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Plant-herbivore interactions across an alpine meadow gradient

by

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Abstract

The Rocky Mountain apollo butterfly, *Parnassius smintheus*, and its host-plant *Sedum lanceolatum*, are endemic to open alpine meadows threatened by the encroachment of trees. I explore variability in interactions between *P. smintheus* and *S. lanceolatum* relative to the treeline-delimited meadow edge, and consider the consequences of continued tree encroachment for these and other species facing similar threats. First, I demonstrate that *S. lanceolatum* distribution and quality vary relative to the meadow edge, with plants near the treeline being both more abundant and more nutritious than those elsewhere in the meadow. Next, I show that this variation influences both oviposition and larval feeding by *P. smintheus* in unexpected ways: females actively oviposit in response to both the abundance and quality of hosts yet show no strong attraction to the meadow edge, while the spatial patterns of host-plants and herbivory upon those host-plants is decoupled (i.e., not ideal) only near the treeline, despite the abundance and apparent suitability of hosts there. I also show that, because larval *P. smintheus* can actively respond to the distribution of their hosts, the spatial pattern of herbivory is likely the product of choice, not chance. Finally, I explore how previous stress, including *P. smintheus* herbivory and flowering history, affect the growth of *S. lanceolatum* relative to the treeline, showing that while flowering is more stressful to *S. lanceolatum* overall than herbivory, herbivory may lead to compensatory growth away from the treeline. I conclude that *P. smintheus*-*S. lanceolatum* interactions vary spatially, that abundant host-plant resources near the meadow edge may in fact not be

available to larvae, and that the extent of actually usable larval habitat may therefore differ from that of apparently suitable habitat. Overall, I propose that a synthetic assessment of habitat for both adults and larvae will give a clearer sense of likely butterfly responses to environmental change and, consequently, aid conservation of Lepidoptera.

Acknowledgements

Soon after I arrived in Edmonton, fresh from a Master's degree in Japanese politics and media studies, I found myself trudging through the winter streetscape of snow and sand to an introductory course in general ecology that was mandatory for all incoming students. Were there any general laws in ecology? Single large or several small? And what was this business about hypervolumes? I had no clue. Studying ecology was an abstract idea, an impulse born of a rural childhood on Vancouver Island and the enjoyment of one or two undergrad courses, but soon it began to take shape. This thesis charts that evolution, from those early days to now, and the appreciation of a discipline made real both intellectually, in experience and study, and corporeally, in the ecologists I've learned from, lived with, and admired. It has been a long, sometimes unscientific process, with many digressions but also many rewards, and for that I owe thanks to many people:

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Chapter 1

Review and synthesis: toward the inclusion of larval resources in butterfly population ecology

1.1 Introduction

The persistence of animal populations in fragmented habitat depends on the potential for individual movement among subpopulations (e.g. Taylor *et al.*, 1993), variation in population dynamics between differently sized patches (e.g. Turchin, 1998; Roland and Matter, 2007), and species-specific habitat requirements that affect emigration from a habitat patch (e.g. Fagan *et al.*, 1999; Ovaskainen and Hanski, 2004). Numerous empirical studies have supported the early predictions of MacArthur and Wilson (1967) that populations in fragmented landscapes suffer an increased risk of local extinction (e.g. Didham *et al.*, 1998; Debinski and Holt, 2000), and much research has addressed the role of habitat edge in mediating dispersal (e.g. Fagan *et al.*, 1999; Schultz and Crone, 2001; Stasek *et al.*, 2008; Ries and Sisk, 2010). Butterflies show promise as indicators of environmental change (Thomas, 2005) and are a popular model organism for studies of dispersal (reviewed in Stevens *et al.*, 2010), especially in the context of climate change and habitat alteration, because of their tractability, well known natural history, and charisma. Many butterfly species are habitat specialists, making it relatively easy to discern their preferred habitat even within a highly heterogeneous landscape (Baguette and Mennechez, 2004), and increasing their sensitivity to habitat edges (Ries and Debinski, 2001; Krauss *et al.*, 2003*a*,*b*; though see Ries and Sisk, 2010). Strong edge avoidance also makes them particularly sensitive to habitat fragmentation (e.g. Ries and Debinski, 2001; Ross *et al.*, 2005*b*; Dover and Settele, 2009).

The importance of host-plant quality and spatial distribution to patterns of butterfly patch occupancy and abundance are well established (e.g. Moilanen and Hanski, 1998; Brommer and Fred, 1999; Haddad and Baum, 1999; Fownes and Roland, 2002; Matter *et al.*, 2003, 2004), as is the necessity of sufficient nectar flower abundance (Matter *et al.*, 2004). Likewise, emigration is more likely where the border between a patch and the surrounding matrix is diffuse or "soft," as compared to abrupt or "hard" edges that preclude movement (e.g. Stamps *et al.*, 1987; Roland *et al.*, 2000; Ricketts, 2001; Berggren *et al.*, 2002; Schooley and Wiens, 2004). Patch shape can also alter the relationship between edge habitat and interior habitat within a patch (summarized in Dover and Settele, 2009). However, habitat patch size and isolation remain the most fundamental metrics for predicting population persistence and dispersal, respectively, for butterflies (Stasek *et al.*, 2008; Dover and Settele, 2009). In this regard, the study of butterfly populations in fragmented landscapes draws on much the same theoretical background as similar studies of other vagile animals.

But there is an important distinction between butterflies and other animals, implicitly accepted but seldom explicitly considered in studies of butterflies in shrinking or fragmented habitat: butterflies have not one but two free-living and mobile life stages, and larvae can differ greatly from adults in their host preferences, habitat requirements, and dispersal ability. A patch's ability to retain and support butterfly larvae—the essential precondition of adult emergence—is as important to its long term occupancy as its ability to retain and support adults *per se*. Indicators of habitat quality may be very different for larvae vs. adult butterflies, and in any case the processes affecting the interaction of larvae or adults with their environment may differ markedly in both nature and scale. There is already evidence that habitat area alone is a poor indicator of likely population persistence, and that close attention must be paid to how populations use their habitat (Shoo *et al.*, 2005). There is also, as I show in Chapter 4 of this thesis, evidence that "effective" habitat area for larvae may be much smaller than "apparent" habitat or adult habitat (Illerbrun and Roland, 2011). These issues have not been integrated.

Here, I highlight ways in which the conceptions of habitat quality and especially habitat edge, as considered in most studies of butterfly response to fragmented habitat, may be restrictive for butterfly systems in light of the larva-adult dichotomy. I assume that larval survival in a patch is a prerequisite of ongoing butterfly persistence in that patch, although I acknowledge that, in some circumstances, such as where immigration from a suitable source population is high, this need not be so. I further assume that in most cases larvae are less mobile than are adult butterflies, and thus more constrained by the conditions of their local environment.

In considering the differences between larva- and adult-centric conceptions of habitat, I acknowledge the considerable body of work addressing butterfly habitat use from an adult perspective, but submit that while this perspective is often sufficient, there may be an additional, less told side to the story. My goal in this chapter is to review and highlight specific ways in which adults and larvae may be affected differently by the characteristics of the patches they inhabit, and speculate upon potential consequences for understanding how butterflies interact with their habitat. In doing so, I first note that habitat edges are most commonly transitional ecotones between habitat and matrix, and that the unique ecology of ecotones may have important implications for larval interaction with their host plants. Second, I argue that both abiotic and biotic factors near habitat edges may decouple effective larval habitat from apparent habitat. In both cases the consequence would be wrongly estimating available larval habitat area. Finally, I propose that a synthetic assessment of habitat for both life stages may provide a clearer sense of the likely responses of butterflies to environmental change and, consequently, give a clearer road map for conservation. I focus primarily on temperate systems where open butterfly habitat is bordered by trees, but argue that the issues I highlight are more broadly relevant.

1.2 Butterflies and butterfly larvae

1.2.1 Larvae and adult butterflies interact differently with their environment

Adult butterflies can mitigate the effects of unfavourable circumstance through emigration, but this response is unavailable to larvae at anything but the most local scale. Because larvae cannot easily emigrate from broadly unfavourable habitat, they are more likely than adults to be constrained by the conditions inherent to the patch they occupy. For example, although most butterflies require nectar flowers to survive, and flower abundance in a given habitat patch therefore mediates their persistence, this requirement is moot if they successfully emigrate to another patch with abundant flowers. Likewise, if population density within a patch becomes too high, an adult butterfly may emigrate to a more favourable patch, while a larva cannot. And while strong edge effects may lead to increased emigration by adult butterflies, larvae must contend directly with these effects. Habitat attributes like flower abundance and edge permeability are important partial determinants of butterfly persistence, but adults nearly always retain some degree of self determination, and may be able to search among several potentially suitable patches. Likewise, larval survival is mediated by, among other things, host plant availability and quality (discussed in Chapters 2 and 4)—but unlike adults, larvae cannot usually move long distances in search of better habitat. Thus habitat attributes are the unavoidable arbiters of larval survival, whereas for adults they are merely the arbiters of persistence within a given patch, but not necessarily of overall survival.

The implications of this for larval interactions with their habitat should not be underestimated.

More generally, butterfly larvae differ from adults in their food requirements, environmental tolerances, and “perspective”—larvae are typically small, close to the ground, and interact with their environment only at an extremely local scale, while adults are more likely to fly over and interact with much larger portions of their environment. Larvae, if they show active host orientation at all, generally rely on chemical cues which are easily confounded by fine-scale local topography and micro-weather (Jermy *et al.*, 1988; Schoonhoven *et al.*, 2005; discussed further in Chapter 5). Adult butterflies, however, often have well-developed eyesight (Scherer and Kolb, 1987; Bernard and Remington, 1991; Kinoshita *et al.*, 1999). Many studies have examined habitat area and quality only from the perspective of adult butterflies, and have consistently shown that butterfly species avoid or otherwise respond to the edges that define the limits of their habitat (Ries and Debinski, 2001; Krauss *et al.*, 2003*a,b*; Ross *et al.*, 2005*b*; Dover and Settele, 2009; discussed further in Chapter 3).

1.2.2 Patch attributes and their importance for butterflies and larvae

Butterflies are particularly useful model organisms for studying dispersal (Stevens *et al.*, 2010), and many studies have examined butterfly movement through, and persistence in, fragmented, patchy landscapes consisting of suitable habitat islands separated by unsuitable matrix. Movement between, and persistence within, patches may be governed by a suite of factors, including patch size, isolation (absolute distance) from other patches, population density within a patch, the nature of the between-patch matrix, patch quality, and patch shape and edge characteristics. Larval and adult butterfly responses to these attributes may differ, but the nature of these differences reveals some consistent patterns.

Some patch attributes affect adults, but have little relevance to butterfly larvae. For example, if a habitat patch is isolated from other patches, it is less likely to receive immigrants, and its population may therefore be more vulnerable to extirpation (MacArthur and Wilson, 1967; Hanski, 1998; Fahrig, 2003). The negative consequences of isolation may be particularly severe for small patches. But though isolation affects the likelihood of adult butterfly dispersal, patch isolation *per se* would likely have no effect on larval interaction with their habitat, because larvae cannot generally respond at a scale where distant alternative habitat patches are relevant. Likewise, the nature of the matrix between habitat patches is of little consequence to larvae, but may significantly affect the rate of successful dispersal by adults (Ricketts, 2001).

Other patch attributes affect both adult butterflies and larvae in similar ways, but with varying consequences. For example, all else being equal, larger patches are likely to contain more total resources and thus support larger populations that are less vulnerable to extirpation (Hanski, 1998; Fahrig, 2003). Assuming resources (nectar flowers and larval host plants) are evenly distributed across the patch, patch size should have a similar effect for both adult and larval butterflies, and support larger populations of each. However, in the more likely case that resources are patchily distributed even within the habitat patch (Watt, 1947), adult butterflies would be better equipped to move and exploit the full benefits of larger patch size. Larvae may instead be constrained by the size of resource patches within the broader habitat patch; that is, they are constrained by patch attributes at a much smaller scale than are adults. Population density could also affect both adults and larvae similarly, with higher densities leading to greater competition for limited resources; however, adults may emigrate from high-density patches before oviposition occurs, lowering larval density (Dethier and MacArthur, 1964; Baguette *et al.*, 1998; but see e.g Kuussaari *et al.* (1998) for an example of an Allee effect on butterfly population density). And the determinants of habitat quality, such as host plant abundance, may be similar for both life stages, though adult butterflies usually respond to additional determinants of quality such as nectar flower abundance (Thomas and Singer, 1987; Odendaal *et al.*, 1989; Schultz, 1998; Fownes and Roland, 2002; Matter *et al.*, 2003; discussed further in Chapter 3).

Finally, some patch attributes may impact both adult butterflies and larvae for very different reasons. For example, edge effects are of interest in assessing butterfly movement among patches, because the characteristics of a patch's edge can affect adult emigration (Haddad, 1999*b*; Cadenasso and Pickett, 2001; Ries and Debinski, 2001; Schultz and Crone, 2001; Schtickzelle and Baguette, 2003; Schtickzelle *et al.*, 2006), while the shape of the patch—and by extension, the spatial arrangement and extent of its edge—affects the frequency with which butterflies encounter edge habitat in the first place. Because larvae cannot disperse at the scale of adult butterflies it may be easy to ignore, as with patch isolation and matrix quality, the implications of habitat edge for butterfly larvae. I make the case, generally in this chapter, and with specific examples in the subsequent chapters, that this is unwise.

Overall, it is clear that patch attributes such as size and isolation—cornerstones of concepts of dispersal and population persistence such as island biogeography (MacArthur and Wilson, 1967) or metapopulation theory (Hanski, 1998)—are of relatively little significance to butterfly larvae. Perhaps this explains why studies of butterfly persistence in a fragmented landscape tend to focus only on adults. However, many studies now incorporate a landscape ecology perspective, where other elements, such as patch quality and edge characteristics, play a role in governing persistence and movement. For example, many studies show that adult butterflies are more likely to emigrate from habitat with an insufficient supply of larval host plants (Thomas and Singer, 1987; Odendaal *et al.*, 1989; Schultz, 1998; Fownes and Roland, 2002), but do not consider the extent to which those host plants are actually used by larvae. Likewise habitat edge is often considered in the context of butterfly emigration (that is, its role in promoting or arresting butterfly dispersal), but the implications of habitat edge for organisms that remain within the patch, such as larvae, are ignored. I argue, here and in subsequent chapters, that edge effects may be equally important to larval persistence, and may in fact mediate other patch attributes such as patch quality.

1.2.3 Two perspectives on habitat edge

From the adult perspective, habitat may be delimited by vertical barriers such as trees (Roland *et al.*, 2000; Jonsen *et al.*, 2001; Ricketts, 2001; Schooley and Wiens, 2004), by unfavourable light (Ricketts, 2001; Ross *et al.*, 2005*b*) or temperature zones (Ricketts, 2001), and by anthropogenic barriers like cropland and motorways (Ries and Debinski, 2001). The distributions of host plants and nectar flowers are important determinants of holistic habitat quality (Schultz and Crone, 2001; Fownes and Roland, 2002; Matter *et al.*, 2004)—hosts and nectar sources must reach some threshold abundance, but their precise distribution appears comparatively unimportant because butterflies can move to particularly favourable areas within a broader region.

By contrast, a larval definition of habitat is delimited by many of the same coarse-scale boundaries, but adds some additional subtleties. For example, while adults favouring open habitat may avoid shady treed edges, larvae contend with the fine scale shade of individual plants, which may be highly variable across their habitat. Similarly, local variation in host plant quality may affect larval foraging far more than would host abundance across the entire habitat. Thus, larvae may be essentially indifferent to habitat edges *per se*, but sensitive to the variations in host plant quality, microclimate, community interactions, and so on, induced by the unique characteristics of edge habitat. Further, considerable research on the effect of edge permeability on the emigration rate of butterflies acknowledges that edges come in many forms (Cadenasso and Pickett, 2001; Haddad, 1999*b*; Ries *et al.*, 2004). Since larvae are typically much less mobile than adults, emigration is of little concern. But where habitat edges are less stark barriers than gradual transitional zones between habitat and matrix, the unique ecology of ecotones may affect larval habitat quality and extent in unexpected ways.

1.3 How edges affect butterfly larvae

While it is well known that a given habitat edge may be more or less permeable to butterflies, there remains the question of where “habitat” ends and “matrix” begins. As indicated above, habitat edge characteristics are variable, and these characteristics can influence butterfly behaviour, including emigration rate. But by focussing on edge permeability and on the starkly binary question of whether a butterfly will remain within or emigrate from a habitat patch, subtler aspects of habitat edge are ignored. Though a case could be made that these aspects may be of little consequence to adult butterflies, the same may not be true for larvae. I outline a larval perspective on habitat edge below.

1.3.1 Edges as ecotones

Although it is convenient to imagine habitat fragments as discrete islands surrounded by a clearly defined matrix, reality is seldom so simple. Habitat edge is rarely a stark boundary. Instead, it more often represents a transition zone between habitat and matrix. In this view, the hardness or softness of an edge is defined by the width of the transitional zone, or ecotone—a wider ecotone means a softer boundary and more transitional habitat. Studies of butterfly movement acknowledge the role of edge attributes when they consider the permeability of an edge with respect to butterfly movement, and many have shown that butterflies respond differently depending on edge characteristics (Stamps *et al.*, 1987; Roland *et al.*, 2000; Ricketts, 2001; Berggren *et al.*, 2002; Schooley and Wiens, 2004). When focussing on butterfly movement, the specific attributes of the edge may be less important than their cumulative effects on butterfly behaviour, and a black-and-white conception of habitat—a butterfly may be either in, or not in, a habitat patch—is appropriate. However, near-edge ecotones may project some distance on either side of the effective edge of a habitat patch.

1.3.2 Abiotic processes acting at the habitat edge

Irrespective of the size of the transitional zone between habitat and matrix, habitat edges are subject to unique micro-environmental conditions that may impact organisms either directly or indirectly. These are generally similar for both butterflies and larvae. Light penetration may vary with overstory cover across the ecotone (Turton and Sexton, 1996; Didham and Lawton, 1999; Dignan and Bren, 2003), concomitantly altering temperature and humidity levels near the edge. Wind patterns may also be affected, especially where open habitat grades into forest (reviewed e.g. in Saunders *et al.*, 1991). In cooler climates where butterfly habitat is bordered by trees, the transitional ecotone may also see deeper over-winter snow accumulation (Walsh *et al.*, 1994), which may effectively prevent access to the ecotone habitat. While these factors may serve to alter both adult butterfly and larval behaviour near edges, their effects on the near-edge biotic community may be especially relevant to larvae.

1.3.3 Biotic processes acting at the habitat edge

One commonly studied aspect of plant-insect herbivore interactions is the role of host plant quality in altering herbivore performance (e.g. Awmack and Leather, 2002; De Bruyn *et al.*, 2002) and distribution in space (e.g. Cates, 1980; Zangerl and Berenbaum, 1993; Loxdale and Lushai, 1999;

discussed further in Chapter 4). And it goes without saying that larvae cannot survive without access to a sufficient supply of their larval host plant(s). Many of the abiotic factors acting at the edge of habitats can affect host plant quality: for example, temperature (Ellis *et al.*, 1977; Richards and Fletcher, 2002; Olsen and Ungerer, 2008) and light (Larsson *et al.*, 1986; Dudt and Shure, 1994) both affect the production of secondary defensive compounds in some plants.

Moreover, since ecotones typically contain taxa from both sides of the transitional zone, they may be home to greater species diversity than the habitats they bisect—the "ecotone effect" (Brown and Kodric-Brown, 1977; Shmida and Wilson, 1985; Stevens, 1992; Schilthuizen, 2000; Lomolino, 2001). Transitional ecotones may display increased plant species richness and diversity, greater heterogeneity in the spatial distribution of plants, and abrupt local-scale changes in plant community composition (Gottfried *et al.*, 1998; Oommen and Shanker, 2005; Camarero *et al.*, 2006; Batllori *et al.*, 2009*a*; but see e.g. Walker *et al.*, 2003 for a dissenting example).

Beginning with the work of Huffaker (1958) and Elton (1958), numerous studies have also shown that heterogeneous plant communities are generally more resistant to specialist herbivores than are simple or uniform communities, and therefore more stable. Feeny (1976) proposed that extremely visible plants need more costly defenses against a broader range of herbivores than unapparent plants, and more recent theories continue to postulate mechanisms by which plants in a heterogeneous community may experience altered herbivory. Others have demonstrated that some plants may derive associational resistance from neighbouring plants (Hamback *et al.*, 2000; Callaway *et al.*, 2005), while Agrawal (2004) proposed the reverse case of associational susceptibility. Still others propose that diverse plant communities complicate and confound herbivore foraging decisions (Bernays, 2001), or that competition with nearby plants may change the costs and benefits of defending against herbivory (Herms and Mattson, 1992; Tiffin, 2002). Agrawal *et al.* (2006) provides a comprehensive review of how plant community heterogeneity may impact herbivores. Though studies give conflicting evidence for whether edge effects favour plants or herbivores (Ries *et al.*, 2004), it seems clear that near-edge habitat can mediate many plant-herbivore interactions by altering the abundance, apparency, and quality of the host plant.

