  is the number of days between the two weight measurements. Upon emergence, adult butterflies were killed using a killing jar, sexed and weighed. The difference between the pupal weight and adult weight was calculated and was termed ‘weight loss’ in the data analysis. If the resulting butterfly was a female, it was dissected to count the number of eggs on emergence. If any of the larvae died before the adult stage was reached, a record was made and a replacement larva was added.

### 5.3.3 Oviposition experiment

An oviposition choice experiment was conducted in the summer of 2010, at the Horticultural Research Area at Lincoln University. Six field cages consisting of a metal frame and a net (BioMesh<sup>TM</sup>, minimum mesh size: 0.28 x 0.78 mm) and measuring 1.8m x 1.8m x 1.8m were erected on a ploughed and unsown field directly south of a shelter belt of pine trees which would provide partial shade over the floor of the cages during the warmest part of the day.

One individual of each of four plant species was planted equidistantly from the cage edge and from each other in the four corners of each cage. The species were randomly allocated to the four corners in order to account for the effect of periodic shade. The plant species were *M. complexa*, *M. astonii*, *M. australis* and *Rumex flexuosus*. A single plant of *C. maximum* was planted in the middle of the four species. On 11 January 2010, a single mated captive-bred female

of *L. salustius* was placed in each cage. Females were considered mated because they were kept in a culturing cage for a maximum of five days prior to the experiment and were observed laying eggs. The eggs laid in the field cages were therefore not the first eggs of the female. Females older than five days were not used in the experiment.

Each day the cages were visited at the same time (18.00h) every evening to water the plants and to count the number of eggs laid on each plant. The experiment was stopped on the fifth day, the eggs were removed from the plants and the experiment was set up again. This process was repeated four times with the same plants so that  $n = 24$ . As not all replicates were carried out on the same day, weather conditions were monitored by recording the minimum and maximum temperatures and noting the predominance of sun or cloud for each day of the experiment.

In this experiment it was considered that the inclusion of *F. esculentum* would make the cages too crowded. Therefore, a separate experiment was established to test *L. salustius* preference between *F. esculentum* and the three *Muehlenbeckia* species above. In six 0.8m<sup>3</sup> nylon mesh cages placed in a greenhouse at Lincoln University, potted plants of the four species of approximately equal size were placed randomly in the corners. To provide a source of nectar, a cut shoot of *C. maximum* held in a vial of water by a dental wick was placed in the middle of the four plants and replaced daily. It was ensured that none of the plants touched each other. On 6 February 2010, a captive-bred and mated female was introduced to each cage, and the number of eggs laid on each plant was counted every day for five days. The eggs were removed at the end of the experiment, and the process was repeated only once ( $n=12$ ) due to time and resource constraints.

#### 5.3.4 Statistical analysis

Measures of larval performance (pupal mass, larval duration, RGR, weight loss, egg number on emergence and adult mass) were unbalanced due to differential survival rates. Therefore to create a balanced data set in each case, data were randomly subsampled. Data were then tested for normality using the Shapiro-Wilks test. Larval duration (days) was not normally distributed and was natural log transformed. Data were analysed using a two way ANOVA, with host plant as the explanatory variable effect. Multiple comparisons were conducted using Tukey's HSD.

Oviposition choice was analysed by summing the eggs laid on each species over the five days, and performing a GLM with a quasi-Poisson error distribution, and plant species, experimental run, cage number and number of sunny days as explanatory variables. Multiple

comparisons were conducted using the Bonferroni correction to Tukey's HSD. A GLM with a quasi-binomial error distribution was also used with the same variables, but by treating the number of eggs laid on each species as a proportion of total eggs laid. Repeated measures analysis of the raw data led to convergence problems, so the eggs laid on each day were also analysed separately. All analyses were performed using R version 2.9.2 (R Core Development Team, 2009).

## 5.4 RESULTS

### 5.4.1 Larval performance

Mean values for the seven components of larval performance are summarised in Table 5.1. There was a significant effect of treatment on relative growth rate (RGR;  $F=8.54$ ,  $df = 75$ ,  $P<0.0001$ ), larval duration ( $F=17.54$ ,  $df=80$ ,  $P<0.0001$ ), pupal mass ( $F=5.9$ ,  $df=95$ ,  $P<0.001$ ), and weight loss ( $F=3.63$ ,  $df=75$ ,  $P<0.01$ ), but not for adult mass ( $F=2.4$ ,  $df=75$ ,  $P=0.058$ ) and number of eggs on emergence ( $F=1.23$ ,  $df=35$ ,  $P=0.32$ ). However, when sex was added to the ANOVA as an explanatory variable, there was a significant effect of both sex and treatment on adult mass (Treatment:  $F=4.16$ ,  $P<0.01$ , sex:  $F=56.13$ ,  $P<0.0001$ ). There was also a significant effect of sex on larval duration (Treatment:  $P<0.0001$ , sex:  $P<0.01$ ) and pupal mass (Treatment:  $P<0.001$ , sex:  $P<0.01$ ), but not RGR. When sex was added to the weight loss ANOVA, there was no effect of treatment, but males lost significantly more weight between the pupal and adult stages than females ( $P<0.0001$ ). This is consistent with the finding that females weighed significantly more than males on emergence (t-test:  $t = 6.215$ ,  $df = 80$ ,  $P<0.001$ ), presumably due to the egg load of females. All multiple comparisons conducted below involved the model with the highest explanatory power (i.e. ANOVAs for treatment and sex for larval duration, pupal mass and adult mass; ANOVAs for treatment only for RGR and weight loss).

There were insufficient data to test for differences between the survival rates of larvae fed the five species. However, the survival rate of larvae fed *F. esculentum* (52%) is much lower than that for the other four species (70-78%). This is supported by the multiple comparisons for RGR (Fig. 5.1a), where there were no significant differences in growth rate of larvae fed the three *Muehlenbeckia* species and *R. flexuosus*, but those raised on *F. esculentum* grew significantly slower than on all ancestral species except *M. australis*. Similarly, larvae raised on *F. esculentum* spent significantly longer in the larval stage than the ancestral species except *M. complexa*, and

those fed only *R. flexuosus* progressed to the pupal stage significantly faster than all other species (Fig. 5.1b).