Further, herbivores' growth and metabolism—although I consider only butterfly larvae here, the principle applies to other herbivores as well—are often limited by N availability, and thus may be strongly influenced by the N content of the plants they consume (Mattson, 1980; Scriber and Slansky, 1981). Particularly in alpine systems N is often limited (Bowman *et al.*, 1993), and may be especially limited where spring snow melt exports nutrients to lower elevations (Brooks *et al.*, 1998; Hood *et al.*, 2003). Areas with deeper snow, such as those near the alpine treeline (Walsh *et al.*, 1994), may suffer reduced N loss because N-retaining soil microbes are better insulated against freezing (Brooks *et al.*, 1997, 1998; Brooks and Williams, 1999; Hood *et al.*, 2003; Schimel *et al.*, 2004; Freppaz *et al.*, 2008; Liptzin and Seastedt, 2009), and as a consequence the alpine treeline ecotone shows considerable variation in nutrient availability (Stevens and Fox, 1991; Körner, 1998), potentially altering the palatability of host plants growing in this region. Snow cover may also directly alter plant defensive chemistry (Ralphs *et al.*, 2002).

Finally, although I have focussed mainly on consequences for herbivores, herbivory has its own effects on plants. For example, it can affect plant population dynamics (Crawley, 1990), alter community structure (Huntly, 1991; Olff and Ritchie, 1998) and, though negative for many plants, moderate levels of herbivory may instead increase plant growth in some species (e.g. Paige and Whitham, 1987; Roland and Myers, 1987; Belsky *et al.*, 1993). Given the factors affecting plant-herbivore interactions near the habitat edge, it is not surprising that both partners would be uniquely affected (Wirth *et al.*, 2008). I consider this issue further in Chapter 6.

1.4 Implications

A heterogeneous plant community, such as is frequently found in transitional ecotones, can significantly alter the relationship of herbivores with their host plants. For butterfly larvae, many of which are monophagous herbivores on a single species or genus, the implications of this are important. Ecotone effects, where present, may vary greatly in scale (Gosz, 1993), and the precise location of the respective habitat boundaries for larvae and adults may differ along the transitional gradient. That is, ecotone effects may be projected some distance beyond the apparent edge of habitat, and so the effective edge of usable habitat may be different from the perspective of larvae and adults. If, for example, host plants within the ecotone are less numerous, less apparent, or less palatable to larvae, then the quality and extent of larval habitat may be considerably more restricted than they would first appear. Where patch size is already small, this effect, like other edge effects, would be especially pronounced. Clearly, the consequences of significantly overestimating available larval habitat could be severe. Conversely, if hosts within the ecotone are instead more susceptible to larval herbivory, then effective larval habitat might exceed first impressions. Perhaps most importantly, both cases would provide a markedly different estimate of available habitat size and quality than when considering adults only.

I noted previously that studies of butterfly populations in fragmented landscapes draw on essentially the same theoretical background as similar studies of other mobile species, and that habitat patch size is considered fundamental to predicting butterfly population persistence and dispersal. (Stasek *et al.*, 2008; Dover and Settele, 2009). Meanwhile many studies demonstrate the importance of abundant larval and adult resources for butterfly conservation (e.g. New *et al.*, 1995; Schultz and Dlugosch, 1999), and others assert that the patch vs. matrix definition of habitat is in fact inappropriate for butterflies, and that more attention should be paid to the details of butterfly behaviour and resource use (Dennis *et al.*, 2006). This sentiment is at the heart of my assertion that the prevailing conception of habitat edge, based as it is in the broader framework of patch-based definitions of habitat, is not a sufficient definition in light of the adult-larva dichotomy in butterflies. Butterfly movement *per se* and the persistence of a butterfly population on a landscape are not the same—persistence requires that both adults and larvae survive in sufficient numbers, and this requires resources that are not only sufficient in number, but also apparent, accessible, and palatable. A more detailed assessment of ecotone effects near habitat edges would provide a better sense of the true nature and extent of those resources.

The relative importance of larval vs. adult habitat quality/area to persistence varies species by species. Regardless of relative importance, the respective habitat requirements of each life stage may be sufficiently different that they bear independent assessment, and may lead to divergent conclusions about the viability of the population. The projection of ecotone-induced edge effects into larval habitat patches may mediate larval interactions with their host-plants in ways undetected by the assessment of host abundance alone. Consequently, studies of butterfly response to habitat variables, particularly in fragmented habitat where edges are more numerous, should also consider as many factors potentially affecting larval habitat as possible. When assessing patch size and viability, I suggest that it would be beneficial to consider “effective” habitat quality and size for both larvae and adults, erring on the side of whichever is more limited.

1.5 The *Parnassius smintheus-Sedum lanceolatum* system

Numerous studies report changes in the growth and distribution of high-altitude and high-latitude forests due to global warming (e.g. Luckman and Kavanagh, 2000; Lloyd and Fastie, 2003; Moen *et al.*, 2004; Danby and Hik, 2007*b*; Wieser *et al.*, 2009; Harsch *et al.*, 2009). Although tree distribution may be governed by a suite of factors, elevated temperatures contribute to raising the treeline—the ecotone where trees give way to shrubs and shorter vegetation—by allowing seedling establishment and persistence in previously inhospitable areas (Körner, 1998; Grace *et al.*, 2002; Wieser *et al.*, 2009). Higher temperatures may also coincide with disruption of natural fire regimes (Grabherr *et al.*, 1994; Luckman and Kavanagh, 2000), changing the frequency with which treeline location is reset. Rising treeline is particularly important to alpine meadow ecosystems, which are usually surrounded by trees. As treeline advances, both size and connectedness of meadows decline (Grabherr *et al.*, 1994; Dirnböck *et al.*, 2003; Fagre *et al.*, 2003; Millar *et al.*, 2004; Parmesan, 2006). Several studies have examined the direct effects of treeline rise on the movement and population dynamics of the alpine meadow dwelling Rocky Mountain Apollo butterfly, *Parnassius smintheus* Doubleday (Lepidoptera: Papilionidae). Roland *et al.* (2000) showed that forest encroachment—causing meadow shrinkage of more than 75%—impedes butterfly movement, reducing connectivity between neighbouring populations, and isolating previously contiguous populations (Roland and Matter, 2007). However, the full range of mechanisms by which rising treeline may affect alpine meadow species is not yet understood, and as discussed above, could include less direct effects. One question is how treeline—its physical proximity, and by extension the consequences of continued encroachment—might alter host-plant-herbivore interactions, and what implications this might have for species facing habitat shrinkage generally.

Working with *P. smintheus* larvae and their host plant, *Sedum lanceolatum* Torr. (Crassulaceae) in the alpine meadows of Jumpingpound Ridge, in Kananaskis Country, Alberta, Canada (50°57'N, 114°55'W), a series of meadows used for the long-term study of *P. smintheus* dynamics (Roland *et al.*, 2000; Keyghobadi *et al.*, 2005*a*; Roland and Matter, 2007) outlined above, this thesis explores precisely this issue. I briefly describe both *P.*

smintheus and *S. lanceolatum* below, before summarizing my objectives in depth.

1.6 Study species

1.6.1 *Parnassius smintheus*

Parnassius smintheus is found from the southern Yukon to New Mexico in well-drained subalpine and alpine meadows and on mountain slopes (Bird *et al.*, 1995; Opler, 1999), although a small, disjunct population also occurs in the Cypress Hills of Saskatchewan (Layberry *et al.*, 1998). A relatively large butterfly at 4.5-6.5 cm in wingspan, males are creamy white with black and scarlet markings, while females are a translucent pewter colour with larger black and scarlet markings than males (Fig. 1.1). The species is univoltine, with peak flight activity from early July to late August, depending on year and location, and adults nectar on a variety of flowers, especially composites (Bird *et al.*, 1995). Eggs are off-white and rounded, but somewhat flattened on both the top and bottom, and have a finely ridged surface. Eggs are laid throughout the flight period, and larvae develop through the fall as pharate first instars before overwintering and hatching soon after spring snow melt. First instars are tiny, black, and covered in short hairs, but develop rows of bright yellow and apparently aposematic dorsal spots as they mature (Guppy and Shepard, 2001), eventually reaching a 5th instar length of around 3cm. The species feeds on a variety of *Sedum* species throughout its range. Larvae pupate from May to late June for a period of approximately 3 weeks, constructing a loose cocoon from particulate material on the ground (Layberry *et al.*,

Figure 1.1: *Parnassius smintheus* adults mating (L), and the ultimate result, a 5th instar larva feeding on *Sedum lanceolatum* (R). In the left pane, the darker, more translucent female is on the left, while the whiter male is on the right. (*Larva photo*: Dave Roth)

1998). Adult males patrol their habitat in search of females and may mate with them even before they are able to fly (Matter *et al.*, 2012), depositing a waxy “mating plug” or sphragis on the female’s abdomen to prevent further mating (Layberry *et al.*, 1998; Matter *et al.*, 2012). Female *P. smintheus* fly less and more furtively than do males, possibly, in part, to avoid unwanted attention from the opposite sex (Matter *et al.*, 2012), and oviposit singly away from the host plant (Scott, 1986). Because of this behaviour, which requires that neonate larvae navigate to a suitable host before feeding can commence, they have been referred to as “haphazard” egg layers (Scott, 1986).

Parnassius smintheus is common in alpine meadows throughout the Rockies, but is restricted to those habitats with a sufficient supply of its larval host plant, *S. lanceolatum* (Fownes and Roland, 2002), and nectar flowers such as *Arnica* spp., *Senecio* spp., *Aster* spp., and other large yellow or yellow-centered composites. *Parnassius smintheus’* life cycle varies along the species' north-south range; on Jumpingpound Ridge adults fly from mid-July until late August and occasionally into September (Guppy and Shepard, 2001), during which time eggs are laid near, but not on, *S. lanceolatum* (Fownes and Roland, 2002). Female *P. smintheus* typically alight on the ground prior to oviposition, and may walk several centimeters before selecting a suitable site and laying a single egg on the underside of vegetation. Eggs hatch after spring snow melt, usually in late April or early May, and larvae feed continuously before pupating in mid- to late June. Although *P. smintheus* larvae feed on several related host species throughout their range (Guppy and Shepard, 2001), on Jumpingpound Ridge they feed almost exclusively on *S. lanceolatum* and only rarely on the less common ledge stonecrop, *Sedum integrifolium* (Roslin *et al.*, 2008).

As with other Apollo butterflies (e.g. *P. apollo, P. phoebus*), *P. smintheus*' species epithet is somewhat tautological. Apollo butterflies are named for the multitalented Greek god of light, healing, truth, music, and myriad other things, and Apollo Smintheus means either Apollo from Sminthe, a city where Apollo was worshipped in pre-Helenic times, or Apollo the mouse god, from the Aeolian "Smintha" for "mouse". Either way, smintheus is simply another name for the god himself, but the latter interpretation is appealing: mice in ancient Greece were thought to have been born of exhalations of the earth that imbued them with prophetic powers, and it is pleasing to imagine that *P. smintheus*, emerging as they do from subterranean cocoons before taking their place in the skies, share some of this magic.

1.6.2 *Sedum lanceolatum*

Sedum lanceolatum is found in exposed rocky sites throughout western North America, from the southern Yukon to New Mexico and from the plains to the high alpine (Kershaw *et al.*, 1998). A perennial succulent, the plant's branched, decumbent stems originate from a central rhizome, sometimes developing roots from nodes in contact with the soil, and bearing compact rosettes of fleshy, lance shaped leaves (Jolls, 1980)—hence both its Latin name and its common name, lance-leaved stonecrop (Fig. 1.2). In mid-summer, fertile rosettes bolt to produce a cyme with leaves along its stem, which bears multiple yellow flowers, each producing seed in dehiscent follicles (Clausen, 1975). Seeds are tiny and, though little is known of their dispersal, they likely fall within a short distance of the inflorescence. Depending on location, they may be dispersed secondarily by rain, meltwater, or soil upheaval (Kurt Illerbrun, *pers. obs.*). The plant can also reproduce vegetatively by means of its creeping rhizome, or by the rooting of severed rosettes. Three subspecies are recognized—the largest and most common *S. lanceolatum* ssp. *lanceolatum*; the smaller alpine form *S. lanceolatum* ssp. *subalpinum* Clausen, and the predominantly western *S. lanceolatum* ssp. *nesioticum* Clausen. Only the most common *S. lanceolatum* ssp. *lanceolatum* is known in Alberta (United States Department of Agriculture, 2013; University of Alberta Herbariums, 2013), where the research for this thesis took place.

Sedum lanceolatum has been used occasionally as a model species, e.g. for paleoclimatic studies (DeChaine and Martin, 2005), but few studies have examined its autecology. Jolls (1980) showed that *S. lanceolatum* natural history varies with altitude, with higher elevation plants allocating more

Figure 1.2: *Sedum lanceolatum* before (L) and during (R) flowering.

biomass to asexual tissues vs. reproductive tissues than lower elevation counterparts. This pattern is in keeping with theories (e.g. Bliss 1971) that vegetative modes of reproduction may be favoured above the treeline, both because sexual reproduction requires heavy investment of resources and may be too costly in a nutrient limited habitat, and because this investment is at greater risk of failing outright if seeds are unable to mature, or if seedlings fail to establish during the short growing season (see Jolls (1984) for a *Sedum*-specific examination of this issue, or e.g. Douglas (1981) for a more general discussion). Because Jolls' (1980; 1984) work took place in Colorado, where both *S. lanceolatum* ssp. *lanceolatum* and *S. lanceolatum* ssp. *subalpinum* occur, its relevance to the Alberta Rockies is unclear. Jolls (1980) suggested that high elevation samples may have been of ssp. *subalpinum*, while lower elevation samples were of ssp. *lanceolatum*, and consequently it is unknown whether this pattern of variable biomass allocation occurs where only ssp. *lanceolatum* is present. Because numerous other species show altered biomass allocation with increasing elevation (e.g. Bliss 1971; Kozlowski 1992; Johnston and Pickering 2004; Hautier *et al.* 2009), I assume that the trend remains relevant, and that *S. lanceolatum* ssp. *lanceolatum* shows some morphological variation with changing elevation.

Similarly, *S. lanceolatum* seedling densities are, on average, much lower above the alpine treeline than at lower elevations (Jolls and Bock, 1983). Further, *S. lanceolatum* distribution above the treeline is markedly patchier than below the treeline, but the spatial heterogeneity of above-treeline populations results in local patches where seedling survivorship and density far exceed those of below-treeline populations. Jolls and Bock (1983) concluded that the alpine environment was not necessarily as stressful to the plant as might be assumed. Overall, *S. lanceolatum* thrives across a wide range of elevations and microhabitats by exhibiting plasticity in its morphology and resource allocation to suit local conditions. One question that remains unanswered is the degree to which this plasticity may affect herbivores that consume *S. lanceolatum*, and I address this question in Chapter 2.

1.7 The pages ahead

Parnassius smintheus and *S. lanceolatum* are endemic to alpine meadows that are threatened by the encroachment of trees. While the shrinkage and fragmentation caused by this encroachment has well-documented consequences for adult *P. smintheus* (Roland *et al.*, 2000; Roland and Matter, 2007), the effects for larvae are unknown. Within the theoretical framework outlined earlier in this chapter, this thesis explores variability in interactions between *P. smintheus* and *S. lanceolatum* relative to the treeline-delimited meadow edge, and speculates about the consequences of continued tree encroachment for these and other species facing similar threats to their habitat.

Chapter 2 examines how proximity to the treeline influences the distribution, abundance, and quality of *P. smintheus* larval food resources, as a prerequisite to understanding spatial variation in *P. smintheus*-*S. lanceolatum* interactions relative to the treeline. Chapters 3 and 4 explore the consequences of this variability through its effects on female oviposition (and by extension larval distribution), and on larval exploitation of the host plant, with important implications for the relationship between apparently available, and actually available, habitat. Chapter 5 digresses slightly but necessarily to validate this finding by testing the capacity of *P. smintheus* larvae to ameliorate their circumstances through directed movement. Finally, Chapter 6 turns the tables by examining the consequences of feeding by *P. smintheus* larvae on *S. lanceolatum* growth and reproductive output, again relative to the treeline, with the aim of understanding if larval activity compounds, mitigates, or leaves unaltered the distribution of *S. lanceolatum* relative to the treeline.

Together, the following chapters (1) demonstrate that proximity to the treeline-delimited habitat edge fundamentally alters the interaction between a herbivore and its host plant, with relevance to other plant-herbivore interactions in shrinking habitats; (2) confirm that such edge effects may confound accurate estimates of available habitat, potentially leading to overestimation; (3) argue for the consideration of larval habitat requirements in any assessment of butterfly habitat; and (4) further contribute to the natural historic understanding of a butterfly species with direct relevance to endangered congeners (*P. apollo*; *P. mnemosyne*).

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Chapter 2

Distribution of *Parnassius smintheus* host plant resources in an alpine meadow

2.1 Introduction and objectives

2.1.1 Background and general ecology of *Sedum lanceolatum*

Few studies have examined *Sedum lanceolatum* Torr. (Crassulaceae) in the context of alpine plant-herbivore interactions, as I do in this thesis. Fownes and Roland (2002) established the importance of the plant as a cue for oviposition by females of the alpine meadow dwelling butterfly, *Parnassius smintheus* Doubleday (Lepidoptera: Papilionidae), whose larvae are locally monophagous on *S. lanceolatum*. Roslin *et al. (2008)* proposed that larvae move rapidly from host to host, consuming only a few leaves per plant, because *S. lanceolatum* produces an induced chemical defense in response to herbivory that increases with time, slowing larval growth. Meanwhile, although it is well-established that rising treeline threatens alpine meadows worldwide (Grabherr *et al.*, 1994; Fagre *et al.*, 2003; Millar *et al.*, 2004; Parmesan, 2006), and that tree encroachment reduces connectivity among meadows (Roland *et al.*, 2000) and decouples subpopulations of *P. smintheus* (Roland and Matter, 2007), isolating previously contiguous populations (Keyghobadi *et al.*, 2005*b*,*a*), it is unknown how *S. lanceolatum* will respond to advancing treeline. Because of its vital importance as food for *P. smintheus* larvae and as a measure of habitat suitability for *P. smintheus* females, examining *S. lanceolatum* growth, spatial distribution, and survival in relation to the treeline is a logical first step towards understanding if, and how, variation in a host plant relative to the treeline may in turn alter interactions between that plant and its herbivore.

2.1.2 Distribution and abundance of *S. lanceolatum*

All else being equal, theory predicts that insect herbivores should follow an ideal free distribution based on the spatial arrangement of palatable host plants (e.g. Cates, 1980; Loxdale and Lushai, 1999) or, where tritrophic interactions exist, on the interaction of host and predator distribution (e.g. Singer and Parmesan, 1993; Williams *et al.*, 2001). Thus, understanding the underlying spatial patterns of host plant abundance, quality, and other physical or growth characteristics is a prerequisite to understanding host plant-herbivore interactions.

2.1.3 Quality of *S. lanceolatum* as food

Interactions between insect herbivores and their host plants are complex and extensively studied, and current theory holds that plant-insect herbivore interactions are a product of a very long associational history (Futuyma and Agrawal, 2009). As an oversimplification, plants evolve ways to mitigate herbivory and its effects, while herbivores evolve ways around those measures. Plant defenses against herbivory may be chemical—either constitutive or induced in response to herbivory—morphological, or phenological (Karban and Baldwin, 1997). Or, depending on the degree to which herbivory reduces plant fitness, plants may instead evolve tolerance to herbivore damage (Strauss and Agrawal, 1999). Regardless, plants contend with an inevitable tradeoff between growth and defense (e.g. Herms and Mattson, 1992). In turn, insect herbivores evolve morphological, physiological, or behavioural means of neutralizing or tolerating the host's defenses, sometimes even turning them to their own advantage (Gullan and Cranston, 2005), and must contend with tradeoffs of their own.

One commonly studied aspect of plant-insect herbivore interactions is the role of host plant quality in altering herbivore performance (e.g. Awmack and Leather, 2002; De Bruyn *et al.*, 2002) and distribution in space (e.g. Cates, 1980; Zangerl and Berenbaum, 1993; Loxdale and Lushai, 1999), and both nitrogen content (percent N) and the ratio of carbon to nitrogen (C:N) in tissues are frequently used as measures of plant nutritional value for herbivores (e.g. White, 1984; Forkner and Hunter, 2000). Herbivores' growth and metabolism are often limited by N availability, and are thus influenced by the N content of the plants they consume (Mattson, 1980; Scriber and Slansky, 1981). While percent N provides an absolute measure of the N contained in a plant's tissue, C:N indicates the degree to which that N is diluted by the accumulation of e.g. carbohydrates during photosynthesis. A high C:N may indicate lowered nutritional quality, or be associated with phenolic plant defenses (Bryant *et al.*, 1983; Agrell *et al.*, 2000), especially when the photosynthetic rate is high. This pattern has been found both in other *Sedum* species (Bachereau *et al.*, 1998) and in unrelated species (Tevini *et al.*, 1991; Day *et al.*, 1993; Veit *et al.*, 1996). Research on the effects of elevated atmospheric CO_2 on plant-herbivore interactions shows that a combination of lowered percent N and increased C:N either reduces herbivore performance (Agrell *et al.*, 2005, 2006; Knepp *et al.*, 2007) or leads to compensatory feeding (Bezemer and Jones, 1998; Coviella and Trumble, 1999; Bidart-Bouzat and Imeh-Nathaniel, 2008), where herbivores must consume more plant matter to acquire sufficient N. Meanwhile, though high percent N and low C:N may indeed represent higher food quality, this may also indicate elevated levels of nitrogenous defensive compounds such as alkaloids (e.g. Mattson, 1980). Therefore, the actual meaning of C:N and percent N may vary with species and habitat type. In alpine systems N and phosphorus (P) are often limited (Bowman *et al.*, 1993), and may be especially limited where spring snow melt exports nutrients to lower elevations (Brooks *et al.*, 1998; Hood *et al.*, 2003). Areas with deeper snow, such as those near the alpine treeline (Walsh *et al.*, 1994), may show reduced N loss because N-retaining soil microbes are better insulated against freezing (Brooks *et al.*, 1997, 1998; Brooks and Williams, 1999; Schimel *et al.*, 2004). Abundant N may increase foliar N content and decrease C:N, but again with unclear consequences for herbivores: some plants also increase N-based defensive chemicals when N is abundant, whereas others do not (Mattson, 1980). Regardless, the leaves of slower growing, longer lived plant species like *S. lanceolatum* tend to be richer in carbon compounds—and thus exhibit higher C:N—than those of faster growing plants (Feeny, 1970; Herms and Mattson, 1992; Cornelissen *et al.*, 1996).

Sedum lanceolatum apparently produces an induced chemical defense in response to herbivory that renders already damaged plants unattractive to subsequent larvae, and results in a relatively diffuse spatial distribution of herbivory (Roslin *et al.*, 2008)—however, the details of such a defense are unknown. As for other alpine plants (e.g. *Delphinium*; Ralphs *et al.*, 2002), there is also anecdotal evidence for increased toxicity—a constitutive defense—during, and for a period after, *S. lanceolatum's* winter dormancy (Guppy and Shepard, 2001). Again, however, the underlying causes of such toxicity are unknown. *Parnassius smintheus* larvae are aposematically coloured and are known to sequester the gamma-hydroxynitrile glucoside sarmentosin, which is found in *S. lanceolatum* (Nishida and Rothschild, 1995; Bjarnholt *et al.*, 2012). However, sarmentosin is not induced (S.F. Matter, *unpublished data*), so any induced defense likely represents an as-yet unidentified compound. Various alkaloids have been identified in other *Sedum* species (Kim *et al.*, 1996), but *S. lanceolatum* has not been tested. Regardless, since snow within the alpine zone accumulates most and thus melts more slowly near the treeline, host plants in this area may be both physically and chemically unavailable to *P. smintheus* larvae for longer than those in more open parts of the meadow and, if N availability varies with snow depth (Brooks *et al.*, 1997, 1998; Brooks and Williams, 1999; Schimel *et al.*, 2004), this variation would be reflected in the percent N and C:N of plants subjected to differing winter snow cover.

2.1.4 Summary of objectives

In Chapter 3 I examine oviposition by *P. smintheus*—and, by extension, distribution of neonate larvae—in relation to distance from the treeline. Likewise, in Chapter 4, I examine *P. smintheus* larval herbivory on *S. lanceolatum*, while in Chapter 6 I explore variation in the consequences of *P. smintheus* herbivory for *S. lanceolatum*, both in relation to distance from the treeline. In this chapter, however, I undertake a comprehensive assay of *S. lanceolatum* growth, distribution, and reproduction, in relation to distance from the treeline, as a necessary prerequisite to addressing these broader issues in the chapters ahead. Further, because nutrient availability is known to vary in relation to the alpine treeline (e.g. Stevens and Fox, 1991; Körner, 1998), and in order to test the extent of overall nutrient limitation in relation to the treeline as an explanation for variation in *S. lanceolatum*'s nutritional quality and growth/distribution, I undertake a simple nutrient supplementation experiment.

Finally, I explore variation in the percent N and C:N of *S. lanceolatum* plants collected from sites with varying winter snow depth, as well as varying distances from the treeline. I hypothesize that plants near the treeline will exhibit higher N content and lower C:N than those growing elsewhere in the meadow, but that this may represent not improved host quality, but in fact elevated levels of nitrogenous defensive chemicals that deter herbivory by larval *P. smintheus*. If this is so, then the extent to which *S. lanceolatum* resources are *actually* available to *P. smintheus* larvae may be rather less than the total of all plants in the meadow, with important implications for the conservation of larval habitat.

2.2 Methods

2.2.1 Study site

Research took place over 5 years (2006-10) in the alpine meadows of Jumpingpound Ridge, in Kananaskis Country, Alberta, Canada (50°57'N, 114°55'W), a series of meadows used for long-term study of *P. smintheus* dynamics (Roland *et al.*, 2000; Keyghobadi *et al.*, 2005*a*; Roland and Matter, 2007). Data were collected in three separate but ecologically similar meadows, one near the northern (G) and two near the southern (L; M) end of Jumpingpound Ridge (Fig 2.1). Both meadow G and meadow L face west, with a steady incline of ~10° from treeline to meadow apex, and a sharp drop-off on the eastern side, while meadow M faces south, with a steeper incline of ~15°. Both G and L are clearly delimited by trees on their western edges, and by both trees and the locally precipitous drop-off on their eastern margin. Meadows L and M are physically connected, with only a diffuse barrier of trees between them. Vegetation is similar among meadows, consisting of *Dryas* spp., *Salix* spp., grasses and wildflowers, interspersed with *S. lanceolatum*. Vegetation gives way to increased amounts of exposed rock towards the meadow apex. Meadow M is grassier overall than meadows G or L, and funnels into a long gully at its base that extends into the subalpine zone. Both meadows are surrounded by subalpine forest dominated by a mix of Engelmann spruce (*Picea engelmannii*), lodgepole pine (*Pinus contorta*) and subalpine fir (*Abies lasiocarpa*). Total meadow area along Jumpingpound Ridge has declined more than 75% over the past 50 years (Roland *et al.*, 2000); forest stands and *krüppelholz* (Holtmeier, 1981) now intervene between some previously connected meadows, and encroach upon others. Treeline is well defined in meadows G and, along the western edge where this study took place, also in L, with little transitional area between trees, *krüppelholz* and lower shrubs/meadow flora. Due to the western aspect, shade from the treeline is negligible until early evening. Treeline in meadow M is locally well defined, but more diffuse and irregularly shaped overall due to the long gully projecting into the subalpine.

Although *P. smintheus* larvae feed on several related host species throughout their range (Guppy and Shepard, 2001), on Jumpingpound Ridge they feed almost exclusively on *S. lanceolatum*, and only rarely on ledge stonecrop, *Sedum integrifolium* (Roslin *et al.*, 2008). Due to the scarcity of *S. integrifolium* at our study site, *P. smintheus* larvae can be considered monophagous on *S. lanceolatum* at our site. Since *S. lanceolatum* is relatively long-lived, its spatial arrangement within meadows remains largely constant among years.

2.2.2 Spatial patterns of *S. lanceolatum* in relation to the treeline

I established 4 transects from the treeline to the meadow apex in meadows G and L. I placed transects at intervals from each other using a random number generator, and established 5m x 5m quadrats at 20m intervals along each transect, beginning at treeline and terminating at the meadow apex. Quadrat size was chosen to provide a balance between the known ~5-10

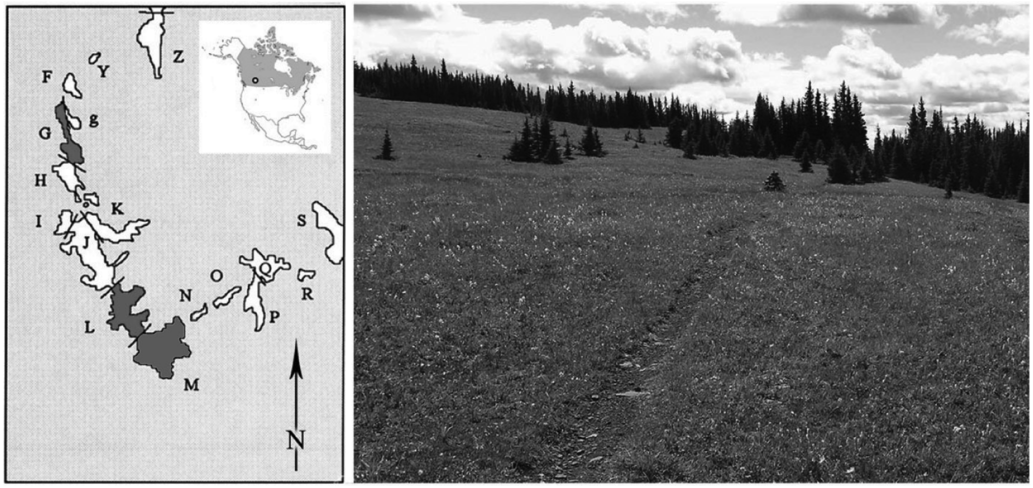

Figure 2.1: Jumpingpound Ridge, showing locations of meadows G and L, and detailed view of meadow L looking south.

m scale of *S. lanceolatum* aggregation (Fownes, 1999; Roslin *et al.*, 2008) and logistical tractability. Thus the number of quadrats in each transect varied in proportion to meadow size, giving a total of 14 quadrats ($350m^2$) in meadow G and 15 quadrats ($375m^2$) in meadow L. In meadow G, this protocol resulted in 4 quadrats near the treeline, 4 near the meadow apex, and 6 in the intervening mid-meadow area. In meadow L, it resulted in 4 quadrats near the treeline, 4 near the meadow apex, and 7 in mid-meadow.

I located every *S. lanceolatum* plant in each quadrat to permit spatial analysis of patterns in the plant's distribution. Rather than record coordinates in the field, I developed a digital method which allowed me to record individual plant locations more precisely in the lab. Within each quadrat I created 30 highly visible reference points to allow assessment of scale during later analysis, and placed colour-coded markers beside each *S. lanceolatum* plant to denote its status as flowering or not. Once every *S. lanceolatum* plant in a quadrat was marked, I took a high-resolution (10mp DSLR) digital image of the entire plot (Fig 2.2), capturing the location of

Figure 2.2: Sample quadrat showing intersecting lines used as reference points, along with pins marking each *S. lanceolatum* plant. Such images were later digitally rectified to reflect the true 5m x 5m shape of each quadrat.

all plants to an accuracy of ~1cm. In the lab, using the reference points as a guide, I orthorectified all images for parallax and perspective error using ESRI ArcGIS, “flattening” each image into a 2D square to best show the spatial relationship among plants, and then recorded the number and coordinates of all plants. Next I subdivided each image into 100 50cm x 50cm squares for later estimation of clumping, as herbivory is known to be clumped at the scale of 20-50cm (Fownes, 1999; Roslin *et al.*, 2008), and obtained count data for *S. lanceolatum* in each square. Data from 3 quadrats in meadow G were excluded due to problems with accurately orthorectifying the images.

To estimate the clumping of *S. lanceolatum* plants (for later comparison with the spatial distribution of herbivory by *P. smintheus* larvae; see Chapter 4) in each of the 29 quadrats, I used SADIE (Spatial Analysis by Distance

IndicEs) (Perry *et al.*, 1999) on the count data for each quadrat. SADIE estimates the distance to regularity (D) of spatial data—the cumulative distance that points in the data set would need to move to produce a regular pattern. A higher D indicates greater clumping. D is associated with a probability, P, the likelihood that a given value of D occurs by chance. High D with low P indicates significant aggregation. I used a 1-way ANOVA and Tukey's HSD post-hoc test to test for differences in the spatial patterns (D) of plants between meadow locations (treeline, mid-meadow and meadow apex). Data from two quadrats were classified as outliers (box plot; >1.5x the interquartile range, either above the upper quartile or below the lower quartile) and excluded from the analysis. I tested for normality and homogeneity of variances wherever applicable and transformed data if necessary; means are reported ±S.D. unless otherwise noted. Statistical analyses were performed with SPSS Statistics 17.0, S-plus 8.0 (Insightful Co., 2007) and R (R Development Core Team, 2012).

2.2.3 *Sedum lanceolatum* abundance and flowering rates in relation to the treeline

Although *P. smintheus* do not oviposit on *S. lanceolatum*, its presence is necessary for *P. smintheus* oviposition (Fownes and Roland, 2002; see Chapter 3 for a further discussion of the issue). I therefore compared overall *S. lanceolatum* abundance between the quadrats from near-treeline, mid-meadow and the meadow apex using a 1-way ANOVA and Tukey's HSD post-hoc test. Next, because *S. lanceolatum* plant size is highly variable, and because the plant's flowers are commonly exploited by adult *P. smintheus* for nectar, I randomly censused *S. lanceolatum* and recorded attributes not captured in the digital photographs, including number of rosettes and inflorescences per plant. The census included the first 200 *S. lanceolatum* plants encountered within 10m of treeline, in mid-meadow, and near meadow apex, for each study meadow. Due to the scarcity of *S. lanceolatum* near meadow apex in meadow G only 122 plants were encountered and censused; this gave a total of 1122 plants in the survey.

2.2.4 The effect of nutrient enrichment on *S. lanceolatum* flowering and survival

Here, my objective was to assess the effects of nutrient supplementation on *S. lanceolatum* in relation to the treeline. Nutrient availability may vary with distance from the treeline, affecting plant chemistry and, consequently, herbivory. Because alpine plant communities may be locally limited by the availability of N or P depending on relatively fine-scale habitat and community heterogeneity (e.g. Bowman *et al.*, 1993; Soudzilovskaia *et al.*, 2005), and because little is known about nutrient availability in our study meadows, I applied a general purpose time-release fertilizer with equal concentrations of N, P, and K (Miracle Grow 10-10-10). In doing so, my goal was to answer the general question of whether nutrient limitation is significant in my study meadows, rather than any specific questions about its nature or precise extent. Where nutrient enrichment resulted in a significant difference in *S. lanceolatum* characteristics—growth, inflorescence production, and so on—compared with unfertilized plants, I assumed that plants were nutrient limited with respect to that characteristic. Conversely, where nutrient enrichment had little effect, I assumed that plants were not nutrient limited with respect to that characteristic.

I created a single transect of paired 5m x 5m plots in meadow L, following the protocol outlined above, resulting in a total of 8 plots (4 fertilizer + 4 paired controls; 200m^2) from the treeline to the meadow apex. I applied fertilizer to one plot of each pair at 75% of the recommended dosage (75 gm^{-2}vs. 100 gm^{-2}; i.e., 7.5gm^{-2}for each of N, P and K specifically) to avoid any potential of over-fertilization. I applied fertilizer 3 times over a one year period: immediately after snow-melt in late May 2009; in October 2009, just prior to the first snowfall; and again immediately after snow-melt in late May 2010. Since the fertilizer released slowly over a period of 4 months, this provided relatively uniform application while the meadows were snow-free and accessible. I counted all *S. lanceolatum* plants and inflorescences in each quadrat at the end of the growing season in both August 2009 and 2010. For each year, I calculated flowering rate as the number of flowering plants over the total number of plants in each quadrat. Between years, I calculated population growth rate as the total number of plants in 2010 over the total number of plants in 2009. I also counted the number of flowers—as opposed to inflorescences—per plant for a randomly selected sample of 20 flowering *S. lanceolatum* in both the fertilized and unfertilized plots, as a measure of potential reproductive output. This latter assay did not take into account distance from the treeline: I counted flowers in the lab, and I sought to minimize the impact on my quadrats, which were to be used for further experiments.

I used a mixed-model ANCOVA, with year as a random effect and distance to treeline as a covariate, to test for an interaction between nutrient enrichment and distance to treeline with respect to inflorescence production, as well as the main effect of nutrient enrichment on inflorescence production. I used an ANCOVA, with distance to treeline as a covariate, to test for an interaction between nutrient enrichment and distance from the treeline with respect to population growth, measured as the number of *S. lanceolatum* plants in 2010 over the number of plants in 2009, as well as the main effect of nutrient enrichment on population growth. I used a *t*-test to compare flower numbers in fertilized and unfertilized plots.

2.2.5 Effects of slope and cover on inflorescence height

Sedum lanceolatum produces tiny seeds that are dispersed only short distances by gravity and wind (Clausen, 1975). However, because the plant is commonly found on exposed, rocky slopes, it is likely that many seeds are secondarily dispersed with soil, either loosened by frost upwellings or carried downhill by gravity or spring meltwater. Most vegetative reproduction by detached rosettes would also result in some downhill dispersal. Even allowing for some secondary upward seed dispersal, e.g. by invertebrates (Chambers and MacMahon, 1994), most new seedlings might be expected to establish down slope of their parent plants (Thompson and Katul, 2009), begging the question: Why do we find *S. lanceolatum* at the meadow apex? Presumably the answer would depend both on a plant's intrinsic ability to disperse seed, as well as interactions with the surrounding environment, such as slope and vegetation cover.

I tested the hypothesis that plants from flat vs. sloping sections of meadow might show morphological differences in inflorescence height, with plants on steep slopes producing taller flowering stalks that facilitate upward seed dispersal (i.e., downhill seed dispersal could occur regardless of inflorescence height, whereas uphill dispersal might be enhanced by taller inflorescencecs). I also examined whether *S. lanceolatum* growing in areas with cover from other plants produced taller inflorescences than those growing in open habitat. I randomly collected inflorescences from *S. lanceolatum* growing in open and covered sites in sloped and flat areas of the adjacent meadows L and M, resulting in a factorial design. In total, I collected 26 inflorescences from sloped, open sites (all irrespective of meadow); 26 from sloped, covered sites; 29 from flat, open sites, and 31 from flat, covered sites, for a total of 112 inflorescences. In the lab, I measured total inflorescence length and counted the number of flowers per inflorescence. I analyzed the effects of slope and cover on inflorescence length using a 2-way ANOVA with interaction.

2.2.6 Nitrogen content analysis

As for other alpine plants (e.g. Ralphs *et al.*, 2002), there is anecdotal evidence of elevated toxicity during and immediately after *S. lanceolatum's* winter dormancy (Guppy and Shepard, 2001), although the exact mechanisms are unknown. Further, since snow within the alpine zone often melts later near the treeline, host plants near the treeline may be both physically and chemically unavailable to *P. smintheus* larvae for longer than those in more open parts of the meadow. If N availability varies with snow depth (Brooks *et al.*, 1997, 1998; Brooks and Williams, 1999; Schimel *et al.*, 2004), this variation should be reflected in the percent N and C:N of plants subjected to differing winter snow cover. Further, if nutrient limitation varies with distance from the treeline, this would also be uniquely reflected in the percent N and C:N of plants growing in each meadow region.

2.2.6.1 Nitrogen content of unfertilized *S. lanceolatum* relative to the treeline

Because snow melts later near the treeline, remaining snow-cover ’zones’ (full cover, partial cover, and open) are spatially analogous to treeline, mid-meadow, and meadow-apex as used in this thesis. For this assay, I collected whole-plant samples of 10 *S. lanceolatum* plants from random locations within each of the same 3 meadow zones on 2 separate occasions, once during spring snow melt and again 4 weeks later during the height of *P. smintheus* larval foraging. During snow melt, the 3 zones represented a progression from full snow cover, where samples were obtained by shovelling away ~1m of snow, to ’just melted,’ to fully open, while 4 weeks later the same locations represented near-treeline, mid-meadow, and near-apex, respectively. During the second sample collection, I collected an additional 11 flowering plants from near the meadow apex. I removed as much soil as possible, placed all samples in an insulated bag with ice packs while in the field, and froze them (-20°C) immediately on return to the lab.

I placed each sample in an individual paper envelope and dried all samples by convection for 72 hours at 50°C. Preliminary testing showed that such an extended period of drying was necessary to overcome *S. lanceolatum*’s impressive water retentiveness. Once dry, I removed roots and any remaining loose soil and ground each sample using a mortar and pestle until it became a consistent, fine powder, cleaning all equipment with water and 99% ethanol between samples.

Percent N and C:N of samples were analyzed by the Department of Biological Sciences Biogeochemical Analytical Laboratory using a thermal conductivity elemental analyzer (CEC (Control Equipment Corporation) Model 440 Elemental Analyzer) using 2.5-3.5mg of each sample.

I used a 2-way ANOVA to test for an interaction between sample periods and meadow regions on C:N or percent N and, within sample periods, 1-way ANOVAs to test for differences in C:N and percent N at varying snow cover and distances from the treeline. When the homogeneity of variances was not improved by transformation, I used Welch's ANOVA (Welch, 1951) to confirm results.

2.2.6.2 Effects of nutrient enrichment relative to the treeline

In addition to examining the effects of nutrient supplementation on *S. lanceolatum* growth and reproductive output (outlined above), I also measured C:N and percent N of plants in the unfertilized control, and fertilized experimental plots used for that assay. Here, again, my goal was to answer the general question of whether variation in nutrient availability affects *S. lanceolatum* nutritional content, and whether these effects vary relative to the treeline, rather than any specific questions about their nature or precise extent. As the sample plots were the same, the nutrient enrichment methodology was as above.