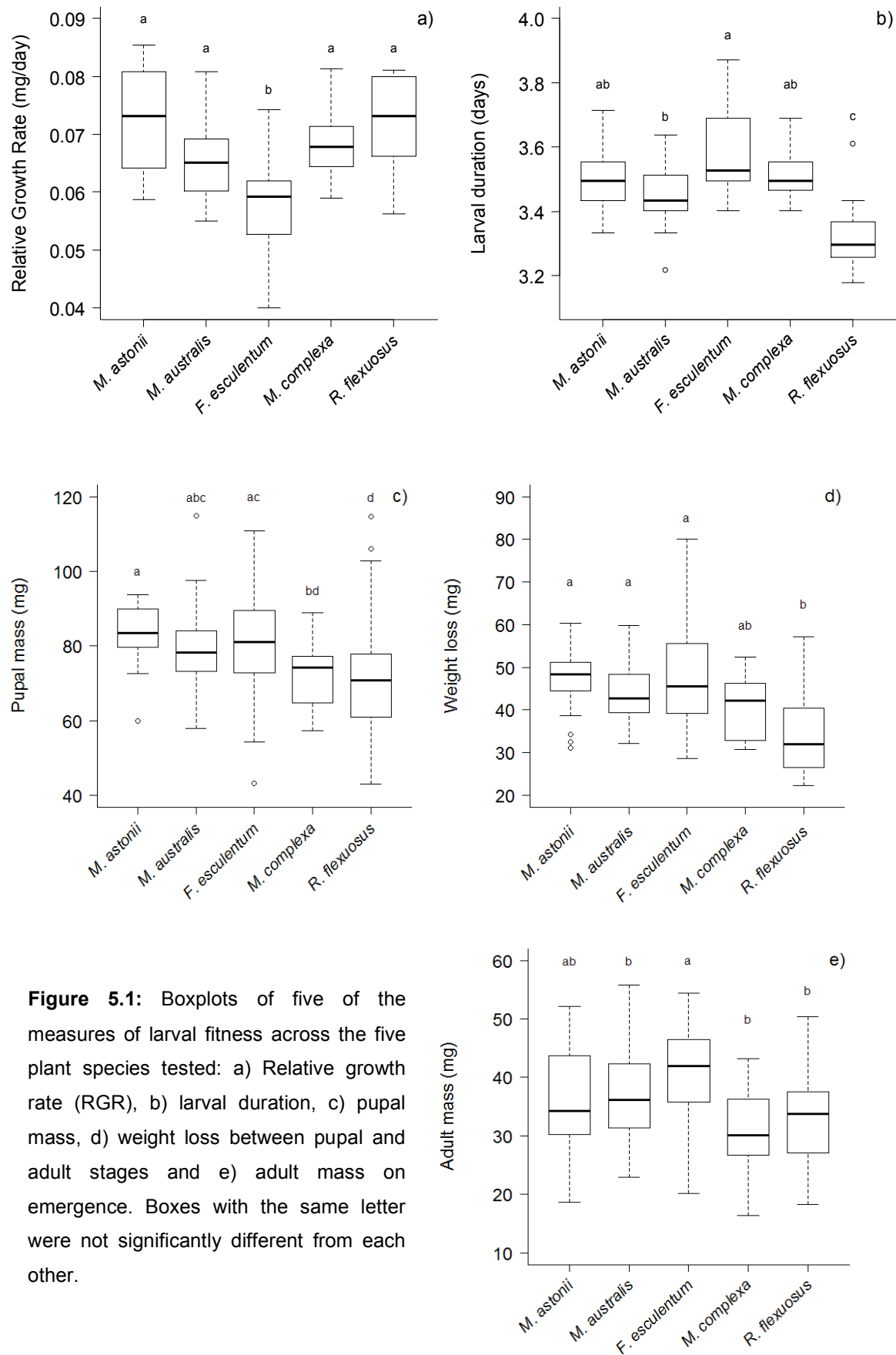
Pupal mass was significantly higher for larvae fed *M. astonii* compared to those fed *R. flexuosus* or *M. complexa*. It was also lower for larvae fed *R. flexuosus* than those fed *F. esculentum* (Fig. 5.1c). Multiple comparisons for weight loss showed that larvae fed *R. flexuosus* lost significantly less weight on emergence than did larvae fed *M. astonii* and *F. esculentum* (Fig. 5.1d). Finally, the adult mass of individuals raised on *F. esculentum* was significantly higher than for *M. complexa* and *R. flexuosus* (Fig. 5.1e). Adult mass was correlated with the number of eggs on emergence (Pearson correlation coefficient = 0.37,  $P < 0.05$ ), but a stronger correlation was found between pupal mass and number of eggs on emergence (Pearson correlation coefficient = 0.47,  $P < 0.01$ ).

**Table 5.1:** The mean values  $\pm$  1 standard error for the six replicated measures of *L. salustius* larval performance across the five plant species tested ( $n = 30$ ). Percentage survival was calculated from the number of larvae out of 30 surviving to the adult stage.

	<i>M. astonii</i>	<i>M. australis</i>	<i>F. esculentum</i>	<i>M. complexa</i>	<i>R. flexuosus</i>
Relative growth rate (mg)	$0.07 \pm 0.002$	$0.07 \pm 0.002$	$0.06 \pm 0.002$	$0.07 \pm 0.002$	$0.07 \pm 0.002$
Larval duration (days)	$32.9 \pm 0.7$	$32.9 \pm 0.7$	$36.8 \pm 1.3$	$33.4 \pm 0.8$	$27.4 \pm 0.7$
Pupal mass (mg)	$84.6 \pm 1.2$	$78.8 \pm 2.1$	$80.0 \pm 3.5$	$72.0 \pm 2.0$	$70.0 \pm 2.9$
Weight loss (mg)	$47.1 \pm 2.0$	$45.4 \pm 2.1$	$46.9 \pm 3.4$	$40.6 \pm 1.7$	$36.0 \pm 2.5$
Adult mass (mg)	$35.5 \pm 2.0$	$36.5 \pm 2.2$	$40.3 \pm 2.3$	$31.5 \pm 1.7$	$33.0 \pm 2.3$
No. of eggs on emergence *	$60.6 \pm 6.2$	$47.6 \pm 4.1$	$62.5 \pm 10.1$	$49.9 \pm 3.8$	$60.2 \pm 10.7$
Survival †	74%	78%	52%	70%	73%

\* no significant effect of plant species; † not tested statistically

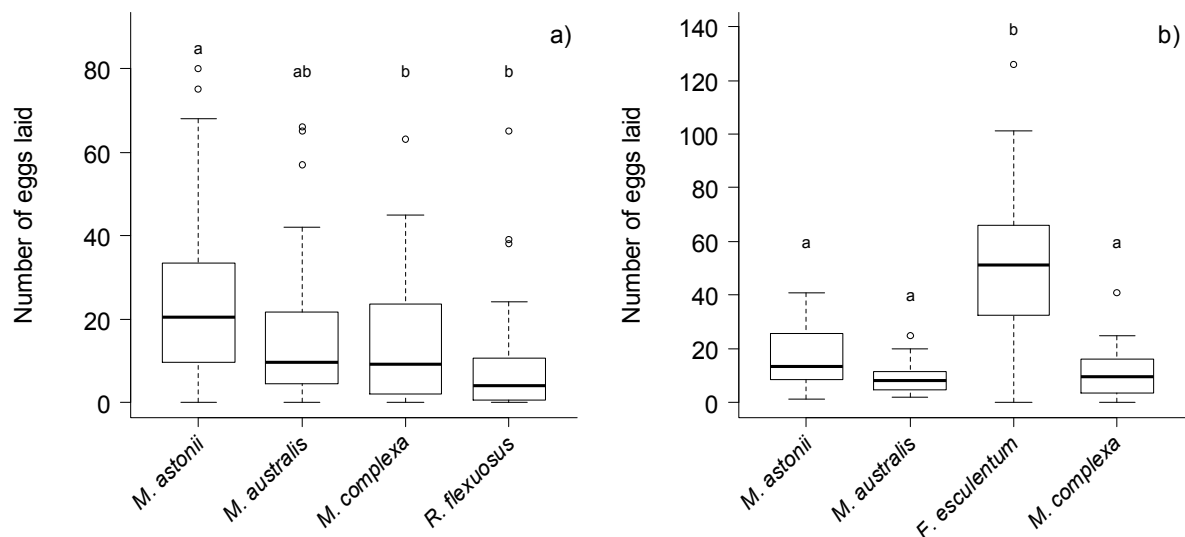




**Figure 5.1:** Boxplots of five of the measures of larval fitness across the five plant species tested: a) Relative growth rate (RGR), b) larval duration, c) pupal mass, d) weight loss between pupal and adult stages and e) adult mass on emergence. Boxes with the same letter were not significantly different from each other.