Differences in C:N and percent N at varying snow cover and distances from the treeline were determined using 2-way ANOVAs with interaction.

2.3 Results

Results are given in detail below, and also summarized in Table 2.6 in the Discussion section of this chapter.

2.3.1 *Sedum lanceolatum* abundance and flowering rates in relation to the treeline

Sedum lanceolatum was most abundant near treeline (631.50 ± 270.10), less so at mid-meadow (86.71 ± 62.81; $p = 0.001$), and least abundant at the meadow apex (20.25 ± 6.13; $p = 0.002$), but the difference between mid-meadow and apex was not significant ($p = 0.886$) (here and below, Fig. 2.3 and Table 2.1; also see Figs 4.3 and 4.4). Thus, there was a significant overall difference in *S. lanceolatum* abundance from treeline to meadow apex in both meadows ($F_{2,24} = 10.74$, $p = 0.001$).

Plant size was highly variable in both meadows, but generally spanned the same range of sizes in all meadow locations: in meadow G, mean *S. lanceolatum* plant size was 4.93 ± 4.23 stems/rosette (n=200) near treeline, 5.51 ± 4.13 (n=200) in mid-meadow, and 4.97 ± 3.48 (n=122) near meadow apex. In meadow L it was 5.30 ± 4.15 stems/rosette (n=200) near treeline, 4.72 ± 4.13 (n=200) in mid-meadow, and 4.35 ± 4.28 (n=200) near meadow apex. There was no effect of location relative to the treeline on stem number in either meadow (meadow G: $F_{2,528} = 1.22$, $p = 0.296$; meadow L: $F_{2,598} = 1.41$, $p = 0.245$).

Despite the difference in overall *S. lanceolatum* abundance relative to the treeline, there was no effect of location relative to the treeline on the number of inflorescences/quadrat for both meadows combined ($F_{2,24} = 1.77$, $p = 0.191$). However, in meadow G, there was an effect of location relative to the treeline on mean inflorescence number/plant ($F_{2,528} = 13.29$, $p < 0.001$). There were significantly more inflorescences/plant at mid-meadow (1.73 ± 1.24 (n=200)) than at either the treeline (1.14 ± 1.30 (n=200); $p < 0.001$) or the meadow apex (1.25 ± 0.95 (n=122); $p = 0.001$), and

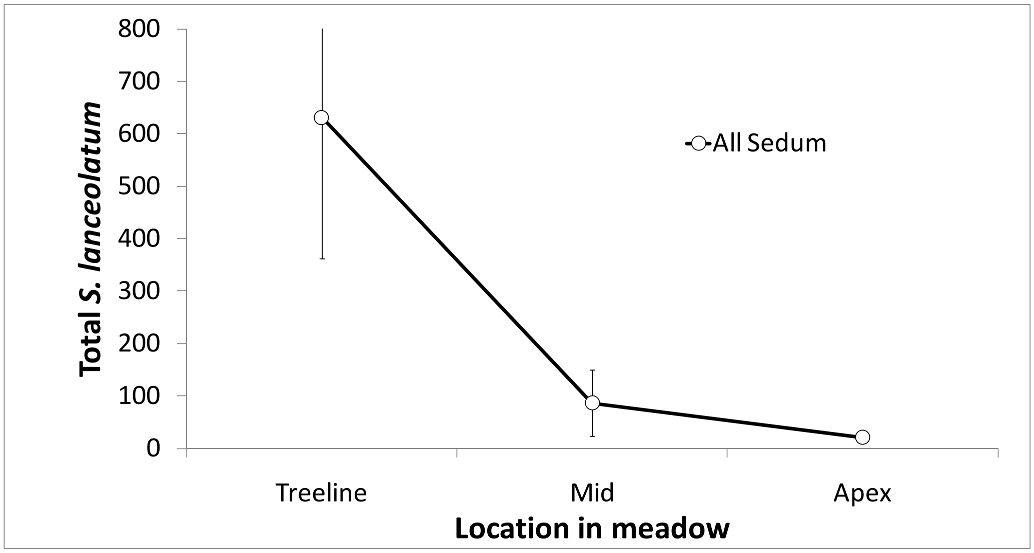

Figure 2.3: Mean *S. lanceolatum* abundance per 25 m^2 quadrat from treeline to meadow apex for both meadows. Standard deviations are indicated.

no difference between treeline and apex (p = 0.695). In meadow L, there were 0.93 ± 1.11 inflorescences/plant (n=200) near treeline, 1.12 ± 1.32 (n=200) at mid-meadow, and 1.09 ± 1.20 (n=200) at the meadow apex; although there was no significant effect of location ($F_{2,598}$ = 1.51, p = 0.222), mid-meadow did show the highest mean inflorescence number of the three locations, and apex was again higher than near-treeline.

2.3.2 Spatial patterns of *S. lanceolatum* in relation to the treeline

Sedum lanceolatum was significantly more clumped near the treeline (D = 3362.67 ± 828.86; Table 2.1; see also Figure 4.6), less so at mid-meadow (1364.09 ± 633.97; $p < 0.001$) and least clumped at the meadow apex (1062.33 ± 401.54; $p < 0.001$), although the latter two are not significantly different from each other (p = 0.631). Specifically, near treeline *S. lanceolatum* was indeed clumped (P = 0.04) while in mid-

Variable	Treeline (a)		Mid-meadow (b)		Meadow apex (c)		Significance		Contrasts
	$\overline{x}$	S.D.	$\overline{x}$	S.D.	$\overline{x}$	S.D.	F	p	
Abundance (both)	631.50	270.10	86.71	62.81	20.25	6.13	10.74	0.001	a-b, a-c
Size (G)	4.93	4.23	5.51	4.13	4.97	3.48	1.22	0.296	-
Size (L)	5.30	4.15	4.72	4.13	4.35	4.28	1.41	0.245	-
Inflorescences/plant (G)	1.14	1.30	1.73	1.24	1.25	0.95	13.29	0.001	a-b, b-c
Inflorescences/plant (L)	0.93	1.11	1.12	1.32	1.09	1.20	1.51	0.222	-
Clumping	3362.67	828.86	1364.09	633.97	1062.33	401.54	24.22	<0.001	a-b, a-c

Table 2.1: The effect of meadow location on the abundance, size, inflorescences/plant, and spatial clumping of *S. lanceolatum*. $\overline{x}$ refers to mean, S.D. refers to standard deviation, and letters (a, b, c) refer to meadow locations used in post-hoc contrasts. For example, "a-b" means that contrasts showed that the variable in question differed between treeline and mid-meadow.

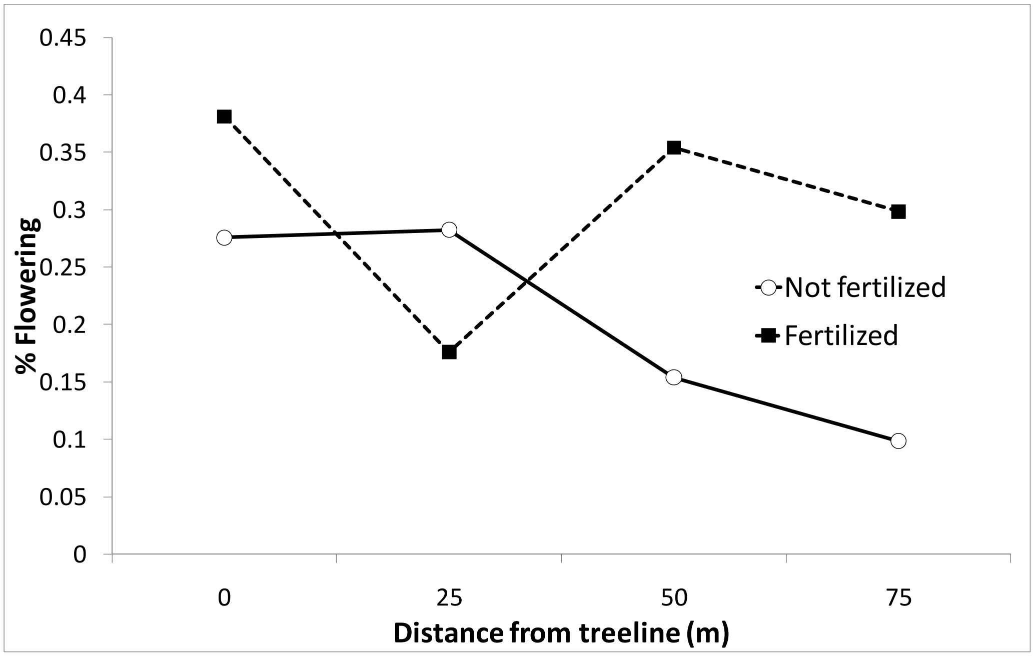

Figure 2.4: The percentage of flowering *S. lanceolatum* from fertilized (closed squares and dashed line) and unfertilized (open circles and solid line) quadrats arranged from the treeline to meadow apex.

meadow ($P = 0.45$) and at meadow apex ($P = 0.21$) it was distributed randomly. Overall, the effect of location relative to treeline on the degree of *S. lanceolatum* clumping was therefore significant ($F_{2,20} = 24.22$, $p < 0.001$). Thus, near the treeline *S. lanceolatum* is both more abundant, and more spatially clumped, than elsewhere in the meadow (I consider this result, and its relationship to *P. smintheus* larval herbivory, in Chapter 4, but I include it here for its relevance to the general discussion of *S. lanceolatum* growth and distribution relative to the treeline).

2.3.3 The effects of nutrient enrichment on *S. lanceolatum* flowering and survival

Nutrient enrichment increased the rate of increase in total number of plants between years ($F_{1,4} = 55.753$, $p = 0.002$), and increased plant number due to nutrient enrichment was more pronounced at greater distance from the treeline (interaction $F_{2,4} = 28.506$, $p = 0.004$; Fig. 2.5 and Table 2.2). Overall, the random sample of fertilized plants produced significantly more flowers than did unfertilized plants (11.00 ± 7.38 vs. 88.20 ± 59.74; $t_{38} = -9.603$, $p < 0.001$), even though there was no main effect of nutrient enrichment on inflorescence (as opposed to flower) production ($F_{1,11} = 0.113$, $p = 0.743$) and no interaction between nutrient enrichment and distance from treeline with respect to inflorescence production ($F_{2,11} = 1.109$, $p = 0.364$; Fig. 2.4).

2.3.4 The effects of slope and vegetation cover on inflorescence height

Inflorescence height was much greater at sloped sites, while ground cover tended to reduce inflorescence height on sloped sites but increase it on flat sites. Flower number was generally similar for all flat sites, regardless of cover, but was greater for the sloped, open sites (Fig. 2.6 and Table 2.3). Specifically, slope and cover both affected inflorescence height (slope: $F_{1,108} = 282.15$, $p < 0.001$; cover: $F_{1,108} = 6.72$, $p = 0.011$), and there was an interaction between the two ($F_{1,108} = 36.05$, $p < 0.001$). Likewise, slope and cover both affected mean flowers/inflorescence (slope: $F_{1,108} = 43.92$, $p < 0.001$; cover: $F_{1,108} = 11.25$, $p = 0.001$), and there was

Sedum variable	*Effects*	Meadow location (covariate)				Significance	
		Treeline (0m)	25m	50m	Apex (75m)	F	*p*
Pop'n growth (λ)	Not fertilized	0.82	1.50	1.33	1.18		
	Fertilized	1.08	1.94	2.61	3.96		
	Fertilizer					55.75	0.002
	Fert x loc					28.51	0.004
Flower rate (%)	Not fertilized	0.28	0.28	0.15	0.10		
	Fertilized	0.38	0.18	0.35	0.30		
	Fertilizer					0.113	0.743
	Fert x loc					1.109	0.364

Table 2.2: The effects of nutrient enrichment on *P. smintheus* population growth and inflorescence production.

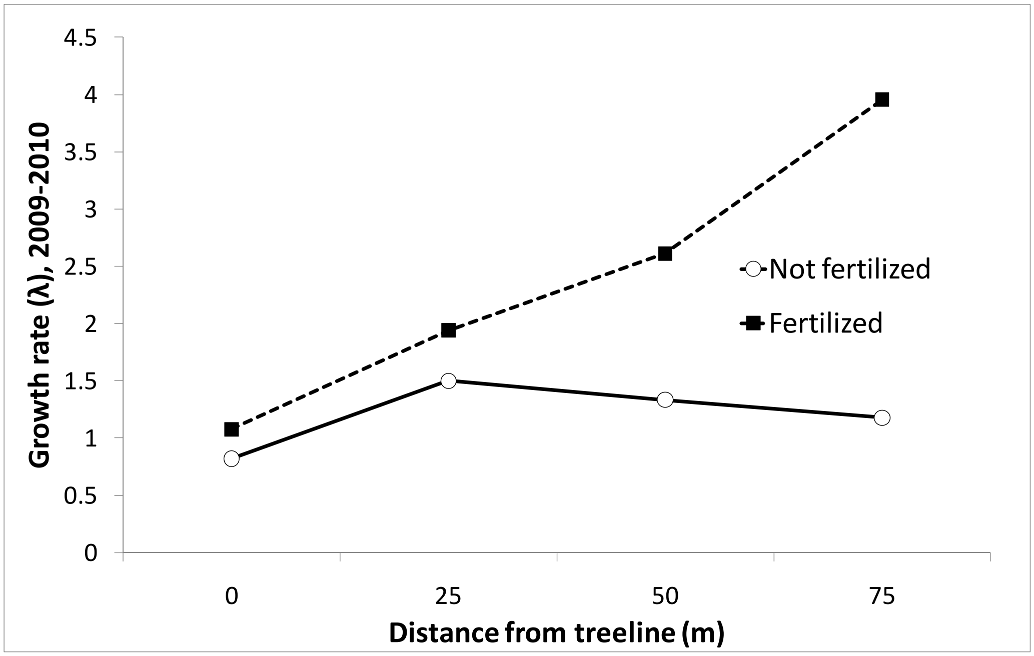

Figure 2.5: Growth rates of populations of *S. lanceolatum* between 2009 and 2010, from fertilized (closed squares and dashed line) and unfertilized (open circles and solid line) quadrats arranged from the treeline to meadow apex.

an interaction between the two ($F_{1,\,108} = 39.03$, $p < 0.001$) (Fig. 2.6 and Table 2.3).

2.3.5 Nitrogen content of *S. lanceolatum* relative to the treeline

In June, there was a trend for low C:N at treeline and higher C:N ratios at the meadow apex. This was associated with high percent N at treeline decreasing toward the meadow apex. This pattern persisted but was reduced by July.

In June, there was a significant difference in C:N between *S. lanceolatum* collected from under the snow, in the melt zone, and in already open meadow ($F_{2,\,27} = 6.59$, $p = 0.005$). Although C:N of *S. lanceolatum* from

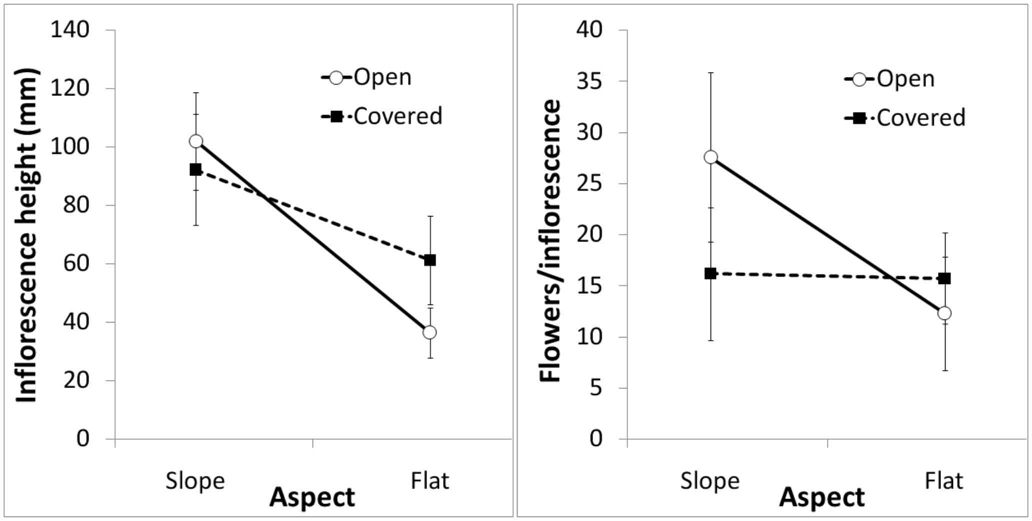

Figure 2.6: The effects of slope and vegetation cover on inflorescence height (left) and number of flowers/inflorescence (right). Standard deviations are indicated.

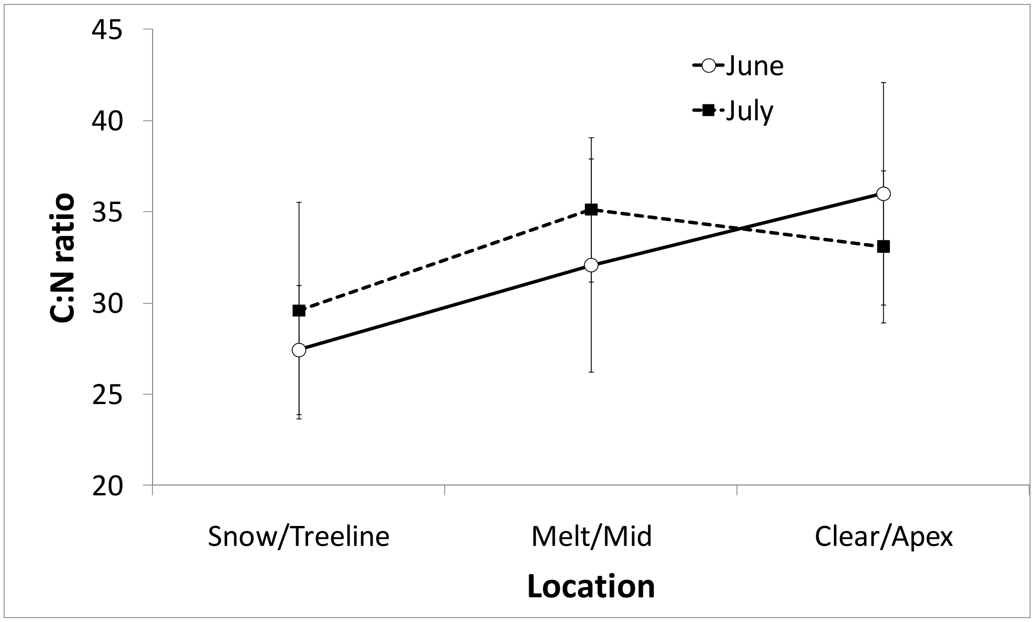

Figure 2.7: C:N ratio of *S. lanceolatum* as a function of snow cover and distance from the treeline. C:N ratio is generally lower near the treeline, but this effect is more pronounced earlier in the season before growth has begun. Standard deviations are indicated.

	Open + Flat		Open + Slope		Cover + Flat		Cover + Slope		Significance	
Variable	$\overline{x}$	S.D.	$\overline{x}$	S.D.	$\overline{x}$	S.D.	$\overline{x}$	S.D.	F	p
Inflorescence height	36.59	8.56	102.12	16.76	61.29	15.05	92.31	18.94		
Slope									282.15	<0.001
Cover									6.72	0.011
Slope x cover									36.05	<0.001
Flower number	12.31	5.54	27.58	8.29	15.74	5.54	16.19	6.51		
Slope									43.92	<0.001
Cover									11.25	0.001
Slope x Cover									39.03	<0.001

Table 2.3: The effects of slope and cover on *S. lanceolatum* flower number and inflorescence height.

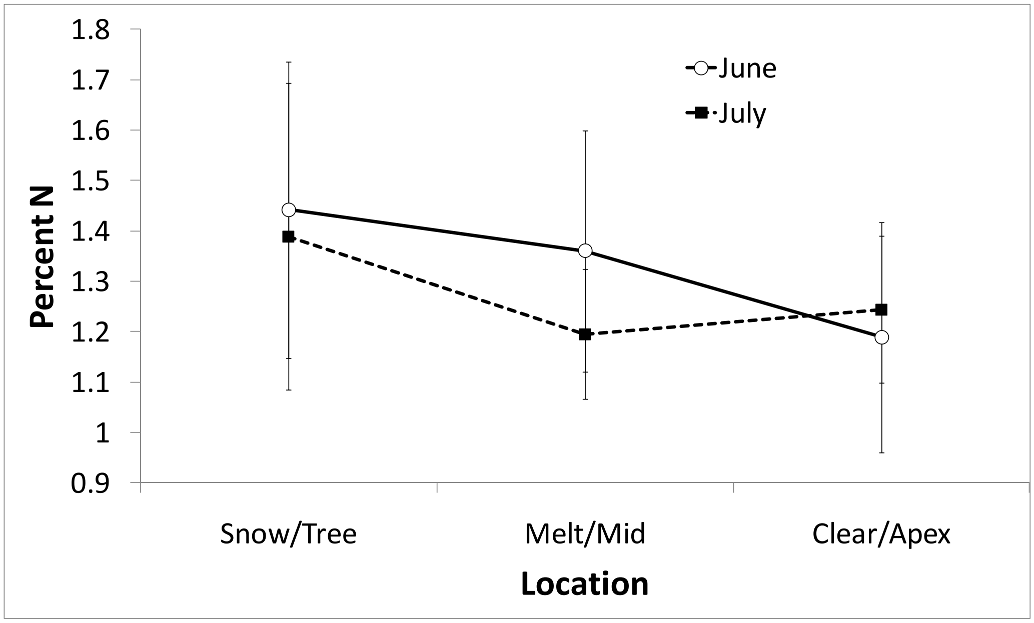

Figure 2.8: Percent N of *S. lanceolatum* as a function of snow cover and distance from the treeline. Overall the data appear to mirror the C:N data above, with percent N declining with increasing distance from the treeline; however, the trend is not statistically significant. Standard deviations are indicated.

the melt zone was not statistically different from that under the snow (Tukey's HSD; $p = 0.141$) or in open meadow ($p = 0.237$), C:N of plants from under the snow was lower than that from plants in open meadow ($p = 0.003$). In July, there was a similar difference in C:N between *S. lanceolatum* collected at the same sites, now labelled treeline, mid-meadow and meadow apex ($F_{2,\,27} = 3.45$, $p = 0.046$). This difference was primarily driven by a significantly lower C:N for *S. lanceolatum* at the treeline vs. plants in mid-meadow ($p = 0.039$), while treeline and meadow apex ($p = 0.245$), as well as mid-meadow and meadow apex ($p = 0.614$), were not significantly different from each other (Fig. 2.7 and Table 2.4).

In June, the trend for percent N closely matched that of C:N, but there was no significant difference among plants collected from the different

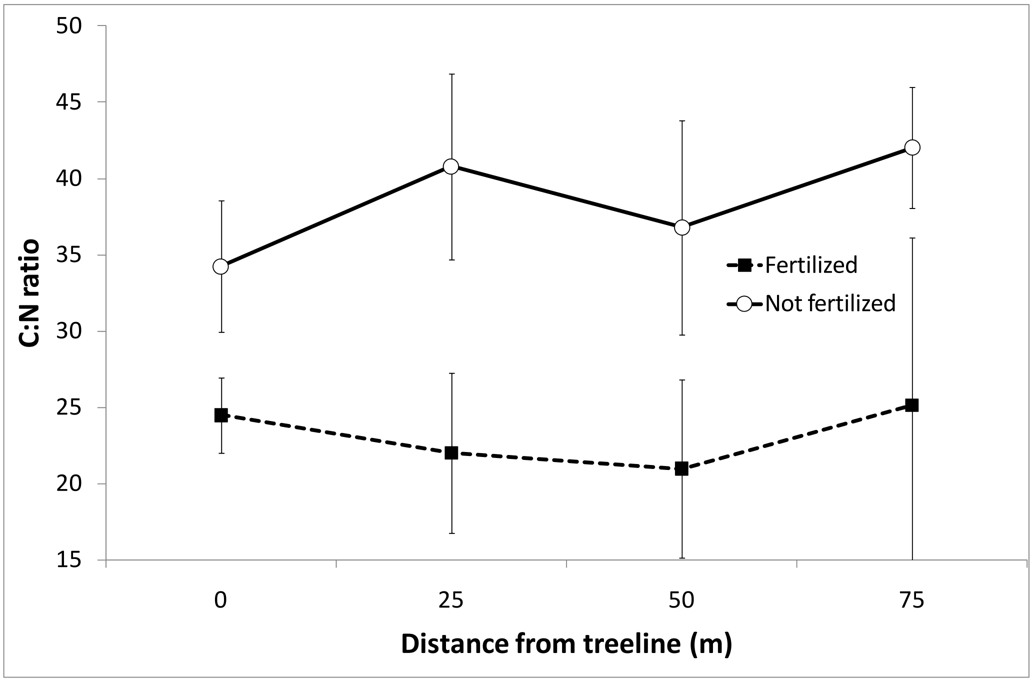

Figure 2.9: C:N ratio of *S. lanceolatum* as a function of fertilizer treatment and distance from the treeline. C:N ratio is generally similar within treatments, but higher in unfertilized plants than in fertilized plants regardless of meadow location. Standard deviations are indicated.

snow-cover treatments ($F_{2,\,27} = 2.45$, $p = 0.105$). In July there was again no difference in percent N among snow cover treatments ($F_{2,\,27} = 2.26$, $p = 0.124$; Fig. 2.8 and Table 2.4), nor was there a significant interaction between snow cover or meadow location and date for either C:N ($F_{2,\,54} = 2.04$, $p = 0.140$) or percent N ($F_{2,\,54} = 1.02$, $p = 0.368$).

C:N ratio did differ between flowering and non-flowering plants collected in July at the meadow apex ($F_{1,\,19} = 8.71$, $p = 0.008$), with the flowering plants showing a much higher C:N (Table 2.4). There was likewise a significant difference between percent N for these same groups ($F_{1,\,19} = 6.31$, $p = 0.021$).

Variable	Effects	Treeline (a)		Mid-meadow (b)		Meadow apex (c)		Significance		Contrasts
		$\overline{x}$	S.D.	$\overline{x}$	S.D.	$\overline{x}$	S.D.	F	p	
C:N	June	27.43	3.55	32.07	5.85	36.00	6.08	6.59	0.005	a-c
	July	29.59	5.94	35.12	3.96	33.09	4.16	3.45	0.046	a-b
	Date							0.35	0.558	-
	Location							8.32	0.001	a-c
	Date x loc							2.04	0.140	-
	Flower					39.15	4.72	8.71	0.008	-
	No flower					33.09	4.16			
% N	June	1.44	0.29	1.36	0.24	1.19	0.23	2.45	0.105	-
	July	1.39	0.30	1.20	0.13	1.24	0.15	2.04	0.140	-
	Date							0.22	0.644	-
	Location							2.60	0.084	-
	Date x loc							1.02	0.368	-
	Flower					1.10	0.12	6.31	0.021	-
	No flower					1.24	0.15			

Table 2.4: The effects of meadow location or snow cover on the C:N and percent-N of *S. lanceolatum*. *Italicized* variables and interactions refer to 2-way ANOVAs, while plain text refers to 1-way ANOVAs. $\overline{x}$ refers to mean, S.D. refers to standard deviation, and letters (a, b, c) refer to meadow locations used in post-hoc contrasts. For example, “a-b” means that contrasts showed that the variable in question differed between treeline and mid-meadow.

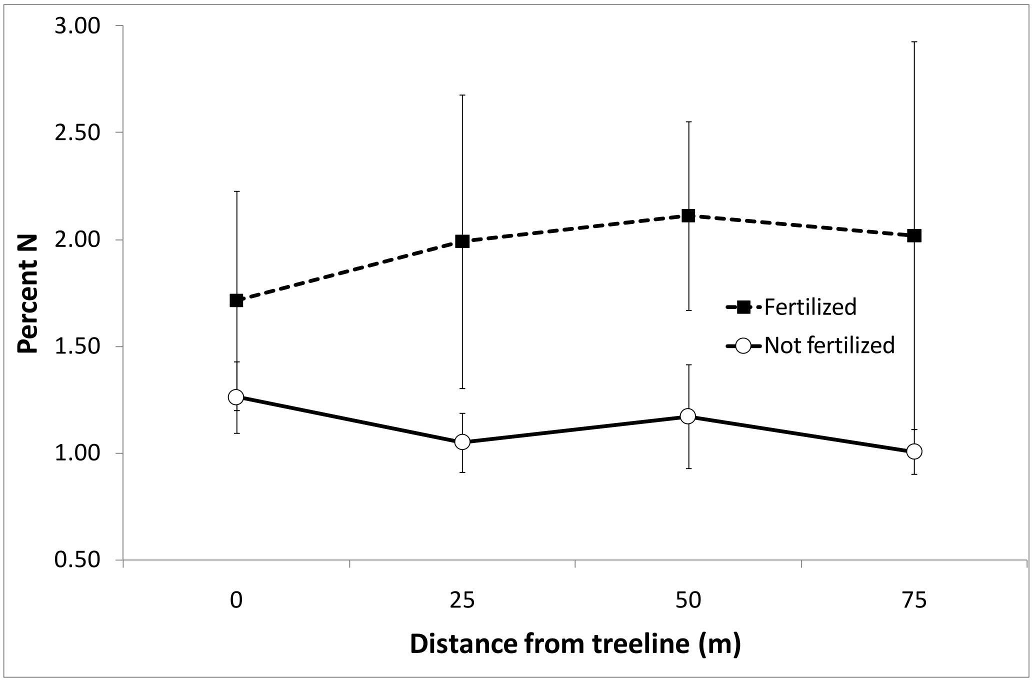

Figure 2.10: Percent N of *S. lanceolatum* as a function of fertilizer treatment and distance from the treeline. Overall the data appear to mirror the C:N data above; percent N is generally similar within treatments, but lower in unfertilized plants than in fertilized plants regardless of meadow location. Standard deviations are indicated.

Variable	Effects	Treeline (0m)		25m		50m		Apex (75m)		Significance	
		$\overline{x}$	S.D.	$\overline{x}$	S.D.	$\overline{x}$	S.D.	$\overline{x}$	S.D.	F	*p*
C:N	Not fertilized	34.24	4.30	40.79	6.07	36.80	7.02	42.01	3.96		
	Fertilized	24.48	2.48	21.00	5.26	20.97	5.82	25.11	11.03		
	Fertilizer									121.69	<0.001
	Location									2.25	0.09
	Fert x loc									2.10	0.108
% N	Not fertilized	1.26	0.17	1.05	0.14	1.17	0.24	1.01	0.11		
	Fertilized	1.71	0.51	1.99	0.69	2.11	0.44	2.02	0.91		
	Fertilizer									124.18	<0.001
	Location									2.28	0.088
	Fert x loc									2.35	0.080

Table 2.5: The effects of meadow location and fertilizer treatments on the C:N and percent-N of *S. lanceolatum*. $\overline{x}$ refers to mean, S.D. refers to standard deviation.

2.3.6 Effects of nutrient enrichment on *S. lanceolatum* nitrogen content relative to the treeline

Fertilized *S. lanceolatum* had significantly lower C:N ($F_{1,75} = 121.69$, $p < 0.001$; Fig. 2.9) and significantly higher percent N ($F_{1,75} = 124.18$, $p < 0.001$; Fig. 2.10) than their unfertilized counterparts irrespective of meadow location. Although a visual examination of the data suggests that nutrient supplementation had less effect near the treeline, the interaction between location and fertilizer treatment was not significant for C:N ($F_{3,72} = 2.10$, $p = 0.108$), and was only marginally significant for percent N ($F_{3,72} = 2.35$, $p = 0.080$).

2.4 Discussion

Near treeline, *S. lanceolatum* grows in greater abundance, and is more densely clumped, than elsewhere in the meadow. Plant size (number of stems), however, is essentially constant throughout my study meadows. These two patterns suggest that most *S. lanceolatum* biomass is near treeline at these sites, although I did not measure biomass directly. While the difference in altitude between my treeline and meadow apex sampling sites is only 10s of meters, my results nonetheless support the general trend identified by Jolls and Bock (1983) of decreasing *S. lanceolatum* abundance with altitude. However, the difference in altitude is correlated with other changes, for example in moisture and cover, and so the pattern is not likely due only to altitude *per se*.

Mean inflorescence number/plant was highest in mid-meadow in both meadows, though significantly so only in meadow G. Likewise, although the differences were not significant, meadow apex had more inflorescences/plant than did near-treeline in both meadows. This likely explains the lack of a significant effect of location relative to the treeline on inflorescence abundance, as the higher inflorescence rate away from the treeline partially offset the greater absolute number of flowers near the treeline.

Unexpectedly, nutrient supplementation had no effect on the rate of *S. lanceolatum* inflorescence production, though it did significantly increase percent N and decrease C:N. Although inflorescence production was greater in the fertilized plot for 3 of my 4 experimental plot pairs, the result is confounded by the results from one plot pair, where the unfertilized plot had greater inflorescence production (Fig. 2.4). In contrast, fertilized plants produced significantly more flowers than did unfertilized plants; this is in keeping with results from other clonal alpine species (Soudzilovskaia and Onipchenko, 2005; Soudzilovskaia *et al.*, 2005). The experiment would need to be repeated with a larger number of plots to better assess the significance of these results, and it is also possible that a longer period of fertilization would produce a greater increase in both inflorescence and flower production, as has been shown in other alpine species (Munoz *et al.*, 2005). In *S. lanceolatum* inflorescences develop from pre-existing stems, and my results therefore imply that this transition is not strictly controlled by nutrient availability, whereas flower production is more nutrient limited. In general, nutrient supplementation produced a slight positive effect on *S. lanceolatum* flowering, but this was primarily through the increased number of flowers. The magnitude of this effect decreases marginally with proximity to the treeline, indicating that nutrients may be more limited away from the treeline, at least with respect to inflorescence production, though again the experiment would need to be repeated to confirm this with more certainty.

More interestingly, there was a greater increase in the number of *S. lanceolatum* plants between 2009 and 2010 in fertilized plots, and this effect was more pronounced with greater distance from treeline. Because *S. lanceolatum* grows slowly and some young plants may be so small that they are hidden and elude censusing, this apparent population increase may be the result of higher overwinter survival of seedlings in fertilized plots, and perhaps also faster growth of seedlings which might have escaped census in 2009.

Taken together, the results of these assays give a sense of the variability in *S. lanceolatum* abundance and growth with relation to the treeline. *Sedum lanceolatum* is found more abundantly close to the treeline, possibly for three reasons. First, near-treeline habitat likely affords more moderate overwinter temperatures and more soil moisture due to deeper snow pack (reviewed e.g. in Wipf and Rixen (2010)), while also offering lower desiccation risk during the growing season as runoff water moves down from the meadow apex. At the meadow apex, by contrast, plants are exposed to drying and cooling winds, more intense sunlight, and ephemeral sources of moisture as rain water quickly filters through the coarse, rocky soil. Near-treeline habitat may also be protected from extreme wind by the forest edge, although edge effects may also lead to more extreme winds in some cases (Holtmeier and Broll, 2005). If seedling survival is higher near the treeline, this may explain the greater clumping observed in near-treeline *S. lanceolatum*, as many seeds may germinate and grow near their parent plant. Likewise, higher seedling survival could explain the low rate of inflorescence production I observed here—many *S. lanceolatum* plants may simply be too immature to flower.

Second, near-treeline habitat appears to be less nutrient-limited than habitat farther from the treeline. Snow tends to accumulate more and melt more slowly near the treeline (Walsh *et al.*, 1994), and snow cover is an important mediator of growth in alpine plants (Stinson, 2005; Huelber *et al.*, 2006; Jonas *et al.*, 2008). Snow cover can affect nutrient cycling (Hood *et al.*, 2003; Freppaz *et al.*, 2008; Liptzin and Seastedt, 2009), insulating and promoting the survival of soil microbes that retain N which might otherwise be washed away during spring melt, and subsequently releasing it to plants (Brooks *et al.*, 1998). In this light, the increased population growth of fertilized *S. lanceolatum* away from the treeline may be because nutrient availability near the treeline is not limiting; that is, *S. lanceolatum* there is limited by the availability of space, and not by microclimate extremes or, as may be the case near the meadow apex, nutrients. Nutrient availability can affect foraging by herbivores (De Bruyn *et al.*, 2002; Cornelissen *et al.*, 2008), suggesting that, if nutrients are less limiting near the treeline, the quality of *S. lanceolatum* as a host plant may be variable relative to the treeline as well. However, while the difference between C:N and percent N of fertilized and unfertilized plants near the treeline appeared, qualitatively, to be less than that elsewhere in the meadow, this was not supported statistically. If fertilization had a similar effect regardless of meadow location, this implies that the baseline state of nutrient availability for *S. lanceolatum* is also similar throughout the meadow. A more detailed analysis with a larger sample size would help to confirm this result. Then again, proximity to treeline might yet affect food quality for herbivores because the winter dormancy induced by snow cover is known to increase toxicity in other plant species (Ralphs *et al.*, 2002), including (anecdotally) in *S. lanceolatum* (Guppy and Shepard, 2001).

Third, if the majority of seeds and vegetative cast-offs come to rest downhill of their origin (Thompson and Katul, 2009), then establishment, and possibly persistence, of *S. lanceolatum* would be greater near treeline. My results show that *S. lanceolatum* growing on slopes may partially counteract this process with taller inflorescences—that is, taller inflorescences, which might increase wind dispersal of seed, may be adaptive on sloped sites. Intriguingly, while *S. lanceolatum* on slopes produced much taller inflorescences than plants from flat areas of the meadow regardless of cover, cover affected inflorescence height differently depending on the slope. In sloped sites, cover was associated with reduced inflorescence height, whereas at flat sites cover was associated with taller inflorescences. Because sloped sites tend to occur at slightly lower elevation than flat sites, which are more common near the meadow apex, it is possible that *S. lanceolatum* on slopes is exposed to more runoff water, and potentially nutrients, than are plants in flat areas. Thus, plants in sloped, open habitat may be able to produce taller inflorescences than those in sloped, covered habitat because they are comparatively free from competition. Conversely, cover may buffer microclimate for *S. lanceolatum* in flat habitat, and any increase in competition with neighbours may nonetheless give a net benefit when compared with exposure to moisture and light stress in open habitats (Klanderud and Totland, 2005*b*). If so, these results would be in line with those of Callaway *et al.* (2002), who showed that positive interactions with neighbours were greater with increasing environmental stress in alpine environments. The significant interaction between slope and cover with respect to flower number appears to be driven almost entirely by the elevated flower counts in sloped, open sites, compared to the flower numbers found in the other 3 treatment combinations. Since I demonstrated a strong effect of nutrient supplementation on flower counts, yet flower numbers did not differ consistently between sloped and flat sites, it seems likely that nutrient availability does not drive my results. As above, one possible answer is that *S. lanceolatum* is outcompeted by other plants, and that the presence of cover (and thus, presumably, competitive interactions with a diversity of other plants) on sloped sites negates the assumed benefits of growing there. However, at flat, covered sites, the protection afforded by cover may essentially cancel out the negative effects of competition. In any event, it remains unclear whether the apparent effect of slope on inflorescence height is the result of developmental plasticity, local adaptation resulting from limited seed dispersal, or microhabitat characteristics such as moisture, soil composition, or nutrient availability. Overall, these results suggest that by producing both taller inflorescences and, at least on open slopes, more flowers on each inflorescence, *S. lanceolatum* growing on slopes may be able to partially offset the expected pattern of seeds moving down-slope toward treeline. Nevertheless, as shown above, *S. lanceolatum* remains considerably more abundant near the treeline than elsewhere in the meadow.

Finally, I show that *S. lanceolatum* food quality is “better” near the treeline, at least with respect to N content. However, whether this represents truly better (i.e., higher available N) or in fact worse (i.e., higher N-based defensive compounds) food quality is unclear. This spatial trend is strongest at the start of the growing season and apparently dissipates with time; given that its samples were collected in mid-summer this may explain why the effect of nutrient supplementation on C:N or percent N did not vary with distance from treeline. In general the pattern I identify of elevated N early in the season is in line with existing research: though N may be limited in alpine systems where meltwater exports N to lower elevations (Brooks *et al.*, 1998; Hood *et al.*, 2003), greater snow accumulation near the alpine treeline can reduce N loss by insulating N-retaining soil microbes against the cold (Brooks *et al.*, 1997, 1998; Brooks and Williams, 1999; Schimel *et al.*, 2004). Likewise, plants growing in stressful environments, such as the more exposed, upper regions of alpine meadows, may have lower foliar N, and thus higher C:N (Mattson, 1980). Further, regardless of N availability, high levels of photosynthesis tend to raise C:N as they produce carbohydrates, thus diluting existing N within plant tissues (Drake *et al.*, 1997; Hughes and Bazzaz, 1997). If *S. lanceolatum* growing in the exposed meadow regions away from the treeline shows increased photosynthesis, this would be yet another potential explanation for the pattern I observed.

Adding a final layer to the complexity, many *Sedum* species represent an intermediate step in the evolution from C_3 to CAM photosynthesis, and display inducible CAM photosynthesis (Groenhof *et al.*, 1986; Lee and Griffiths, 1987; Martin *et al.*, 1988*b*,*a*; Borland and Griffiths, 1990, 1992; Gravatt and Martin, 1992; Conti and Smirnoff, 1994). C_3 photosynthesis, while efficient with respect to carbohydrate yield, results in extremely high transpirative water loss (Raven and Edwards, 2001), and is therefore less suited to hot or moisture-limited environments. In CAM photosynthesis the leaf stomata open at night, allowing CO_2 to enter and be fixed as malic acid, which is then stored until daytime (Cushman, 2001). During the day this stored source of carbon is released to the light-requiring Calvin-Benson-Bassham cycle and photosynthesis proceeds with the stomata closed, thereby conserving water. Inducible CAM plants, although they may be incapable of full-efficiency CAM photosynthesis, can facultatively switch between C_3 (where moisture is abundant) and near-CAM (under moisture stress) photosynthesis (Cushman, 2001; Black and Osmond, 2003; Herrera, 2009). A plant's photosynthetic pathway results in unique internal chemistry, and may alter the costs and benefits of responding to environmental stress. For example, in several species inducible CAM is associated with elevated reproductive output under drought stress (summarized in Herrera, 2009). In any case, it is possible that *S. lanceolatum* growing near the meadow apex is undergoing CAM photosynthesis, while plants growing near the treeline are instead undergoing C_3 photosynthesis. Further, as in other plants (Tevini *et al.*, 1991; Veit *et al.*, 1996), the congener *S. album* produces phenolic compounds in response to excessive light as a kind of "sunscreen" (Reuber *et al.*, 1996; Close and McArthur, 2002), altering the plant's chemistry both directly (Roberts and Paul, 2006), and indirectly through its effects on the CAM pathway (Bachereau *et al.*, 1998). This raises the possibility that *S. lanceolatum* growing in open meadow, where drought may be severe during the summer, may exhibit markedly different internal chemistry, as well as different temporal variation in that chemistry, from *S. lanceolatum* growing near the treeline. There is, however, still evidence to suggest that *S. lanceolatum* near the treeline is of lower food quality for *P. smintheus* larvae at least some of the time: an anecdotal study on *P. smintheus* showed uniquely high mortality in larvae fed a diet of *S. lanceolatum* collected immediately after snow melt (Guppy and Shepard, 2001).