### 5.4.2 Oviposition choice

For the summed oviposition choice data, there was a significant effect of both plant species ( $P < 0.05$ ) and experimental run ( $P < 0.01$ ), but not of cage or number of sunny days which were both removed from the analysis. Subsequent multiple comparisons showed that there were no differences in oviposition choice between the three *Muehlenbeckia* species, but *R. flexuosus* was chosen significantly less often than *M. astonii* ( $P < 0.05$ ) (Fig. 5.2). Data analysed for each individual day showed no differences. When the data were analysed as the proportion of eggs laid on each plant species using a quasi-binomial error distribution, the effect of plant species was more significant ( $P < 0.001$ ), and Bonferroni-adjusted multiple comparisons showed that a significantly greater proportion of eggs were laid on *M. astonii* than on both *M. complexa* ( $P < 0.05$ ) and *R. flexuosus* ( $P < 0.001$ ). There were insufficient replicates to test whether the position of the plants within each cage made any difference to choice, although this would have been accounted for to some extent by the inclusion of the variable ‘cage’, as plant positions were not changed within each cage between runs. For the smaller study of choice between the three *Muehlenbeckia* spp. and *F. esculentum*, both quasi-Poisson and quasi-binomial GLMs returned the same result. The species of plant was significant in both models ( $P < 0.0001$ ), and Bonferroni-adjusted multiple comparisons showed that females chose to lay significantly more eggs on *F. esculentum* than on any of the *Muehlenbeckia* spp. ( $P < 0.0001$ ) (Fig. 5.3).



**Figure 5.2:** a) Boxplot of the number of eggs laid by *L. salustius* females on each of the four experimental plants in field cages, b) Boxplot of the number of eggs laid by *L. salustius* females on three species of *Muehlenbeckia* and *F. esculentum* in greenhouse cages. Boxes with the same letter did not differ significantly based on a Generalised linear model using a quasi-binomial error structure.

## 5.5 DISCUSSION

Thompson (1988) notes that in laboratory studies the components of butterfly larvae performance (e.g., growth rate, survival, pupal mass, larval duration) are not always positively correlated with each other. This is a problem because different criteria of larval performance of a herbivore can give differing conclusions about the fitness hierarchy of potential host-plant species. In this study, a lack of correlation between larval performance components has been found for *L. salustius*, which consequently affects the conclusions concerning the performance-preference relationship. The plant species imparting the best performance to larvae depends on the performance criterion chosen and any of those plant species considered could be concluded as being superior (Table 5.2).

**Table 5.2:** A list of the performance criteria measured and the plant species that conferred the best performance based on each criterion alone.

Performance criterion	Plant species
Relative growth rate	<i>M. astonii</i>
Larval duration	<i>R. flexuosus</i>
Pupal mass	<i>M. astonii</i>
Weight loss	<i>M. complexa</i>
Adult mass	<i>F. esculentum</i>
Eggs on emergence *	<i>F. esculentum</i>
Survival †	<i>M. australis</i>

\* no significant effect of plant species

† not tested statistically

In oviposition preference assays, female *L. salustius* significantly preferred *F. esculentum* over the four native plant species. This is assuming that *R. flexuosus*, the least favoured plant when tested alongside the *Muehlenbeckia* species, would not have been chosen if it was included in a five-way choice test. This assay was also conducted with a small number of replicates. However, this preference hierarchy in itself may not be surprising. Butterfly species have been

recorded ovipositing on plants they have never before encountered (Warren 1987a). Preference for plants related to native hosts which have similar chemistry has been recorded for other species of butterfly, for example Edith's checkerspot (*Euphydryas editha* Boisduval (Nymphalidae)) on English plantain (*Plantago lanceolata* L. (Plantaginaceae)) (Thomas *et al.* 1987), and may eventually be included into the host range if behavioural or physiological pre-adaptation permits it (Tabashnik 1983). The similarity of the chemistry between *F. esculentum* and ancestral *Muehlenbeckia* spp. hosts is not known, but similarity may be inferred because *L. salustius* adults have rejected other species in the same family (*R. acetosa*) in previous work (M. Gillespie, unpublished).

### 5.5.1 Negative preference/performance correlations

Nylin and Janz (1993) suggested that the most clear-cut components of performance are relative growth rate and survival in a study on the comma butterfly (*Polygonia c-album* L. (Lycaenidae)), and these are considered here first. On the basis of these two criteria, *F. esculentum* was the least suitable plant species in terms of fitness for larvae of *L. salustius* in this study. This therefore represents a negative correlation between larval performance and oviposition preference, if evidence from the small greenhouse bioassay is considered. Such a relationship has been found for a number of other species (Courteney 1982, Valladeres and Lawton 1991, Singer *et al.* 1994, Berdegue *et al.* 1998, Bossart 2003, Keeler and Chew 2008), and the reasons for this lack of congruence have been related to the evolution of a species' host-plant range. A poor correlation is considered to be evidence of the importance that factors other than nutritional quality play in the evolution of host plant choice. While optimal oviposition theory suggests that natural selection should favour those females that oviposit on the plant species that confer the greatest fitness benefits to their offspring (Jaenike 1978), a number of alternative hypotheses based on empirical evidence have been put forward to try to understand the adaptive basis of deviance from this theory. The most relevant are considered here.

The 'time' hypothesis posits that populations need time to adapt to new potential host species. In this case, selection may not have had time to either reduce the tendency of females to oviposit on poor novel hosts or to increase larval performance and survival on them (Futuyma 1983). Use of *F. esculentum* has not been recorded in the field; therefore if the population from which *L. salustius* were collected for this study had encountered *F. esculentum* for the first time in these experiments, oviposition preference may have been based on basic parameters such as

nitrogen content and secondary chemistry (Porter 1992). *Rumex flexuosus* is also not known to occur in the habitats from which the experimental insects originate, but this ‘novel’ host was not selected in the same way as *F. esculentum*. The reasons for this are not known but again may relate to some basic nutrition parameters or secondary chemistry.

The ‘enemy free space’ hypothesis (e.g., Bernays and Graham 1988, Murphy 2004) suggests that that performance and survivorship of larvae in the absence of natural enemies may differ to that when they are present and that adults may oviposit on inferior hosts if the latter constitute enemy free space (Murphy 2004). However, the opposite may be the case in this study. While *F. esculentum* is preferred by *L. salustius* in the greenhouse in the absence of natural enemies, ancestral hosts may be preferred in the field. As a flowering plant used to attract the natural enemies of pests, *F. esculentum* may attract parasitoids and predators of *L. salustius*. If adult *L. salustius* lay eggs on *F. esculentum*, and they are known to deviate from habitat patches in search of nectar (Gibbs 1980, Chapter 2, this thesis), it may place the larvae at a higher risk of attack than the ancestral *Muehlenbeckia* spp. hosts, which may actually be well suited to assist with crypsis and other forms of predator avoidance. More detailed fieldwork is required to determine which host plant, if any, constitutes enemy-free-space (Berdegue *et al.* 1998, Murphy 2004). In addition, the slower growth rate of larvae on *F. esculentum* which may be related to the metabolic demands relating to detoxification processes (Keeler and Chew 2008), also leaves larvae potentially vulnerable to predation for a longer time than those feeding on alternative hosts, if the ‘slow-growth-high-mortality’ hypothesis holds true (Clancy and Price 1987).

An alternative to the time hypothesis is the ‘confusion’ hypothesis which suggests that if plants are similar in terms of cues that an insect uses for recognition, the two plants might be perceived as identical (Larsson and Ekbom 1995). Thus, a poor plant may be included in the diet of the larvae because selecting against it may mean selecting against the similar, but superior plant (Fox and Lalonde 1993). Testing this hypothesis is difficult in practice, but simulation models suggest that species with short life spans and limited dispersal abilities may suffer only small penalties in exhibiting a non-discriminatory oviposition strategy, particularly if resources are scarce (Jaenike 1990, Larsson and Ekbom 1995). Thus oviposition on additional plants may be a way of spreading the risk (Jaenike 1990) and in the enclosed environment of a small cage, the behaviour of female *L. salustius* may be a function of the size of leaves of *F. esculentum*, an absence of natural enemies in the cage, a similarity of oviposition cues and a general state of

‘confusion’. However, the lack of preference for the large leaved *R. flexuosus* in experiments suggests that any confusion may be based on chemical cues rather than leaf and plant size.

Host choice may also be explained by optimal foraging rather than optimal larval performance (Schiers *et al.* 2000, Mayhew 2001). For example, Forister *et al.* (2009) found that adults of the lycaenid *Lycaeides melissa* Edwards altered oviposition behaviour in the presence of flowers of a novel host, *Medicago sativa* L. In foliage-only tests, the native host *Astragalus canadensis* L. (Fabaceae) was preferred, and was the better host for larvae. However, when offered plants with flowers in no-choice experiments, equal numbers of eggs were laid on the two hosts. Thus, plants may be chosen for adult fitness at the expense of larval fitness (Mayhew 2001, Forister *et al.* 2009). Analysis of the oviposition data from a study on floral nectar preferences (Chapter 4; not shown) also showed that between flowering and non-flowering *F. esculentum*, adults laid more eggs on flowering plants, although the number of replicates was insufficient to test this statistically. Adults of *L. salustius* are also known to feed on the nectar of *M. complexa* flowers (Chapter 4), but the effect of flowers on oviposition behaviour was not tested here. Nonetheless, the fact that adults can feed and oviposit on the same species indicates a high spatial overlap between two different resources, the searches for which are usually spatially and temporally separated (Wiklund 1977, Stanton 1984). By utilising such plants, a female can increase search efficiency for both resources (Janz 2005), a strategy employed by the lycaenid *Polyommatus icarus* (Janz *et al.* 2005). In the case of both *F. esculentum* and *M. complexa*, the costs involved in this combined search are larval survivorship and growth and general poor performance, respectively. In the field such costs may be acceptable if nectar feeding increases fecundity sufficiently. Again, field studies would be required to establish such a strategy in *L. salustius*.

### **5.5.2 Positive preference/performance correlations**

In addition to these hypotheses, it could be considered that the correlation between performance and preference is positive by looking at other performance criteria. The fitness components of pupal mass, adult mass and number of eggs on emergence had high mean values for larvae fed *F. esculentum*. These components of performance are important factors in the fecundity and longevity of the next generation of adults (Boggs 1997) and have been used to evaluate performance in a number of studies (e.g., Berdegue *et al.* 1998, Forister 2004, Forister *et al.* 2009, Keeler and Chew 2008). A positive correlation between preference and performance is

also common in other species (Via 1986, Bossart 2003, Forister 2004, Janz 2005, Forister *et al.* 2009) and suggests that nutrition is more important in host association evolution than other ecological factors (Nylin and Janz 1993, Berdegue *et al.* 1998), and the above hypotheses are not required to explain the novel host use. Furthermore, in *L. salustius* the quality of offspring, i.e. heavier and more fecund emerging adults, may be more important than the quantity and speed of development. Further tests are needed to confirm this.

Overall, if the correlation between preference and performance in the field is a poor one, i.e. survivorship and growth rate are most important to long-term persistence, evolutionary theory predicts that *F. esculentum* would be incorporated into the host range only if natural selection acts to increase survivorship and growth rate more rapidly than it acts against oviposition preference (Futuyma 1983). If the preference/performance correlation is positive, e.g., pupal mass is more important in *L. salustius* persistence, *F. esculentum* may be incorporated into the host range (Futuyma 1983). In addition in this latter case, any ancestral host that leads to poorer larval performance may be eliminated in the long term if preference for the new host continues to evolve more rapidly than performance on the poorer host (Karowe 1990). Use of field studies would add important information to address the likelihood of these scenarios (Thompson 1988, Mayhew 1997).

Removing *F. esculentum* from consideration to focus on the four native species results in alternative conclusions. Among the native and endemic plant species, *M. astonii* was preferred by ovipositing adults. In addition, this plant species conferred high or intermediate fitness benefits in six out of the seven criteria (weight loss was the second highest behind *F. esculentum*). In this case, there is again a positive correlation between oviposition preference and larval performance. As Nylin and Janz (1993) point out, and as discussed above, this suggests that plant quality characteristics such as nutrition are important in larval performance and this helps to limit host plant range in nature. However, this species was the rarest of the three *Muehlenbeckia* species in surveys of vineyard areas in Waipara (Chapter 2), although it was not so rare to define it as a novel host. *Muehlenbeckia astonii* is also given little importance for *L. salustius* populations in the literature (Gibbs 1980), which may be because it is a difficult plant on which to physically search for larvae and eggs, and is rare in the field to the point of being nationally threatened (Rogers *et al.* 2005). In contrast, *L. salustius* adults are most often found in association with the most common of the host plants, *M. complexa*. Larvae fed this species in this study showed high performance only by the measure of weight loss, a lesser-used component of larval performance

but which may reflect digestive efficiency. Thus, despite the general low larval performance on *M. complexa* in bioassays, the relative abundance of the plant in the field may demonstrate a trade-off that the female *L. salustius* makes. Thompson's (1988) patch dynamics hypothesis predicts that females will oviposit disproportionately more on hosts that are more abundant, even if they are inferior. For example, *Euphydryas chalcedona* Doubleday (Nymphalidae) prefers to oviposit on *Scrophularia californica* Cham & Schldl. (Scrophulariaceae) in choice trials, but in coastal Californian habitats uses the more abundant host *Diplacus aurantiacus* Curtis (Scrophulariaceae) (Williams *et al.* 1983). *Muehlenbeckia complexa* may represent a 'safe' option, in that costs to all components of performance are relatively low compared to host plant species that lead to higher performance in some respects but low performance in others (e.g., *R. flexuosus*: rapid development but low pupal mass; *M. astonii*: high pupal mass but low adult mass), and these costs are negligible in overall survival and fecundity when the plant is more readily located.