Considering these lines of evidence, I conclude that *S. lanceolatum* shows considerable variation in its growth, spatial distribution, response to nutrient supplementation, and quality as food, with respect to distance from treeline, and that near-treeline habitat appears to be suitable for its growth. The general patterns of this variation are summarized in Table 2.6. I further suggest that because of its high density near the treeline and its importance as the larval host plant of *P. smintheus*, *S. lanceolatum* shows promise for the study of plant-herbivore interactions in relation to the treeline, and to a dynamic alpine treeline advancing into open meadows. In the chapters that follow, I examine how these patterns of *S. lanceolatum* abundance, quality, and growth, influence *P. smintheus* oviposition (Chapter 3), larval herbivory (Chapter 4), and in turn, how they may be influenced by larval herbivory (Chapter 6). Throughout, my overarching goals are to examine variation in a plant-herbivore interaction in relation to distance from the alpine treeline, to speculate upon the potential consequences of continued tree encroachment for insect herbivores, and to explore the importance of considering larval needs when assessing habitat for Lepidoptera in general.

	S. lanceolatum attribute									
	Not fertilized						Fertilized			
Location	Abund.	Clumping	Quality	Inflor.	Flowers	Growth	Quality	Inflor.	Flowers	Growth
Treeline	✪	✪	✪	▽	▽	▽	✪	☆	✪	▽
Middle	▽	▽	☆	☆		☆	✪	☆		☆
Apex	▽	▽	▽	☆		☆	✪	☆		✪

Table 2.6: Summary of variation in *S. lanceolatum* attributes as a function of distance from the treeline. Circled stars (✪) represent high levels of a given attribute, clear stars (☆) represent medium levels, while clear triangles (▽) represent low levels. This representation is intended to provide a visual overview of broad patterns, and is not meant to accurately represent the exact relationships between the variables, the significance of any differences, and so on.

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Chapter 3

Oviposition preferences of the haphazard egg laying butterfly, *Parnassius smintheus*, in relation to host plant abundance and quality

3.1 Introduction

The ability of female phytophagous insects to identify, and lay eggs upon, plants suitable for their offspring is well documented (e.g. Thompson and Pellmyr, 1991). This pattern of oviposition has often been explained in the context of the preference-performance hypothesis (Jaenike, 1978), which proposes that offspring will survive and grow best, and thus female fitness will be maximized, when oviposition preference aligns with host suitability.

Hence, insects such as the Yucca moth (Aker and Udovic, 1981; Pellmyr, 2003) or heliiconine butterflies (e.g. Benson, 1978), where females selectively oviposit not only on the host but on those specific parts of the host most suitable for larval performance, come as no surprise. For many species, females choose hosts on which larval performance is, on average, better than that on hosts that they do not choose (Thompson, 1988). Conversely, a minority of phytophagous insects oviposit preferentially on non-host plants (e.g. Chew and Robbins, 1984; Mitter *et al.*, 1991; Finch and Collier, 2000)—apparently making oviposition "mistakes." Explanations for such "sub-optimal" behaviour include the neural constraints hypothesis (Bernays, 2001; Janz, 2003), which holds that females' ability to finely differentiate among potential oviposition targets is impaired by other, conflicting demands on their limited neural capacity. Alternately, "sub-optimal" oviposition strategies may not actually be sub-optimal: ovipositing away from the host plant may prevent predators or parasitoids from using the host plant as a "rendezvous" point (Dethier, 1959*a*; Singer, 1984; Mappes and Kaitala, 1995), or the host plant itself may have anti-egg defenses (Petzold-Maxwell *et al.*, 2011). Likewise, for species with herbaceous host plants that senesce over winter, oviposition on the host plant may be less important or even counterproductive (Scott, 1986). Again, many of these behaviours have been explained in the context of preference-performance theory: optimal oviposition behaviour is that which maximizes female fitness as it maximizes larval survival.

Surprisingly, many studies show a weak correlation between host preference in females, and offspring performance (Mayhew, 1997), suggesting that additional factors may further influence female oviposition behaviour.

Scheirs *et al.* (2000) document a Dipteran herbivore that oviposits where it feeds, and feeds on hosts which enhance adult, not offspring, performance. Scheirs and De Bruyn (2002) go on to propose that optimal foraging behaviour may be as important to oviposition as optimal oviposition behaviour. In other words, an oviposition strategy that maximizes maternal performance, resulting in a great many eggs laid in sub-optimal locations, may be equivalent in fitness terms to a strategy where maternal sacrifice results in a smaller number of eggs laid optimally. This notion highlights the potential conflict between maximizing maternal performance and maximizing offspring performance.

For butterflies, oviposition behaviour typically results in a close association between host plant and adult butterfly distributions. Numerous studies have shown altered butterfly behaviour in, or emigration from, habitats lacking the preferred host plant (Thomas and Singer, 1987; Odendaal *et al.*, 1989; Schultz, 1998; Matter *et al.*, 2003). However, studies on the determinants of oviposition behaviour in so-called haphazard egg layers (Scott, 1986)—species who lay their eggs "sub-optimally" off the host plant—are lacking. Is their oviposition, in fact, haphazard? Fownes and Roland (2002) showed that females of one such species, the Rocky Mountain apollo butterfly, *Parnassius smintheus* Doubleday (Lepidoptera: Papilionidae), were more likely to oviposit when caged in the presence of their host plant *Sedum lanceolatum* Torr. (Crassulaceae), than in control cages without the host plant. On the other hand, a second study that manipulated host plant abundance while holding nectar flower abundance constant, showed no effect on emigration or oviposition by *P. smintheus* females (Fownes and Roland, 2002; Matter *et al.*, 2003). Likewise, movement of the closely related *P.*

apollo was constrained by the spatial arrangement of both host plant (larval resource) and nectar flower patches (adult resource) (Brommer and Fred, 1999).

As a further complication, early instar *P. apollo* larvae show little host-finding ability (Fred and Brommer, 2010), suggesting that the chances of larvae successfully finding their host plant would be enhanced if females oviposited in dense host plant patches. In Chapter 2 I showed that *S. lanceolatum* grows most abundantly near the treeline, implying that near-treeline habitat may be an ideal location for oviposition—yet a previous study shows that at least *P. smintheus* males avoid the forest delimited meadow edge (Ross *et al.*, 2005*a*)—might females behave similarly? Further, herbivory may produce an induced defense in *S. lanceolatum* (Roslin *et al.*, 2008) that may remain between years. *S. lanceolatum* is also known to produce the gamma-hydroxynitrile glucoside sarmentosin, which is sequestered by *P. smintheus* larvae and persists in adults as well (Nishida and Rothschild, 1995; Bjarnholt *et al.*, 2012). Given that plant defensive compounds act as oviposition stimulants in other Lepidoptera (Honda, 1986; Feeny *et al.*, 1988; Nishida *et al.*, 1987; Pereyra and Bowers, 1988; Roessingh *et al.*, 1992), the balance of olfactory cues available to female *P. smintheus* may be altered in damaged plants, deterring oviposition. Regardless, previous herbivory persists as noticeable scarring which itself may be a deterrent (I discuss this issue further in Chapter 4). Thus, ovipositing females likely must consider the abundance, location, and quality of the larval host plant.

Together, these findings suggest that *P. smintheus* respond to potentially complex and conflicting cues from the host plant (larval resource), nectar flowers (adult resource), and the broader environment (both meadow and surrounding forest) during oviposition. Here, I attempt to elucidate these cues in detail for *P. smintheus*. I ask: (1) What are the relative contributions of host plant and nectar flower abundance in making the decision to oviposit? (2) Given that first instar larvae are unlikely to show strong host orientation ability, while adults are highly mobile, at what scale do host plant and nectar flower abundance guide oviposition behaviour? (3) How do previous herbivory, and the presence of other plant species, affect oviposition behaviour? And finally, (4) I relate these to proximity to the treeline. Better understanding oviposition cues advances the general understanding of how butterflies, and haphazard egg layers in particular, respond to their environment, and helps to elucidate the determinants of habitat quality. Although *P. smintheus* is not currently endangered, it is experiencing habitat loss from encroaching treeline (Roland *et al.*, 2000) such that the relative amount of habitat near and far from the treeline is changing rapidly. My results will have implications for the endangered European congeners *P. apollo* and *P. mnemosyne*, which also oviposit off their host plants.

3.2 Methods

3.2.1 Study site and species

Research took place during the summer of 2010 in the alpine meadows of Jumpingpound Ridge, in Kananaskis Country, Alberta, Canada (50°57'N, 114°55'W), a series of meadows used for long-term study of *P. smintheus* dynamics (Roland *et al.*, 2000; Keyghobadi *et al.*, 2005*a*; Roland and Matter, 2007). Data were collected in two separate but ecologically similar meadows (meadows L and M), near the southern end of Jumpingpound Ridge (Fig 3.1). Meadow L faces west, with a steady incline of ∼10° from treeline to meadow apex, and a sharp forested drop-off on the eastern side, while meadow M faces south and east with a steeper incline of ∼15°. Meadows L and M are physically connected, with only a diffuse barrier of trees between them. Vegetation is similar among meadows and consists of *Dryas* spp., *Salix* spp., grasses and wildflowers, interspersed with *S. lanceolatum*. Vegetation gives way to increased amounts of exposed gravel and rock towards the meadow apex, but species composition remains similar throughout. Meadow M is grassier overall than meadow L, and funnels into a number of long gullies around its base that extend into the subalpine zone. Both meadows are surrounded by subalpine forest dominated by a mix of Engelmann spruce (*Picea engelmannii*), lodgepole pine (*Pinus contorta*) and subalpine fir (*Abies lasiocarpa*). Total meadow area along Jumpingpound Ridge has declined more than 75% over the past 50 years (Roland *et al.*, 2000); forest stands and *krüppelholz* (Holtmeier, 1981) now intervene between some previously connected meadows, and encroach into others. Treeline is well defined in meadow L, with little transitional area between trees, *krüppelholz* and lower shrubs/meadow flora. Due to the western aspect, shade from the treeline is generally negligible until early evening. Treeline in meadow M is locally well defined, but more diffuse and irregularly shaped than in meadow L due to its long gullies.

Parnassius smintheus is common in alpine meadows throughout the Rockies, but is restricted to those habitats with a sufficient supply of its larval host plant, *S. lanceolatum* (Fownes and Roland, 2002), and nectar flowers such as *Arnica* spp., *Senecio* spp., *Aster* spp., and other large yellow or yellow-centered composites. *Parnassius smintheus'* life cycle varies along the species' north-south range; on Jumpingpound Ridge adults fly from mid-July until late August and occasionally into September (Guppy and Shepard, 2001), during which time eggs are laid near, but not on, *S. lanceolatum* (Fownes and Roland, 2002). Female *P. smintheus* typically alight on the ground prior to oviposition, and may walk several centimeters before selecting a suitable site and laying a single egg on the underside of vegetation. Eggs hatch after spring snow melt, usually in late April or early May, and larvae feed continuously before pupating in mid- to late June. Although *P. smintheus* larvae feed on several related host species throughout their range (Guppy and Shepard, 2001), on Jumpingpound Ridge they feed almost exclusively on *S. lanceolatum* and only rarely on the less common ledge stonecrop, *Sedum integrifolium* (Roslin *et al.*, 2008). Since *S. lanceolatum* is relatively long-lived, its spatial arrangement within meadows remains largely constant between years—in other words, host plant distribution at the time of oviposition closely matches that encountered by newly hatched larvae. Similarly, herbivory by all but very early instar *P. smintheus* larvae is unique in character and visible as physical damage to the plant's leaves, remaining visible from previous years. Larvae, especially in later instars, are quite mobile, moving 10s of meters in a single day in search of food (Roslin *et al.*, 2008), and show no antagonism toward each other. Larvae of the congener *P. apollo*, however, show limited mobility in their first instar (Fred and Brommer, 2010). The *P. smintheus* population was at an intermediate density during this study, compared to the 18-year average (Roland *et al.*, 2000; Matter *et al.*, 2003; Roland and Matter, 2013).

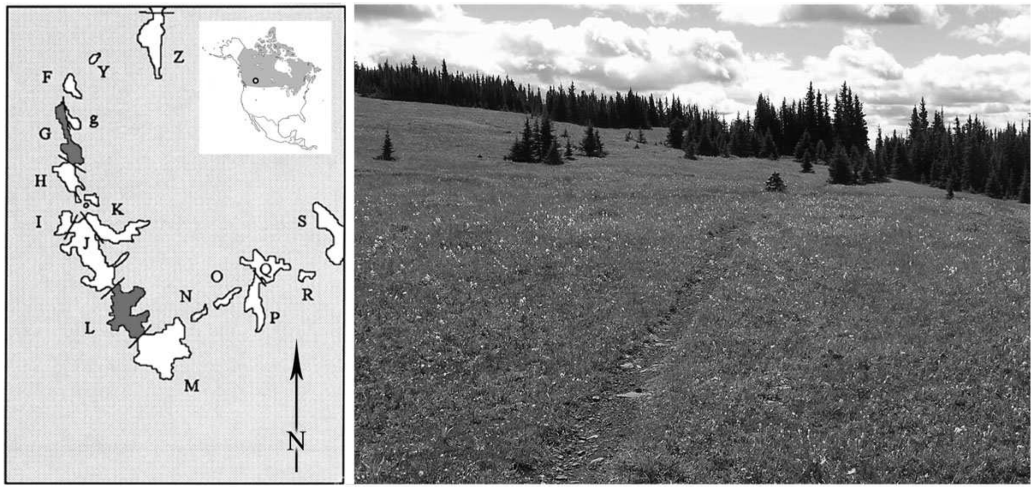

Figure 3.1: Jumpingpound Ridge, showing locations of meadows M and L, and detailed view of meadow L looking south.

3.2.2 Oviposition site preference and previous herbivory

3.2.2.1 Oviposition circles

I addressed all 4 research questions by recording the locations of *P. smintheus* eggs and relating these locations to the distribution, abundance, and quality of host plants, the distribution and abundance of other vegetation, and the distance from the meadow edge (treeline). Over a two week period I captured 20 *P. smintheus* females for this study, working only on sunny, warm, relatively calm days. Because female *P. smintheus* are especially prone to erratic "escape" behaviour after capture, I cooled each butterfly for 10 minutes in a small (12cm x 12cm x 5 cm), opaque, plastic container surrounded by ice packs. I replaced each "calmed" butterfly where it had been captured and waited until activity resumed. Once a butterfly resumed activity, I followed it at a discrete distance; after the butterfly had been active for 10 minutes I assumed that it was no longer under the influence of the cooling treatment and began to record oviposition events. I placed a marker beside each egg as soon as possible after it was laid, taking care not to disturb the butterfly, to a maximum total of 10 eggs per female or until I either lost track of the butterfly or compromised my observations by disturbing it. Because female *P. smintheus* fly quickly, are well camouflaged, and are generally challenging to follow without disturbing, the actual number of ovipositions recorded varied from 1 to 9 per female, with most yielding 3-4 observations. In total, I marked the locations of 75 eggs.

During non-ideal observation periods—cooler or windier days, cloudy periods, etc.—I made a comprehensive census of the habitat immediately surrounding each of the 75 eggs. I did this at 3 scales by creating concentric circles around each egg: a small circle, of radius 15 cm; a medium circle, of radius 30 cm; and a large circle, of radius 50cm. I chose these sizes to encompass the known scale ($\leq$ 50 cm) of *P. smintheus* response to host plant and nectar flower abundance (Fownes, 1999). For each scale I counted all rosettes of the host plant, *S. lanceolatum*, and its less common congener, *S. integrifolium*, as well as all *S. lanceolatum* rosettes visibly damaged by previous *P. smintheus* larval herbivory (by the time this work took place, the larval feeding period had been over for $\sim$1 month). Based on location on the plant, most damage was caused by feeding earlier in the year of the study (most feeding occurs near the tip of the rosette, such that damaged leaves appear to move down the rosette as it ages). However, this work took place in August, by which time all damaged leaves had developed scars, and it is therefore possible that a small number of the damaged plants were damaged in the previous year, or in both the current and previous year. I also counted all inflorescences of every flower species observed to be a nectar source (K. Illerbrun, S. Matter, and others, *pers. obs.*) for *P.*

smintheus: *Agoseris glauca*; *Arnica angustifolia*; *Erigeron* spp.; *Hedysarum* spp.; *Potentilla* spp.; *Sedum lanceolatum*; *Senecio* spp.; *Solidago multiradiata*. Next, I recorded the presence of every other plant species within each circle. Plants were identified to species where possible and ecologically relevant, and to genus in all other cases. For example, while there are several ground covering *Dryas* species that may be found in the meadows of Jumpingpound Ridge, they are broadly similar in growth form and ecological relevance as ground cover and potential oviposition sites for *P. smintheus*. Finally, I recorded the distance to treeline from each egg, and the substrate (plant species, leaf litter, rocks, etc.) upon which the egg was laid.

3.2.2.2 Control circles

To serve as a benchmark against which to compare the "oviposition" circles, I established and censused 30 control locations using the same method as for the egg circles, but randomly chosen as follows: First, I overlaid a 20m x 20m coordinate grid on maps of both study meadows and recorded the coordinates of all intersection points that fell within known *P. smintheus* habitat. For example, meadow L contains a small, bog-like region where *P. smintheus* is seldom seen, and this area was excluded from the coordinates. Next, I randomly drew 30 coordinate pairs out of a hat. Finally, I used visual references to go to each location thus chosen, blindly threw a butterfly net, and established my census circles as indicated by the net's tip.

3.2.2.3 Species censused

In total, between both the experimental and control circles, I encountered 74 distinct species or, where identification to species was either not possible

List of all species/taxa censused (experimental and control circles)

- *Achillea millefolium*
- *Agoseris glauca*
- *Allium* spp.
- *Anaphalis margaritacea*
- *Androsace chamaejasme*
- *Antennaria* spp.
- *Arabis* spp.
- *Arctostaphylos* spp.
- *Arnica angustifolium*
- *Aster* spp.
- *Astragalus* spp.
- *Besseya alpine*
- *Botrychium lunaria*
- *Campanula* spp.
- *Cardamine pennsylvanica*
- *Carex* spp.
- *Castilleja* spp.
- *Cerastium* spp.
- *Crepis elegans*
- *Delphinium* spp.
- *Dodecatheon pulchellum*
- *Draba* spp.
- *Dryas* spp.
- *Epilobium latifolium*
- *Equisetum* spp.
- *Erigeron* spp.
- *Eriogonum umbellatum*
- *Fragaria virginiana*
- *Galium* spp.
- *Gentiana* spp.
- *Geranium* spp.
- Grasses
- *Hedysarum* spp.
- *Juniperus* spp.
- *Lathyrus* spp.
- Lichens
- *Minuartia* spp.
- *Mitella nuda*
- Mosses
- Mushrooms
- *Myosotis asiatica*
- *Oxyria digyna*
- *Oxytropis sericea*
- *Penstemon procerus*
- *Phacelia* spp.
- *Picea engelmannii*
- *Plantago* spp.
- *Polemonium pulcherrimum*
- *Polygonum viviparum*
- *Populus* spp.
- *Potentilla fruticosa*
- *Potentilla gracilis*
- *Potentilla nivea*
- *Pulsatilla patens*
- *Rhinanthus minor*
- *Salaginella* spp.
- *Salix* spp.
- *Saxifraga bronchialis*
- *Saxifraga cernua*
- *Saxifraga occidentalis*
- *Sedum integrifolium*
- *Sedum lanceolatum*
- *Senecio* spp.
- *Sibbaldia procumbens*
- *Silene* spp.
- *Smelowskia calycina*
- *Solidago multiradiata*
- *Taraxacum officinale*
- *Thalictrum* spp.
- *Vaccinium* spp.
- *Valariana* spp.
- *Vicia americana*
- *Viola adunca*
- *Zigadenus elegans*

Table 3.1: A complete list of all species censused for the project.

or not ecologically relevant to *P. smintheus* oviposition, genera. These species are listed in table 3.1.