### 5.5.3 Conservation implications

Preference/performance trade offs can take many forms in the field. Ovipositing adults can be less discriminatory in nature when preferred hosts are rare (Williams *et al.* 1983), when oviposition-site search time is limited (Tabashnik 1983), or as egg-load or adult age increase (Stanton and Cook 1983). Studies have also suggested that a greater range of hosts are used in the presence of natural enemies, competitors or both (Thompson 1988, Valladeres and Lawton 1991, Kursar *et al.* 2006). In addition to these complications, adult butterflies take a number of decisions at a number of different levels when ovipositing, assessing the topography and geography of habitat patches, the density, age and phenology of host plants (Porter 1992), plant maturity and availability (Kursar *et al.* 2006), microclimate (Thomas 1983) and proximity of nectar (Murphy *et al.* 1984). Larval performance may be further affected by interactions with competitors, natural enemies, other trophic levels and abiotic conditions (Thompson 1988). The removal of many of these factors in bioassays may have significantly altered both adult and larval behaviour. It is therefore imperative to conduct field studies on oviposition preferences and larval performance if this laboratory work is to be validated (Thompson 1988, Mayhew 1997).

Despite this complexity and need for further study, a number of hypotheses and recommendations may be tentatively put forward relating to the resource requirements of *L. salustius*. Of the ancestral, native food plants, *M. astonii* fits with the theory of optimal nutrition



where female oviposition preferences are apparently based on the optimal nutritive value of plants. *Muehlenbeckia astonii* is a plant incorporated into Greening Waipara plantings on vineyards, so the encouragement of further plantings of this species in remnant habitats may have beneficial effects on *L. salustius* populations. In addition, using this plant in corridors linking remnant habitats to other planted areas may assist in the butterfly's dispersal to these areas. The presence of *M. australis* and *M. complexa* is also likely to be beneficial to the butterfly, however, in that it allows ovipositing adults to 'spread the risk' associated with assigning eggs to host plants (Porter 1992). The use and importance of these plants in the field are not fully understood in light of the results of this study, but in practice conserving all species in the butterfly's range is more likely to be beneficial to this and other related species in creating the habitat mosaic that most butterfly species require (Chapter 2).

The planting of *F. esculentum* in corridors between source populations and areas such as gardens and biodiversity trails may help to attract the butterfly to these areas, particularly as adults can use the plant for both foraging and oviposition. However, at this early stage of research, it is not recommended for *F. esculentum* to be incorporated into the remnant habitats, because it is not known how the butterfly will react to the plant in nature. It may have the potential to act as a population sink because it is likely to require maintenance in terms of watering in the hot dry Canterbury summers. If adults are drawn to oviposit on *F. esculentum* in the manner shown in the bioassays and the plants are not maintained, large proportions of the progeny will not survive if they cannot move onto alternative hosts. However, the discovery of a related plant species that *L. salustius* is unlikely to have encountered in nature but will accept in laboratory assays, opens up a potential system for study to contribute towards our understanding of how insects adapt to exploit novel hosts.

## Chapter 6 General Discussion

### 6.1 Introduction

Although the relationship between ecosystem function and biodiversity is not fully understood (Landis *et al.* 2000), there is likely to be a positive association between the number and diversity of organisms and the provision of ecosystem services in a landscape (Costanza *et al.* 1997, Cardinale *et al.* 2006). However, there may be redundancy in the relationship or even negative aspects in intra-guild predation and other such interactions. Nevertheless, the current state of many mono-cultural landscapes is of concern because the associated low levels of biodiversity reflect the paucity of ecosystem services in such land uses (Daily 1997, Cardinale *et al.* 2006, Fiedler *et al.* 2008). This concern is widespread and measures are being explored to address the problem. These include habitat manipulation techniques (Landis *et al.* 2000), and plans to promote them in the form of agri-environment schemes in Europe and the USA (Kleijn and Sutherland 2003, Tschardtke *et al.* 2007), and projects such as the Greening Waipara project in New Zealand (Fiedler *et al.* 2008). These projects and schemes attempt to link research with landowners by encouraging them to adopt environmentally friendly farming practices (Kleijn and Sutherland 2003, Merckx *et al.* 2009). They therefore have the potential to halt biodiversity declines (Tschardtke *et al.* 2007, Warren *et al.* 2008), and even increase biodiversity and thus ecosystem function (Cardinale *et al.* 2006, Fiedler *et al.* 2008). However, such enhancements are not always effective (Kleijn *et al.* 2006) and require monitoring and continued research to ensure the desired effects are being realised and to continue to improve them (Whittingham 2007, Merckx *et al.* 2009), ideally leading to an experimentally derived service providing unit (Luck *et al.* 2003).

This thesis is an example of this evaluative research. The objective was not focussed on specific enhancements that target individual, economically important organisms. These studies are widespread; for example, the study of the effects of providing flowering plants to natural enemies of agricultural pests (Berndt and Wratten 2005, Berndt *et al.* 2006, Robinson *et al.* 2008), and the application of mulches beneath vines to reduce fungal infection (Jacometti *et al.* 2007, 2010). Less common in the southern hemisphere is research into the ‘stacked ecosystem services’ of ecosystem enhancements (for northern hemisphere examples see Dover 1991, Fiedler and Landis 2007). If biodiversity and ecosystem function are positively linked, it is reasonable to expect that the addition of targeted habitat improvements would help to enhance populations of

non-target organisms. For example, in the UK, restoration coppice management for the heath fritillary, *Melitaea athalia* Rottemburg (Nymphalidae), also benefitted woodland ground flora (Warren 1987b, Pullin 1996) and conservation headlands that are aimed at improving farmland bird species have been shown to increase butterfly populations (Dover 1990, 1991). As butterflies are known to be an indicator of ecosystem 'health' (Erhardt and Thomas 1991, Oostermeijer and van Swaay 1998, Pywell *et al.* 2004), this is a positive endorsement for conservation headlands. In this thesis, for the first time in New Zealand, ecosystem enhancements that were not aimed at butterflies were assessed for their impact on butterfly populations. In addition, measures that could be incorporated with such enhancements to further improve butterfly populations were sought and indentified, and in the process a better understanding of some aspects of the New Zealand butterfly fauna was gained.

Comparatively little is known about the butterfly fauna of New Zealand, while in Europe for example, a long history of butterfly monitoring and autecological work (Thomas 1984, Warren 1987a,b, Bourn and Thomas 1993, Bergman *et al.* 2001, Eichel and Fartman 2007), provides a detailed framework which can be vital to enhancing conservation through habitat management (Thomas 1984, Pullin 1996). It was necessary therefore for this thesis to both draw on this wealth of information to make inferences on closely related New Zealand species and similar habitats, and to fill the gaps in the knowledge about New Zealand butterfly species in the process. The aims of the thesis were therefore addressed in three ways. Chapter 2 consisted of initial survey work and information on broad habitat associations, Chapter 3 sought to ensure the identity of a taxonomically difficult species found in the surveys and Chapters 4 and 5 investigated the adequacy of current resource provision for butterflies in Waipara vineyards. The results of these studies can be interpreted in terms of their contribution to the assessment of the effectiveness ecological enhancements, the inferences they make about future conservation efforts and the contribution made to the knowledge of butterflies in New Zealand.

## **6.2 The importance of ecosystem enhancements to butterfly populations in Waipara**

The results of the four experimental chapters suggest that vineyard landscapes in Waipara are currently poor habitats for native New Zealand butterflies. While ecosystem enhancements were not aimed at improving butterfly populations, in the current stage of development they are little used by butterflies. In Chapter 2 it was shown that only eight species of butterfly visited vineyards in Waipara, of which only five species were encountered regularly. By far the most

abundant butterfly was *Z. oxleyi* (southern blue), a relatively sedentary and weakly flying butterfly that uses members of the Fabaceae as host plants. The ubiquity and contiguous cover of these host plants, particularly *T. repens* (white clover) ensured the abundance of this species. The other four species consisted of *P. rapae* (cabbage white), an agricultural pest species, and three endemic and sedentary species of Lycaenidae that all relied on host plants restricted to remnant pockets of native vegetation on relatively marginal land or gravelly river beds. The Greening Waipara plantings were not used by most butterflies, despite one of the most important environmental factors to butterfly abundance being native plant species cover.

The low number of species in Waipara vineyards can be explained in part by the small New Zealand fauna compared to that of other countries. In a similar study in Germany, vineyards accommodated 34 species, 8 of which were in the country's Red Data Book (Schmitt *et al.* 2008), and in the UK, studies have recorded at least 32 species in arable field margins (Dover 1994). These figures are greater than the total butterfly fauna of New Zealand. Despite this, it is possible to draw parallels in the butterfly habitat associations of European farmland with those on New Zealand farmland, just as it is possible to identify similar patterns of land use and agricultural intensification between the two regions. Numerous studies on farmland in Europe have shown that environmental factors such as flower abundance (Summerville and Crist 2001, Clausen *et al.* 2001, Tudor *et al.* 2004, Shepherd and Debinski 2005, Schmitt *et al.* 2009), plant diversity (Steffan-Dewenter and Tscharntke 2002, Shepherd and Debinski 2005), shrub cover (Dover 1997, Pywell *et al.* 2004), and host plant cover (Munguira and Thomas 1992, Smallidge and Leopold 1997, Clausen *et al.* 2001) are important in explaining butterfly abundance variation. Similar factors were also important in Chapter 2: butterfly abundance variation was explained by nectar abundance, the cover of *Z. oxleyi* host plants, and native plant cover, a category that represented host plants of the Lycaenidae, other shrubs and a greater diversity of plants in general. The importance of these factors implies that resources such as host plants and nectar may be the limiting factors for butterfly populations (Thomas 1984, Bourn and Thomas 1993, Dover 1997, Dover and Sparks 2000). Although many complex factors can limit the carrying capacity of butterfly habitats (see Bergman *et al.* 2001 for a review), the restriction of such resources and the majority of butterfly species in Waipara vineyards to small parcels of land support this suggestion. For some of the more mobile species that might visit vineyards, host plants are virtually absent. The factors outlined above also help to explain why the Greening Waipara plantings are not as important to butterflies as are the remnants of native vegetation. Although the

plantings were not designed with the aim of promoting butterfly populations, and have not been evaluated for other forms of biodiversity, in the vineyards visited these plantings were too small, young and isolated and did not include sufficient host plants and nectar sources to be of use to butterflies.

The results presented in Chapter 3 are relevant to this section of the discussion mainly because they raise more questions about the conservation status of an endemic butterfly (*Z. oxleyi*). While the species of *Zizina* present in Waipara was identified as *Z. oxleyi* by both molecular and morphological markers, hybridisation between the two species remains a plausible scenario. It is currently thought that the main difference in ecology between *Z. oxleyi* and *Z. labradus* is that only the endemic *Z. oxleyi* feeds on species in the *Carmichaelia* (Fabaceae) genus which are taken to be its ancestral host plant range (Gibbs 1980). This potential niche separation between the two species remains unsubstantiated, but if it applies, this group of plants may provide a habitat to *Z. oxleyi* that is not occupied by *Z. labradus* in zones where the ranges of the two species overlap. Such ecological differentiation can continue the divergence of recently separated species into less overlapping niches (Schluter 2000). An absence of *Carmichaelia* species in habitats such as Waipara vineyards, and lowland agricultural habitats in general, may prevent this occurring, leaving *Z. oxleyi* more vulnerable to hybridisation. However, this suggestion is dependent on a number of assumptions as discussed in Chapter 3 and requires much more work to be substantiated.

The findings in Chapter 4 have also highlighted that for *L. salustius*, optimal sources of nectar are not present in Waipara vineyards. This species is an opportunistic nectar feeder that uses the perennial ‘weed’ species available within a short distance of host plants. However, one such species that is frequently used by nectaring *L. salustius* (*Achillea millefolium*) led to fitness levels that were not significantly greater than those provided by water. The superior fitness benefits imparted on females by the top ranking flower species, *Hebe salicifolia* suggests that this, and perhaps other *Hebe* species, were used for nectar prior to European settlement. Their absence from remnants and plantings suggests that *L. salustius* is using, and may be limited by, inferior nectar sources (Dover 1997, Dover and Sparks 2000).

Finally, Chapter 5 showed that the plant species most common in *L. salustius* habitats in Waipara (*Muehlenbeckia complexa*) was not the most preferred host plant by ovipositing females in cages, or the most important for larval growth. There were differences between the female preference hierarchy and that of larval performance, but in both cases the best plants were species

not currently present, or present in low numbers in the Waipara vineyards studied. Female *L. salustius* laid significantly more eggs on *F. esculentum* when it was present and on *M. astonii* when it was not. While larvae could complete their life cycle on the novel host *F. esculentum*, both survival and growth rate were low, suggesting it is an inferior host, but survival and growth on *M. astonii* were significantly higher. While *M. astonii* was present in Greening Waipara planting areas, its young age and isolation from source populations is currently unlikely to be of benefit to this butterfly species. Butterfly species richness and abundance is negatively affected by small habitat patch size (Saunders *et al.* 1991, Steffan-Dewenter and Tscharntke 2002) and isolation from other habitat patches (Thomas *et al.* 1992, Hanski 1999, Haddad and Baum 1999).

Overall, while native plantings may in time provide some resources of use to butterflies and other ecosystem enhancements such as planting strips of *F. esculentum* to attract natural enemies of pests may benefit butterflies to some extent by providing of adult food, significantly improved populations of butterflies are unlikely to occur with piecemeal measures. Specific resource provision combined with larger scale habitat management such as hedgerow conservation, extension and connection of remnant habitats and the promotion of landscape heterogeneity as encouraged by agri-environment schemes in the northern hemisphere, are far more likely to enhance butterflies and biodiversity in general. These measures are discussed below in more detail.

### **6.3 Future conservation of butterfly populations in New Zealand agricultural landscapes**

The above discussion makes the direction that future conservation efforts for Waipara butterflies must take relatively clear. In general, butterfly populations in vineyard landscapes are probably limited by suitable resources unless those resources are acceptable parts of, or incidental to modern agricultural operations (e.g., *T. repens*). In particular, mobile species such as *Bassaris gonerilla* (New Zealand red admiral) and *B. itea* (yellow admiral) are limited by a lack of host plants (Chapter 2), and sedentary species such as *L. salustius* are limited by the absence of optimal nectar sources, relying instead on readily available but inferior nectar sources (Chapter 4), and possibly by inferior larval food plants (Chapter 5). Therefore the addition of these basic resources should be considered in future plantings if butterflies are to be targeted.