Additionally, the following were included as continuous variables: abundance of intact *Sedum lanceolatum*, abundance of *S. lanceolatum* damaged by previous herbivory, abundance of nectar flowers listed above both collectively (irrespective of species) and individually, and distance in meters to the meadow edge (treeline).

3.2.3 Analysis

3.2.3.1 Oviposition circles

To control for the uneven number of ovipositions made by individual females I averaged habitat values from all eggs laid by each female, giving a single, integrated measure of habitat for each of the 20 butterflies tested. Thus, my experimental group consisted of 20 estimated habitat variables, compared to a control group of 30 estimated habitat variables. I used logistic regression with a combination of backwards and forwards model selection, validated with Akaike's Information Criterion (AIC), to estimate the probability that a plot is an "egg" plot or a "control" plot, at each scale (S, M, L). To maintain tractability, I omitted from the analysis any variable that appeared in fewer than 5 of the 50 total plots at the small scale, 10 of the total at the medium scale, and 15 of the total at the large scale; variables included in the analysis for each scale are summarized in Table 3.3. Analyses were performed using R (R Development Core Team, 2012).

3.2.3.2 Oviposition substrate preference

Of 75 eggs, 72 remained clearly visible where they had been oviposited at the time of the vegetation census. Eggs were laid on a variety of species as summarized in Table 3.6. I used Ivlev's Electivity Index (Ivlev, 1961) and Strauss's Linear Index (Strauss, 1979) to determine female preference for oviposition substrate materials. Ivlev's index was initially conceived to estimate the degree to which a predator prefers, or avoids, a given prey species. It is defined as:

$$E = \frac{r_i - p_i}{r_i + p_i}$$

where E is the measure of electivity, r_i is the relative proportion of prey *i* in the predator's diet, and p_i is the relative proportion of prey *i* in the environment. In this case, the proportion of observed oviposition that occurred on substrate *i* is represented by r_i, while the percent-cover of substrate *i* in the environment, determined by analyzing the photos described in Chapters 2 and 4, and supplemented by 24 additional survey plots exclusive to this analysis, is represented by p_i. Ivlev's index, *E*, can range from -1 to +1, with positive values denoting active selection, near-zero values random selection, and negative values avoidance or inaccessibility. One criticism of Ivlev's index is that it has wide confidence intervals where values of p_i are very low (e.g. Strauss, 1979). Since several of the oviposition substrates considered here are very uncommon, I also calculated Strauss's Linear Food Selection Index (*L*), which addresses this concern (but, conversely, has wider confidence intervals for high values of p_i). Strauss's index is defined as

$$L = r_i - p_i$$

and, like Ivlev's index, its output can range from -1 to +1, with near-zero values indicating random selection. Strauss's index produces more conservative output (that is, it gives values near -1 and +1 only in extreme cases where the prey item is abundant but almost never consumed, or *vice versa*) than does Ivlev's index.

In calculating both electivity indices I considered 72 of the 75 eggs from the 20 females (3 eggs disappeared in the time between oviposition and vegetation censusing) and, since individual females oviposited on a variety of substrates, I did not average the preferences of females that laid multiple eggs.

	Initial model?			*p* if retained in final model		
Species/family name	S	M	L	S	M	L
Achillea millefolium	✔	✔	✔	-	-	-
Agoseris glauca	✔	✔	✔	-	-	0.994
Androsace chamaejasme	✔	-	-	(0.043)	-	-
Arnica angustifolium	-	✔	✔	-	0.118	-
Besseya spp.	-	✔	-	-	-	-
Carex spp.	-	✔	-	-	0.044	-
Cerastium spp.	✔	✔	✔	-	(0.045)	-
Dryas spp.	✔	✔	-	-	-	-
Epilobium latifolium	✔	-	-	-	-	-
Erigeron spp.	✔	-	-	-	-	-
Galium spp.	✔	-	-	-	-	-
Grasses	✔	✔	-	0.994	-	-
Hedysarum spp.	✔	✔	✔	-	-	-
Lichens	✔	✔	✔	0.026	-	-
Mosses	✔	✔	-	-	-	-
Oxytropis sericea	✔	✔	✔	-	-	0.014
Polygonum viviparum	✔	✔	-	(0.042)	(0.024)	-
Potentilla fruticosa	✔	✔	✔	-	-	-
Potentilla gracilis	✔	✔	-	-	-	-
Sedum lanceolatum	✔	✔	✔	0.058	0.059	-
S. lanceolatum (damaged)	✔	✔	✔	(0.041)	-	-
Senecio spp.	-	✔	✔	-	-	-
Smelowskia calycina	✔	✔	✔	-	-	(0.015)
Solidago multiradiata	✔	✔	✔	-	-	-
Zigadenus elegans	-	✔	-	-	-	-
Distance to treeline	✔	✔	✔	(0.058)	(0.260)	(0.207)
Nectar flower abundance	✔	✔	✔	-	0.063	-

Table 3.3: Summary of variables included in the initial model for each scale (S, M, and L). Underlined text indicates a continuous variable. Tick marks (✔) denote inclusion in the initial model for a given scale. For variables retained in the final models, *p*-values in parentheses indicate variables with negative effects (irrespective of significance) on the likelihood of oviposition, while variables without parentheses denote a positive effect.

Model	AIC
Initial (SMALL): 22 variables	75.35
Final (SMALL): *Androsace chamaejasme* + Grasses + Lichens + *Polygonum viviparum* + *Sedum lanceolatum* + *S. lanceolatum* (damaged) + Distance to treeline	53.77
Initial (MEDIUM): 23 variables	68.49
Final (MEDIUM): *Arnica angustifolium* + *Carex* spp. + *Cerastium* spp. + *Polygonum viviparum* + *Sedum lanceolatum* + Distance to treeline + Nectar flower abundance	54.52
Initial (LARGE): 15 variables	54.28
Final (LARGE): *Agoseris glauca* + *Oxytropis sericea* + *Smelowskia calycina* + Distance to treeline	49.48

Table 3.4: AIC values for initial and final models for each oviposition scale (small, medium, large). Underlined variables are continuous.

3.3 Results

3.3.1 Oviposition circles

At the smallest scale (≤ 15 cm from the egg), abundance of lichen had a significant positive effect, and abundance of *Sedum lanceolatum* had a marginally significant ($p < 0.10$) positive effect, on the likelihood of oviposition (Tables 3.3, 3.4, and 3.5). Abundance of *S. lanceolatum* previously damaged by herbivory, presence of *Polygonum viviparum*, and presence of *Androsace chamaejasme* all had significant negative effects, while distance from the treeline had a marginally significant negative effect (that is, females were more likely to lay eggs near treeline), on the likelihood of oviposition. Presence of grasses had no significant effect but was retained in the model.

Final model	Null deviance	Residual deviance	Explained deviance
Small scale	67.30	37.77	0.44
Medium scale	66.27	38.53	0.42
Large scale	66.27	39.48	0.40

Table 3.5: Deviance explained by the final model for each scale.

At the medium scale (15-30 cm from the egg), presence of *Carex* spp. had a significant positive effect, and abundance of both *S. lanceolatum* and nectar flowers had marginally significant positive effects, on the likelihood of oviposition. Presence of both *Polygonum viviparum* and *Cerastium* spp. had significant negative effects on the probability of oviposition. Neither presence of *Arnica angustifolia* (positive) nor distance to the treeline (again, negative) had significant effects, but both were retained in the final model.

At the largest scale (30-50 cm from the egg), presence of *Oxytropis sericea* had a significant positive effect, and presence of *Smelowskia calycina* had a significant negative effect, on the likelihood of oviposition. Neither presence of *Agoseris glauca* (positive) nor distance to the treeline (once again negative, as at the other two scales) had significant effects, but both were retained in the final model. Interestingly, at the largest scale, the abundance of *S. lanceolatum* did not affect the probability of oviposition, suggesting that it is a relatively fine-scale cue.

3.3.2 Oviposition substrate preferences

Female *P. smintheus* displayed the strongest preference for ovipositing on grasses and *Cerastium* spp., and the strongest aversion to ovipositing on moss, loose leaf litter and, to a lesser degree, *S. lanceolatum* (Table 3.6).

Substrate	Eggs (obs/exp)	r_i	p_i	E	L
Grasses	26 / 21	0.361	0.286	0.116	0.075
Dryas spp.	15 / 16	0.222	0.228	-0.013	-0.006
Cerastium spp.	8 / 2	0.111	0.028	0.597	0.083
Hedysarum spp.	4 / 3	0.056	0.047	0.087	0.009
Potentilla fruticosa	4 / 3	0.056	0.040	0.167	0.016
Potentilla gracilis	3 / 5	0.042	0.068	-0.236	-0.026
Solidago multiradiata	3 / 2	0.042	0.024	0.273	0.018
Misc. leaf litter	2 / 12	0.028	0.166	-0.711	-0.138
Agoseris glauca	1 / 2	0.014	0.033	-0.378	-0.019
Androsace chamaejasme	1 / 0	0.014	0.005	0.474	0.009
Arnica angustifolium	1 / 0	0.014	0.005	0.474	0.009
Lichen	1 / 3	0.014	0.031	-0.409	-0.014
Mitella nuda	1 / 0	0.014	0.005	0.474	0.009
Moss	1 / 8	0.014	0.117	-0.786	-0.103
Oxytropis sericea	1 / 1	0.014	0.016	-0.067	-0.002
Sedum lanceolatum	0 / 3	0	0.045	-1.000	-0.045

Table 3.6: *Parnassius smintheus* oviposition substrates and preferences. Positive values of both E and L indicate preference, while negative values indicate avoidance.

They showed very mild preference for ovipositing on *Potentilla fruticosa* and *Solidago multiradiata*, and very mild avoidance of *Potentilla fruticosa*, *Agoseris glauca* and lichen. They oviposited almost randomly on *Dryas* spp., *Hedysarum* spp., *Androsace chamaejasme*, *Arnica angustifolia*, *Mitella nuda*, and *Oxytropis sericea*. As expected, Strauss's index gives a much more conservative estimate of electivity for cases of only 1 egg laid on a plant that is itself very uncommon (e.g. *Mitella nuda*). Cases such as moss or leaf litter likely do represent real electivity, however, as the substrates themselves are relatively common. Because neither index ascribes a significance level to its output, these results, at least in cases where both eggs and substrate are extremely rare, should also be interpreted conservatively.

3.4 Discussion

My results confirm that *P. smintheus* does not lay its eggs on *S. lanceolatum*, but rather on a variety of other plant species coincident with *S. lanceolatum*. I also show that despite laying eggs off the host plant, female *P. smintheus* respond most strongly to the abundance and quality of *S. lanceolatum* (Fig. 3.3), and less so to maternal nectar resources, when choosing where to oviposit.

Of 72 eggs examined, 57 were laid on either grasses (26 eggs), *Dryas* spp. (15), or on the stems of *Cerastium* spp. (8), *Hedysarum* spp. (4), or *Potentilla fruticosa* (4). While female *P. smintheus* showed varying degrees of preference for these species (only *Dryas* spp. was electively neutral), they are also united in that, whether dead (Grasses; *Cerastium* spp.; *Hedysarum* spp.) or alive (*Dryas* spp.; *P. fruticosa*), their structures are generally sturdy enough to overwinter without significant decomposition or crushing by snow (Kurt Illerbrun, *pers. obs.*). This may increase the chances of overwinter egg survival. Similarly, though litter contained leaves and twigs from species preferred for oviposition, it is easily blown or washed away, and females avoided it as a substrate for oviposition. These results suggest that, at the very least, *P. smintheus* oviposition is not truly haphazard. Females may oviposit preferentially on substrates that improve the chances of overwinter egg survival, but more work will be needed to understand the nature of, and mechanisms behind, this apparent preference. One possibility is that the act of oviposition itself, where female *P. smintheus* curl their abdomens around to oviposit on the underside of suitable leaves and stems, requires a target that has sufficient structure and is suspended sufficiently off the ground to provide access, and such conditions may be most commonly met by sturdy plants.

That female *P. smintheus* choose to oviposit where larval resources are abundant is perhaps not surprising, given that larvae of *P. apollo* show no ability to locate their hosts at distance (Fred and Brommer, 2010), which would likely favour oviposition in areas of high host plant density. That they prefer to oviposit where *S. lanceolatum* is abundant not only at the finest scale ($\leq$ 15 cm from the egg)—presumably the scale relevant to the earliest instar larvae—but also at a broader scale (15-30 cm from the egg) that would be more relevant for later, more mobile instars, is intriguing. Although only marginally significant, these results are in line with previous results (Fownes, 1999; Fownes and Roland, 2002), and further suggest that females select oviposition sites that provide not only the best odds of initial host plant location by newly hatched larvae, but that also provide for the longer term needs of their offspring.

More surprisingly, at the 15 cm scale—presumably the scale most relevant for newly hatched larvae, which have limited mobility—the abundance of *S. lanceolatum* previously damaged by *P. smintheus* herbivory had a significant negative effect on the likelihood of oviposition. A previous study (Roslin *et al.*, 2008) suggests that *S. lanceolatum* produces an induced defense in response to herbivory that increases with time, although Doyle (2011) was unable to detect any negative effect of herbivory in the previous year on larval growth. While it remains unclear whether previous herbivory induces a defense rendering *S. lanceolatum* less attractive in subsequent years, herbivory does cause lasting scarring and, when defoliation is severe or there is significant damage to a plant's meristem, may retard its subsequent growth. Both chemical defenses and leaf toughness are particular challenges for first instar Lepidoptera (Zalucki *et al.*, 2002) and it is probable that, whether due to greater leaf toughness or defensive chemicals, previously damaged *S. lanceolatum* represents a lower quality food source for neonate *P. smintheus* than do undamaged plants. In this light, it is logical that female *P. smintheus* should avoid such plants when ovipositing. Further, that damaged *S. lanceolatum* has a significant effect only at the smallest scale may reflect differences in mobility between first- and later instar larvae: neonates may be unable to assess and orient towards higher quality hosts (or, more fundamentally, may be physically incapable of moving towards distant hosts even if they can detect them), making maternal avoidance of damaged plants particularly important at the fine scale. Finally, if deterrent volatile cues from damaged plants are involved in female oviposition behaviour, these would probably be most concentrated at the finest scales.

This behaviour is in line with existing research. In general the process by which female Lepidoptera find suitable targets for oviposition may involve orientation towards visual or olfactory cues from the host plant at a distance, as well as assessment of the texture, chemical cues, and other attributes of potential host plants once encountered (Renwick and Chew, 1994). Once a potential host has been encountered, oviposition by many specialist Lepidoptera is stimulated by leaf allelochemicals (Honda, 1986; Feeny *et al.*, 1988; Nishida *et al.*, 1987; Pereyra and Bowers, 1988; Roessingh *et al.*, 1992). However, oviposition may rely on both quantitative and qualitative assessments of host suitability. Many Lepidoptera oviposit in response to a combination of stimulant and deterrent cues from the host plant (Renwick and Radke, 1981; Huang and Renwick, 1993; Huang *et al.*, 1993; Haribal and Feeny, 2003), and this appears to be true of *P. smintheus* as well. Chemical cues released in response to damage of the host plant itself (Renwick and Radke, 1981; De Moraes *et al.*, 2001), or evidence of conspecific feeding (Hilker and Klein, 1989; Li and Ishikawa, 2004; Xu *et al.*, 2006) or merely presence (Liu *et al.*, 2008), may act as a further deterrent to oviposition. In short, females generally select host plants that provide neonate larvae with the best chance of success. However, most studies have examined these issues with respect to species that oviposit directly upon their host plant, and can therefore assess and respond to its specific chemistry via contact chemoreception (Renwick and Chew, 1994).

Species that oviposit directly on their larval host plant must first find potential hosts, and subsequently must assess the quality of that host; however, because eggs are laid directly on the host and neonate larvae are thus at a distinct advantage in locating it, overall host abundance may be a secondary concern. In contrast, species which oviposit off the host must assess the suitability of a *region* for oviposition, taking into account both the abundance and the suitability of hosts within that region. *Parnassius smintheus* oviposits off its host plant, and rarely oviposits on the host (Kurt Illerbrun, *pers. obs.*). In line with predictions, my results show that female *P. smintheus* can assess both the abundance and the quality of *S. lanceolatum* within a small region of the meadow without physical contact with the host plant. Given that *S. lanceolatum* is low-growing and is often surrounded by a diversity of other alpine species, it is unlikely that female *P. smintheus* locate *S. lanceolatum* visually, at least at distance. Rather, as has been shown in many phytophagous insects including Lepidoptera, olfactory cues from the host plant likely guide females on their initial approach (Renwick and Chew, 1994; Bruce *et al.*, 2005). For example, another Papilionid butterfly, *Eurytides marcellus*, oviposits in response to the *relative* concentrations of stimulant and non-stimulant compounds in the host plant, allowing an assessment of host quality that mitigates seasonal variation in host chemistry (Haribal and Feeny, 2003). Perhaps *P. smintheus* oviposits in response to a similar interplay between stimulant (e.g., sufficiently strong olfactory cues from undamaged *S. lanceolatum*) and deterrent (e.g., sufficiently weak olfactory cues from damaged *S. lanceolatum*) cues. Such cues would presumably be airborne and pervade the area being considered for oviposition. Little is known about volatile or other cues released by *S. lanceolatum*, or the ability of *P. smintheus* to detect such cues (I experimentally examine the ability of larval *P. smintheus* to detect and orient towards the host plant in Chapter 5). However, *S. lanceolatum* is known to produce the gamma-hydroxynitrile glucoside sarmentosin, which is sequestered by *P. smintheus* larvae and persists in adults as well (Nishida and Rothschild, 1995; Bjarnholt *et al.*, 2012), and which may *decline* in response to herbivory (S.F. Matter, *unpublished data*). Given that similar plant defensive compounds act as oviposition stimulants in other Lepidoptera (Honda, 1986; Feeny *et al.*, 1988; Nishida *et al.*, 1987; Pereyra and Bowers, 1988; Roessingh *et al.*, 1992), it is possible that the presence of previously damaged plants alters the balance of olfactory cues available to female *P. smintheus*, deterring oviposition, and potentially explaining the avoidance of damaged *S. lanceolatum*.

While female *P. smintheus* oviposited in locations that maximized both the abundance and quality of larval resources, adult resources (nectar flower abundance) had a marginally significant positive effect on oviposition only at the medium scale. Likewise, the presence of a small number of additional potential nectar species, irrespective of whether or not they were flowering, was retained for only the medium and large scales (15-30 cm, and 30 cm-50 cm from the egg). At the medium scale the presence of *Arnica angustifolium* was retained in the model, but non-significant. At the large scale only the presence of *Oxytropis sericea*, which is rarely exploited by *P. smintheus*, had a significant positive effect on oviposition, while the presence of *Agoseris glauca* was retained but non-significant. Aside from overall nectar flower abundance, the individual nectar flower variables that were retained considered only the presence or absence of a given species within a given circle size, and the effect was for both flowering and non-flowering individuals of the species combined. In fact, most individuals of these species do not flower in a given season and would have been visible only as foliage, not nectar resources. Thus their retention in the model may be because their habitat requirements are similar to those of *S. lanceolatum*. Because nectar resources were marginally significant only at the medium scale, while *S. lanceolatum* abundance and quality had significant and marginally significant effects, respectively, on oviposition at the small scale, maternal resources do not appear to be a primary consideration in fine-scale egg location. Moreover, *S. lanceolatum* abundance retained a marginally significant effect on oviposition likelihood at the medium scale as well. However, given the mobility of adult *P. smintheus* compared with neonate larvae, it seems logical that adult resources should influence oviposition decisions more at larger scales. It is also possible that even the large, 50 cm scale used in this study was too small to adequately measure the effect of maternal resources on female location choice. For example, nectar flower abundance is known to influence the movement and emigration of both *P. smintheus* (Fownes and Roland, 2002; Matter and Roland, 2002) and *P. apollo* (Brommer and Fred, 1999), and it is therefore probable that nectar flower abundance influences a female's decision to remain in a given meadow region and commence oviposition behaviour in the first place. Thus, while my results suggest that the abundance and quality of larval, and not maternal, resources guides the choice of a specific, fine-scale location for oviposition, they do not imply that maternal resources are subordinate at all scales.

Several other species that influenced the likelihood of oviposition are more challenging to explain. For example, presence of lichen had a significantly positive effect at the smallest scale, even though female *P. smintheus* showed mild avoidance of lichen as a substrate for oviposition. Likewise, presence of *Cerastium* spp. had a significantly negative effect at the medium scale, despite females' preference for it as an oviposition substrate. Lichen is quite common in open areas throughout the meadow habitat and, as *P. smintheus* females can only oviposit in places that are open enough to be physically accessible to them, it may be that these places are coincidentally home to lichen as well. *Cerastium* spp., by contrast, is quite uncommon, and *P. smintheus* females appear to prefer it as an oviposition substrate. Given this preference, and the fact that *Cerastium* spp. represents only a small proportion of overall vegetation cover, it may be that *Cerastium* spp. at the medium scale would normally be detected, and potentially sought out, by females. In other words, *Cerastium* spp. would not be expected to negatively influence oviposition at the smallest scale, because

P. smintheus females favour it for oviposition, while it might likewise have little effect on oviposition at the largest scale because it may be too far away to be detected, and its presence or absence would not influence oviposition. Additionally, presence of *Carex* spp., *Androsace chamaejasme, Polygonum viviparum*, and *Smelowskia calycina* all had significant effects on the likelihood of oviposition, but have no known or obvious ecological relevance to *P. smintheus*. Again, their retention in the models may be because their habitat requirements are related (either positively or negatively) to those of either *S. lanceolatum* or, less likely, nectar flowers.