However, the literature abounds with evidence concerning the importance of other resources in the provision of habitats to butterflies (e.g., Hardy and Dennis 2007) and the resultant effects on biodiversity in general (Sparks and Parish 1995, Smallidge *et al.* 1996, van

Swaay and Warren 1999, Fleishman *et al.* 2000, van Swaay 2002). Butterfly species require varying resources due to different requirements between life stages and species (Waltz *et al.* 2004). Non-consumable resources such as shelter (e.g., Dover 1996), insolation (e.g., Clausen *et al.* 2001), roosting and basking structures (e.g., Dennis 2004) and structures to promote dispersal (e.g., Haddad and Baum 1999) have all been shown to be important to butterfly conservation. The present thesis has not investigated the importance of such resources, but as Hardy and Dennis (2007) advocated, in the absence of detailed species-specific data, high levels of landscape heterogeneity should be provided, including vegetative structures that allow butterflies to make the most of diurnal and seasonal variation in insolation and temperature. This suggestion receives support from many studies that show the importance of habitat heterogeneity to butterfly species richness and abundance (Erhardt 1985, Sparks *et al.* 1994, Pullin *et al.* 1995, Steffan-Dewenter and Tschardt 2002, Shepherd and DeBinski 2005, Kivinen *et al.* 2006, Nelson *et al.* 2008).

The negative effects of habitat fragmentation and isolation can be alleviated through habitat connection and much work has considered appropriate forms of corridors such as hedgerows (reviewed by Dover and Sparks 2000) that provide host plants, nectar sources and flyways between habitat patches. Conservation headlands, strips of land at field margins which are selectively sprayed with specific pesticides, also act in this way and are excellent sources of adult butterfly food and shelter (e.g., Dover 1991). Though these features were not included in the current study due to their absence, they are nevertheless suggested as potential ways to improve not only butterfly habitat provision but biodiversity in general (Rands 1986, Pullin 1996, Merckx *et al.* 2009) including beneficial insects (Bugg and Pickett 1998, Lovell and Sullivan 2006, Bianchi *et al.* 2006).

In addition to these factors, recent studies have demonstrated the importance of considering biodiversity enhancement at the landscape scale. Agri-environment schemes have not always been successful (Kleijn *et al.* 2006) but their success can be increased significantly if farms adopting such schemes are not isolated, but cover much of the landscape (Kivinen *et al.* 2007, Rundlof *et al.* 2008, Merckx *et al.* 2009). In Waipara, the situation is encouraging as over 50 vineyards and other properties are involved in the Greening Waipara project (Fiedler *et al.* 2008). However, plantings are isolated from remnants and each other, and significant improvements for butterflies and other organisms are likely to require landscape-scale consideration. The Greening Waipara project and studies involved in the project's assessment and improvement (Scarratt *et al.* 2004, this thesis, Tompkins 2009), have started to raise awareness of this importance. Further

studies in this vein are likely to help to encourage landowners to continue ecosystem enhancement work by demonstrating the resultant long-term economic benefits, and assist in convincing funders and policy makers of the importance of landscape scale improvements and associated monitoring and evaluation.

#### **6.4 Future research into enhancing butterfly populations in agricultural landscapes**

The population dynamics of *L. salustius* shown in Chapter 2 demonstrated the wide variation in phenology of this species when compared to the findings of Craw (1975). As Gibbs (1980) suggested, the comparison of phenology of species in a number of regions is important for understanding and establishing species' conservation status. The information for *L. boldenarum* and *Z. oxleyi* represents the first of such data in New Zealand and invites further research on these species. Data has not been collected on relationships between these species and ants because New Zealand lycaenid species are not thought to require such an association, which is common in other parts of the world, to complete their life cycle (Gibbs 1980). However, such a relationship remains a possibility and future research could address this.

While regular population monitoring of New Zealand species like that carried out in Europe (Pollard and Yates 1993, van Swaay 2002) and the USA (e.g., Swengel 1995) may be unrealistic, such studies as conducted in Chapter 2 provide the foundation for comparisons and the construction of trends in abundance. Additional work on a regular basis is therefore encouraged. Identifying finer scale species specific requirements is also vital in prescribing beneficial habitat improvements (Pullin 1996). The results of Chapter 2 identified broad requirements that were similar to those of European species. However, more detailed autecological work and bioassay work like that of Chapters 4 and 5 are suggested for all species of the New Zealand butterfly fauna.

The findings in Chapters 3-5 also have evolutionary significance. The taxonomy of *Z. oxleyi* was resolved for the Waipara region at least, and evidence for possible hybridisation was found. Regardless of the two scenarios presented in the discussion of Chapter 3, the evolutionary origin of the *Zizina* species in New Zealand is fascinating and the current study has highlighted the possibilities and insights to be gained from further study, which can also contribute to global understanding of butterfly evolution. Chapters 4 and 5 also raise evolutionary questions related to co-evolution of *L. salustius* and nectar sources and host plants that invite further research,



particularly using field studies to corroborate the findings and fine-tune the recommendations made.

Finally, research projects can be formulated with the assistance of vineyard owners to investigate a wide range of habitat management practices on many aspects of biodiversity, including whether the depauperate butterfly fauna in New Zealand can meaningfully be used as bio-indicator species. The impacts of the management techniques discussed above and in biodiversity-ecosystem function literature (e.g., Landis *et al.* 2000), require investigation on a number of scales, particularly given recent emphasis on the importance of landscape-scale adoption (Merckx *et al.* 2009). This, however, would require almost unprecedented collaboration between landowners at a landscape-scale (Fiedler *et al.* 2008). Payment for ecosystem services may be needed to achieve this (FAO 2007). The potential for these measures to complement each other with a range of outcomes also needs to be considered in future work (Fiedler *et al.* 2008). As different groups of species respond differently to measures aiming to enhance diversity (Dauber *et al.*, 2003; Jeanneret *et al.*, 2003), research should seek to establish the most appropriate ecosystem enhancements to develop balanced and beneficial assemblages of species that will deliver ecosystem services without requiring additional financial input. Ultimately, linking these enhancements to meaningful and economic benefits to landowners using evidence based research is likely to be vital in increasing landowner adoption of both PES and other non-financial projects.

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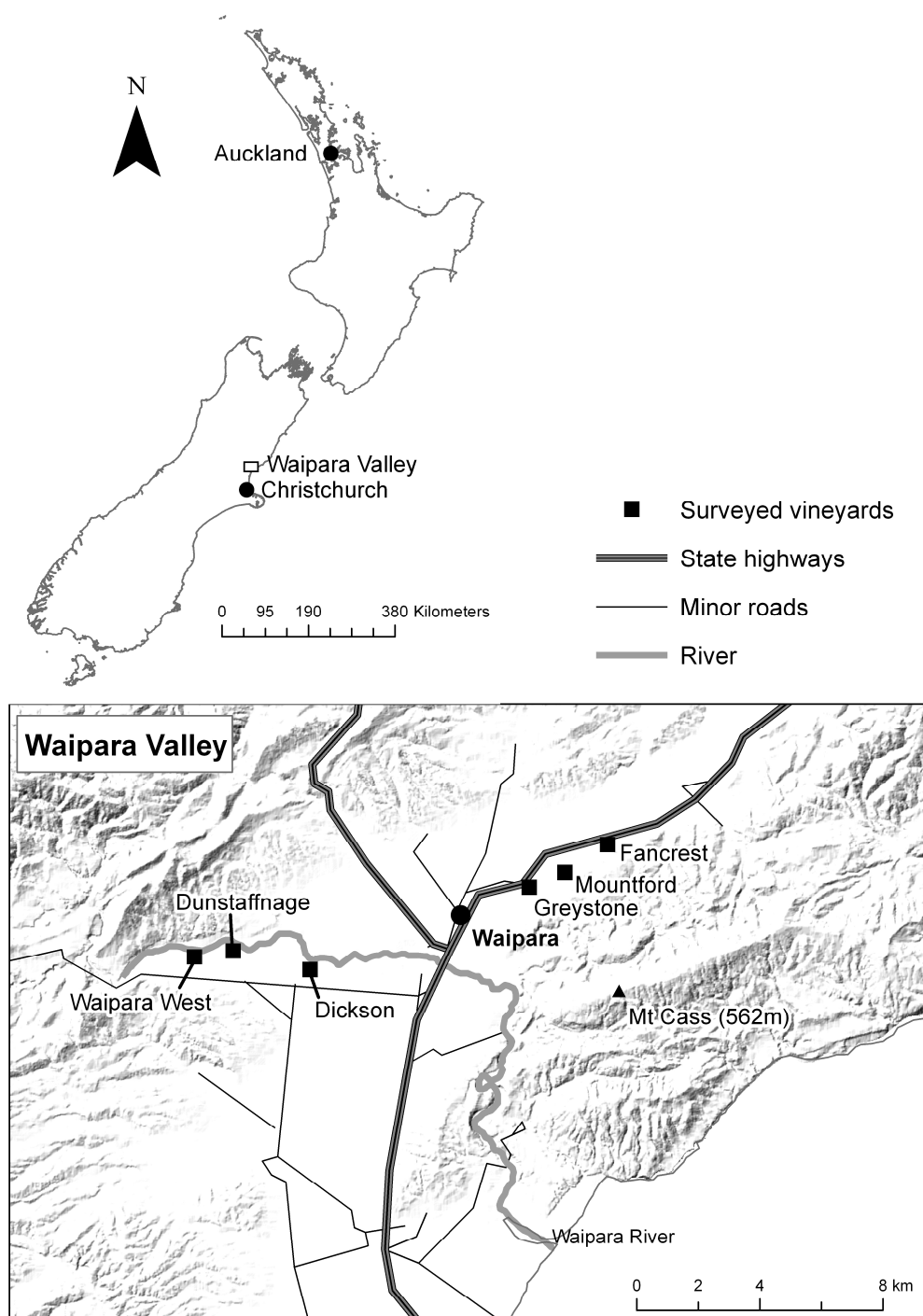
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## APPENDIX 1      Location of transects for butterfly surveys in Waipara



**Figure A1:** Location of the Waipara Valley within New Zealand (top) and the vineyards used for butterfly surveys in the Waipara valley (bottom).

**Table A1:** Breakdown of transect section types, length and GPS co-ordinates for each vineyard surveyed in Waipara. Section type classifications are those specified in Table 2.1. The GPS co-ordinates mark the beginning of each section and are given in World Geodetic System 1984 decimal degrees. Sections in bold are those removed due to zero counts of all butterflies.

Vineyard	Section Number	Section type	Section length (m)	GPS co-ordinates (decimal degrees)	
				East	North
Dickson	<b>1</b>	<b>pasture</b>	<b>180</b>	<b>172.7007</b>	<b>-43.0735</b>
	2	margin	100	172.6998	-43.0724
	3	track	240	172.6996	-43.0713
	4	remnant	35	172.6996	-43.0703
	5	pasture	100	172.6998	-43.0694
	6	river	220	172.6996	-43.0686
	<b>7</b>	<b>pasture</b>	<b>100</b>	<b>172.6983</b>	<b>-43.0688</b>
	8	remnant	490	172.6956	-43.0698
	9	remnant	200	172.6978	-43.0695
	10	track	130	172.6993	-43.0695
	11	vinerows	385	172.7006	-43.0715
	12	planting	10	172.7008	-43.0707
Dunstaffnage	1	margin	370	172.6701	-43.0650
	2	remnant	80	172.6711	-43.0654
	3	pasture	90	172.6716	-43.0651
	4	river	460	172.6707	-43.0640
	5	pasture	130	172.6686	-43.0632
	6	remnant	380	172.6687	-43.0643
	7	vinerows	230	172.6710	-43.0667
	8	margin	250	172.6696	-43.0677
	9	margin	470	172.6671	-43.0656
	<b>10</b>	<b>planting</b>	<b>10</b>	<b>172.6743</b>	<b>-43.0705</b>
Waipara	1	margin	150	172.6546	-43.0672
West	2	pasture	30	172.6551	-43.0666
	3	pasture	240	172.6565	-43.0664
	4	track	60	172.6567	-43.0671
	5	vinerows	170	172.6569	-43.0680
	6	margin	100	172.6576	-43.0687
	7	remnant	550	172.6558	-43.0690
	8	track	160	172.6536	-43.0673
	<b>9</b>	<b>pin</b>	<b>100</b>	<b>172.6534</b>	<b>-43.0666</b>

				GPS co-ordinates (decimal degrees)	
Vineyard	Section Number	Section type	Section length (m)	East	North
Waipara West (contd)	10	track	100	172.6530	-43.0662
	11	pasture	160	172.6511	-43.0652
	12	river	215	172.6491	-43.0657
	13	margin	180	172.6500	-43.0659
	14	planting	20	172.6448	-43.0675
Fancrest	1	margin	180	172.8195	-43.0372
	2	remnant	380	172.8181	-43.0385
	3	pasture	160	172.8162	-43.0379
	4	planting	80	172.8187	-43.0379
	5	vinerows	140	172.8191	-43.0378
	6	margin	90	172.8178	-43.0371
	7	vinerows	180	172.8176	-43.0368
	8	margin	70	172.8166	-43.0355
	9	track	220	172.8175	-43.0363
Mountford	1	track	650	172.8024	-43.0453
	2	pasture	290	172.8080	-43.0473
	3	remnant	260	172.8100	-43.0480
	4	remnant	200	172.8095	-43.0476
	5	pasture	50	172.8094	-43.0471
	6	margin	120	172.8095	-43.0467
	7	vinerows	130	172.8086	-43.0455
	8	margin	100	172.8078	-43.0442
	9	pasture	450	172.8062	-43.0424
	10	pasture	600	172.8012	-43.0428
	11	planting	200	172.7974	-43.0431
Greystone	1	planting	70	172.7882	-43.0496
	2	track	260	172.7881	-43.0503
	3	pasture	300	172.7906	-43.0514
	4	pasture	200	172.7913	-43.0535
	5	track	420	172.7914	-43.0545
	<b>6</b>	<b>track</b>	<b>20</b>	<b>172.7933</b>	<b>-43.0539</b>
	7	pasture	340	172.7938	-43.0551
	8	vinerows	160	172.7926	-43.0568
	9	margin	70	172.7931	-43.0574
	10	remnant	780	172.7948	-43.0600