Finally, distance to the treeline did not consistently predict oviposition likelihood, although it had a marginally significant negative effect at the smallest scale and it was retained in the model with a negative but non-significant effect for the medium and large scales. Ross *et al.* (2005*b*) previously showed that male *P. smintheus* avoid the forest-delineated meadow edge, but my results suggest that females show no such avoidance, and may in fact choose to oviposit *closer* to the treeline. My result implies that, all else being equal, *P. smintheus* eggs should be laid where larval resources are of sufficient abundance and quality to initiate oviposition, but not with any systematic bias away from the meadow edge. Regardless, whether it represents a true affinity to the treeline is unclear, but it seems reasonable to conclude that male and female *P. smintheus* interact with treeline-delimited meadow edges differently. This result, and its implications for *P. smintheus* habitat use, is considered further in Chapter 4.

Overall, I conclude that, though maternal resources likely play an important role in attracting *P. smintheus* females to, and retaining them in, a given meadow region, they are capable of detecting and ovipositing in response to fine scale variations in both the abundance and quality of *S. lanceolatum*. In doing so they appear to not only provide neonate larvae with the best chance of encountering a suitable host but, by also ovipositing in response to host abundance at a larger scale than may be necessary for neonate survival, they may promote the longer term success of their offspring as well. My results support the notion that haphazard egg layers may not be haphazard at all.

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Chapter 4

Treeline proximity alters an alpine plant-herbivore interaction

4.1 Introduction

The effects of climate change on ecosystems around the world are extensive and well documented (e.g. Parmesan *et al.*, 1999; Walther *et al.*, 2002; Parmesan and Yohe, 2003; Kelly and Goulden, 2008; Parmesan, 2006) and numerous studies report changes in the growth and distribution of high-altitude and high-latitude forests due to global warming (e.g. Luckman and Kavanagh, 2000; Lloyd and Fastie, 2003; Moen *et al.*, 2004; Danby and Hik, 2007*b*; Harsch *et al.*, 2009; Wieser *et al.*, 2009). Elevated temperatures contribute to raising the treeline, where trees give way to shrubs and shorter vegetation, by allowing seedling establishment in previously inhospitable areas (Körner, 1998; Grace *et al.*, 2002; Wieser *et al.*, 2009), and by altering natural fire dynamics (Grabherr *et al.*, 1994; Luckman and Kavanagh, 2000). This phenomenon is particularly important to alpine meadow ecosystems, which are typically surrounded by trees. As treeline has advanced, both size and connectedness of meadows have declined worldwide (Grabherr *et al.*, 1994; Fagre *et al.*, 2003; Millar *et al.*, 2004; Parmesan, 2006).

Though alpine treeline rise is well documented, its consequences for alpine ecosystems are less understood, and research has largely focussed on its potential to reduce and/or fragment alpine habitat. To this end, several studies addressed the effects of treeline rise on the movement and population dynamics of the alpine meadow dwelling Rocky Mountain apollo butterfly, *Parnassius smintheus* (Lepidoptera: Papilionidae). Roland *et al.* (2000) showed that forest encroachment creates physical barriers to butterfly movement, reducing connectivity between populations in neighbouring meadows, decoupling population dynamics (Roland and Matter, 2007) and genetically isolating previously contiguous populations (Keyghobadi *et al.*, 2005*a*). Dirnböck *et al.* (2003) predicted similar effects on alpine vegetation. However, the full range of mechanisms by which rising treeline may affect alpine meadow species is not yet understood, and could include less direct effects. Alpine treeline represents more than an advancing physical barrier: moisture and nutrient gradients, biotic processes, local microclimate (e.g. Stevens and Fox, 1991; Körner, 1998) and snow accumulation (Walsh *et al.*, 1994) vary across the treeline ecotone, altering species richness, spatial pattern, and quality of near-treeline plant communities (Schilthuizen, 2000; Holtmeier and Broll, 2005; Batllori *et al.*, 2009*a*). Increase in plant community heterogeneity, in turn, alters the performance of herbivores (summarized e.g. in Agrawal *et al.*, 2006) which may influence predator and parasitoid species, and so on. Treeline probably casts a wider ecological shadow than is typically considered.

Whereas the response of adult *P. smintheus* to tree encroachment is established, interactions between *P. smintheus* larvae and their host plant in relation to the treeline provide a good opportunity to look for further, perhaps less obvious but no less important, treeline effects. *Parnassius smintheus* larvae feed on lance-leaved Stonecrop (*Sedum lanceolatum*; Crassulaceae), a small perennial succulent common in alpine meadows throughout the Rockies whose abundance and distribution correlate strongly with adult *P. smintheus* population size and dispersal (Fownes, 1999; Matter *et al.*, 2003). *Sedum lanceolatum* appears to produce an induced chemical defense in response to herbivory, rendering already damaged plants unattractive to subsequent larvae, and leading to a relatively diffuse spatial distribution of herbivory (Roslin *et al.*, 2008). As for other alpine plant species (e.g. Ralphs *et al.*, 2002), there is also anecdotal evidence for increased toxicity—a constitutive defense—during, and for a period after, *S. lanceolatum*'s winter dormancy (Guppy and Shepard, 2001); moreover, snow cover (and, therefore, weather) may mediate plant growth in alpine ecosystems (Stinson, 2005; Huelber *et al.*, 2006; Jonas *et al.*, 2008). Since snow accumulates most and thus melts more slowly near the treeline compared to in open meadows, host plants in this "tree zone" may be both physically and chemically unavailable to *P. smintheus* larvae for longer than those in more open parts of the meadow. Adult male *P. smintheus* actively avoid treeline-delimited meadow edges (Ross *et al.*, 2005*a*) but, as I showed in Chapter 3, females display no such aversion, at least during oviposition (Tables 3.3 and 3.4). However, eggs laid near the meadow edge would likely hatch later and neonates would be forced to contend with any edge-effects directly. These factors would serve to isolate larval and adult *P. smintheus* from the full extent of available food resources, and any further rise in treeline should therefore increase the proportion of *S. lanceolatum* which grows in the "tree zone" as overall meadow area decreases. That *S. lanceolatum* also grows most densely near the treeline and in suitable small meadows (Matter *et al.*, 2003) lends broad support to this expectation. The consequence of such a pattern would be reduction in available food for *P. smintheus* larvae as treeline rises, at a rate faster than overall habitat area declines, due to the larger perimeter-to-area ratio found in smaller meadows.

Here, I investigate the potential impacts of rising treeline on larval *P. smintheus* feeding patterns by exploring how larvae interact with the distribution of their host resources, with the broader goal of elucidating likely consequences of continued treeline rise. First, I examine feeding intensity by *P. smintheus* larvae in relation to the treeline, and establish an index of herbivory near treeline, in mid-meadow, and at meadow apex, with the expectation of an "ideal" distribution of herbivory in relation to host-plant abundance across all meadow regions (Fretwell and Lucas, 1969; Bernstein *et al.*, 1991). Second, I examine the spatial patterns of *S. lanceolatum* growth and *P. smintheus* herbivory from the treeline to the meadow apex (the degree to which they are random, clumped, etc., but more importantly, the degree to which the spatial patterns are congruent with each other), with the goal of identifying spatial variation in the species' interaction. There is already evidence that proximity to forest edge may alter plant-herbivore interactions across multiple taxonomic groups, including Lepidoptera (reviewed in Wirth *et al.*, 2008), but many studies report positive effects for the herbivore, and few consider these effects in the context of forest encroachment. More generally, there is evidence of plant-herbivore interactions varying in response to habitat and landscape structure (e.g. Coughenour, 1991; Kruess, 2003; de la Pena *et al.*, 2011). Establishing a trend of differential host plant use by *P. smintheus* larvae in relation to distance from the alpine treeline would suggest another mechanism by which treeline rise may influence populations of an alpine plant species, and its primary insect herbivore, even before the direct effects of tree encroachment are apparent—the decoupling of population size and habitat area (Shoo *et al.*, 2005). My findings will aid habitat assessment and conservation decisions for both *P. smintheus* and endangered congeners elsewhere (e.g. *P. apollo*; *P. mnemosyne*), and are relevant to alpine plant-herbivore interactions generally.

4.2 Methods

4.2.1 Study site and study species

Research took place over 3 years (2006-08) in the alpine meadows of Jumpingpound Ridge, in Kananaskis Country, Alberta, Canada (50°57'N, 114°55'W), a series of meadows used for long-term study of *P. smintheus* dynamics (e.g. Roland *et al.*, 2000; Fownes and Roland, 2002; Roland and Matter, 2007). Data were collected in two separate but ecologically similar meadows, one near the northern (G) and one near the southern (L) end of Jumpingpound Ridge (Fig. 4.1). Both meadows face west, with a steady incline of $\sim 10^{\circ}$ from treeline to meadow apex, and a sharp drop-off on

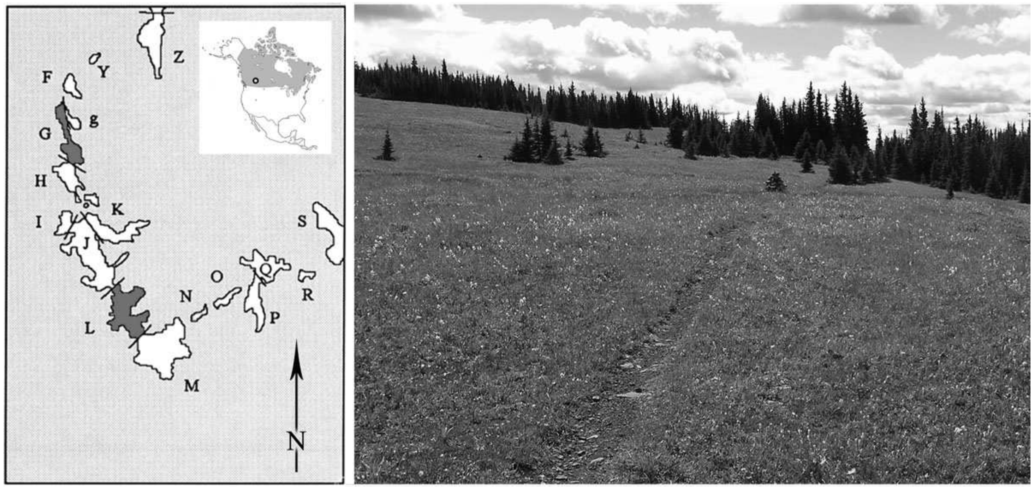

Figure 4.1: Jumpingpound Ridge, showing locations of meadows G and L, and detailed view of meadow L looking south.

the eastern side. Vegetation is essentially the same in the two meadows, consisting of *Dryas* spp., *Salix* spp., grasses and wildflowers, interspersed with *S. lanceolatum*. Vegetation gives way to increased amounts of exposed rock towards meadow apex, but species composition remains broadly the same throughout. Both meadows are surrounded by subalpine forest dominated by a mix of Engelmann spruce (*Picea engelmannii*), lodgepole pine (*Pinus contorta*) and subalpine fir (*Abies lasiocarpa*). Total meadow area along Jumpingpound Ridge has declined more than 75% over the past 50 years (Roland *et al.*, 2000); forest stands and climatically stunted *krüppelholz* (Holtmeier, 1981) now intervene between some previously connected meadows, and encroach upon others. Treeline is well defined in both meadows, with little transitional area between trees, *krüppelholz* and lower shrubs/meadow flora; due to the western aspect, shade from treeline is generally negligible until early evening.

Parnassius smintheus is common in alpine meadows throughout the Rockies, but is limited to areas with a sufficient supply of its host plant, *S.*

lanceolatum (Fownes and Roland, 2002), and nectar flowers like *Arnica* spp., *Senecio* spp., *Aster* spp., and other large yellow or yellow-centered composites. *Parnassius smintheus'* life cycle varies along the species' north-south range, and many of its details remain unknown. On Jumpingpound Ridge adults fly from mid-July until late August and occasionally into September (Guppy and Shepard, 2001), during which time eggs are laid near, but not directly on, *S. lanceolatum* (Fownes and Roland, 2002). Eggs hatch after snow melt, usually in April, with larvae feeding continuously before pupating in mid- to late June. Larvae, especially in later instars, may be quite mobile, moving 10s of meters in a single day in search of food (Roslin *et al.*, 2008), and show no antagonism toward each other. *Parnassius smintheus* population levels were similar in all 3 years of this study (Roland and Matter, 2013).

Although *P. smintheus* larvae feed on several related host species throughout their range (Guppy and Shepard, 2001), on Jumpingpound Ridge they feed almost exclusively on *S. lanceolatum*, and only rarely on ledge stonecrop, *Sedum integrifolium* (Roslin *et al.*, 2008). Since *S. lanceolatum* is relatively long-lived, its spatial arrangement within meadows remains largely constant between years.

Herbivory by all but very early instar *P. smintheus* larvae is visible as physical damage to the host plant's leaves, and can be distinguished from other damage (e.g. trampling or desiccation) by its characteristically clean scars, and by its orientation perpendicular to each leaf's axis (Fig. 4.2). Although some generalist insect or mammalian herbivores may occasionally feed on *S. lanceolatum*, the damage caused by these is negligible compared to herbivory by *P. smintheus*. Recent herbivory produces fresh, green scars, which gradually harden to grey by mid-summer; in this way it is possible to estimate the age of feeding damage—for instance, a hardened, grey scar in spring or early summer indicates herbivory in the previous growing season. Typical herbivory can range from small nibbles to near complete defoliation, but stems are rarely, if ever, eaten (Kurt Illerbrun, *pers. obs.*).

4.2.2 Spatial patterns in *S. lanceolatum* and herbivory

To understand the spatial patterns of *S. lanceolatum* growth and larval herbivory in relation to treeline, I established 4 transects from treeline to meadow apex in each study meadow. I placed transects at arbitrary intervals from each other using a random number generator, and established 5m x 5m quadrats at 20m intervals along each transect, beginning at treeline and terminating at the meadow apex. Quadrat size was chosen to provide a balance between the known scale ($\leq$ 50 cm) of *S. lanceolatum* aggregation (Fownes, 1999; Roslin *et al.*, 2008) and logistical tractability. Thus the number of quadrats in each transect varied in proportion to meadow size, giving a total of 14 quadrats (350m^2) in meadow G and 15 quadrats (375m^2) in meadow L. In meadow G, this protocol resulted in 4 quadrats being placed near treeline, 4 near meadow apex, and 6 in the

Figure 4.2: Late instar *Parnassius smintheus* larva feeding on *Sedum lanceolatum*. Telltale signs of herbivory are clearly visible as blunt scars directly beneath the larva's head. (*photo*: Dave Roth)

intervening mid-meadow area. In meadow L, it resulted in 4 quadrats near treeline, 4 near meadow apex, and 7 in mid-meadow.

After *P. smintheus* larval pupation, I located the position of every *S. lanceolatum* plant in each quadrat to permit spatial analysis, using herbivory amount and pattern as a proxy for larval abundance and distribution. Rather than record coordinates in the field, I developed a digital method which allowed us to record individual plant locations more precisely in the lab. For each quadrat I created 30 highly visible reference points to allow assessment of scale during later analysis, and placed colour-coded markers beside each *S. lanceolatum* plant to denote its herbivory status—herbivory or no herbivory. Once every *S. lanceolatum* plant in a quadrat was marked, I took a high-resolution (10mp DSLR) digital image of the entire plot, capturing the location of all plants to an accuracy of ~1cm. In the lab, using the reference points as a guide, I orthorectified all images for parallax and perspective error using ESRI ArcGIS, "flattening" each image into a 2D square to best show the spatial relationship between plants. I recorded the totals and coordinates of all plants, then further subdivided each image into 100 50cm x 50cm squares, as herbivory is known to be clumped at the scale of 20-50cm (Fownes, 1999; Roslin *et al.*, 2008), and obtained count data for *S. lanceolatum* in each square. Data from 3 quadrats in meadow G were excluded due to problems with accurately orthorectifying the images.

4.2.3 Host abundance and larval feeding intensity

I compared overall *S. lanceolatum* abundance between quadrats from near treeline, mid-meadow and meadow apex using a 1-way ANOVA and Tukey's HSD post-hoc test.

Next, I censused *S. lanceolatum* and recorded attributes not captured in the digital photographs: number of stems (rosettes) and flowers per plant, as well as number of leaves fed upon per plant. I qualitatively assessed herbivory severity, as distinct from the number of points of herbivory, on each plant using a 3-point scale, where 0 = no damage, 1 = light or normal damage, e.g. to tips of leaves, and 2 = heavy damage likely to impair the plant's future development because of its extent (>25% of leaves damaged) and/or location (e.g. completely removing the meristem). The census included the first 200 *S. lanceolatum* plants encountered within 10m of treeline, in mid-meadow, and near meadow apex, for each study meadow, based on the locations of the quadrats discussed in 4.2.2. Due to the scarcity of *S. lanceolatum* near meadow apex in meadow G only 122 plants were encountered and censused; this gave a total of 1122 plants in the survey. Results provided clear estimates of mean number of points of herbivory per host plant. Means are reported ±S.D.

To establish an overall measure of herbivory intensity (herbivory m^{-2}) from treeline to meadow apex, I grouped quadrats as being either near treeline, mid-meadow, or meadow apex, and used the corresponding results of the *S. lanceolatum* survey to estimate actual herbivory in each quadrat with the formula:

$$Amount\,eaten/m^2 = \frac{(\#\,eaten\,plants)(mean\,herbivory\,points/plant)}{25m^2}$$

I also estimated proportion of plants eaten for each quadrat and meadow region using the formula:

$$Proportion\,eaten = \frac{\#\,eaten\,plants/quadrat}{\#\,total\,plants/quadrat}$$

In this way I estimated the number of individual points of herbivory and overall herbivory intensity per unit of area, for both meadows and across my tree-to-apex transects. If *S. lanceolatum* is equally attractive to *P. smintheus* larvae throughout the meadow, amount eaten should be in proportion to host plant density in a given area, and proportion eaten should be similar among areas.

4.2.4 Differential patterns of resource use

I used SADIE (Spatial Analysis by Distance IndicEs) (Perry *et al.*, 1999) to assess the spatial distribution of both *S. lanceolatum* plants and *P. smintheus* herbivory in each of the 29 quadrats. SADIE estimates the distance to regularity (*D*) of spatial data—the cumulative distance that points in the data set would need to move to produce a regular pattern. Thus *D* indicates the degree to which spatial data are aggregated, with a higher *D* indicating greater clumping. *D* is associated with a probability, *P*, the likelihood that a given value of *D* occurs by chance. High *D* with low *P* indicates significant aggregation. In performing this analysis, my objective was not to focus on clumping of hosts or herbivory *per se*, but rather to compare their respective spatial distributions at varying distances from the treeline. With an "ideal" distribution of herbivory, the spatial distributions of *S. lanceolatum* and *P. smintheus* herbivory would be expected to match. That is, I would expect to see similar levels of herbivory per *S. lanceolatum* plant throughout the meadow, resulting in much more *S. lanceolatum* biomass being consumed near treeline, where the host plant is most abundant. I used a 2-way ANOVA to test for significant differences in the spatial patterns (*D*) of hosts and herbivory between locations (treeline, mid-meadow and meadow apex), and orthogonal contrasts to detect specific differences among locations. For this analysis only the presence or absence of herbivory, and not the amount eaten, were considered. If *P. smintheus* larvae respond to their host plants equally across the meadow, the degree of congruence between the spatial patterns of *S. lanceolatum* and herbivory should be similar among locations.

4.3 Results

4.3.1 Host abundance and larval feeding intensity

Sedum lanceolatum abundance decreased from treeline to meadow apex, while percent herbivory increased and actual amount eaten remained similar, regardless of local host abundance (Figs.4.3 and 4.4).

For meadow G, amount eaten was 1.31 individual points of herbivory m^{-2} near treeline, 1.53 points m^{-2} in mid-meadow, and 0.31 points m^{-2} near meadow apex. Herbivory rate was 22% near the treeline (out of a total of n=321 plants; n quadrat^{-1}= 107±43.40), 55% in mid-meadow (n=75; n quadrat^{-1}= 15 ±6.88), and 83% near meadow apex (n=6; n quadrat^{-1}= 3±4.24).

For meadow L, amount eaten was 1.59 individual points of herbivory m^{-2} near treeline, 1.85 points m^{-2} in mid-meadow, and 1.17 points m^{-2} near meadow apex, and herbivory rate was 6% (out of a total of n=2526 plants; n quadrat^{-1}= 631.5±270.10), 32% (n=607; n quadrat^{-1}= 86.71±62.81), and 42% (n=81; n quadrat^{-1}= 20.25±6.13) respectively. Again, sample size was lower near meadow apex, reflecting the relative lack of *S. lanceolatum* plants in this zone.

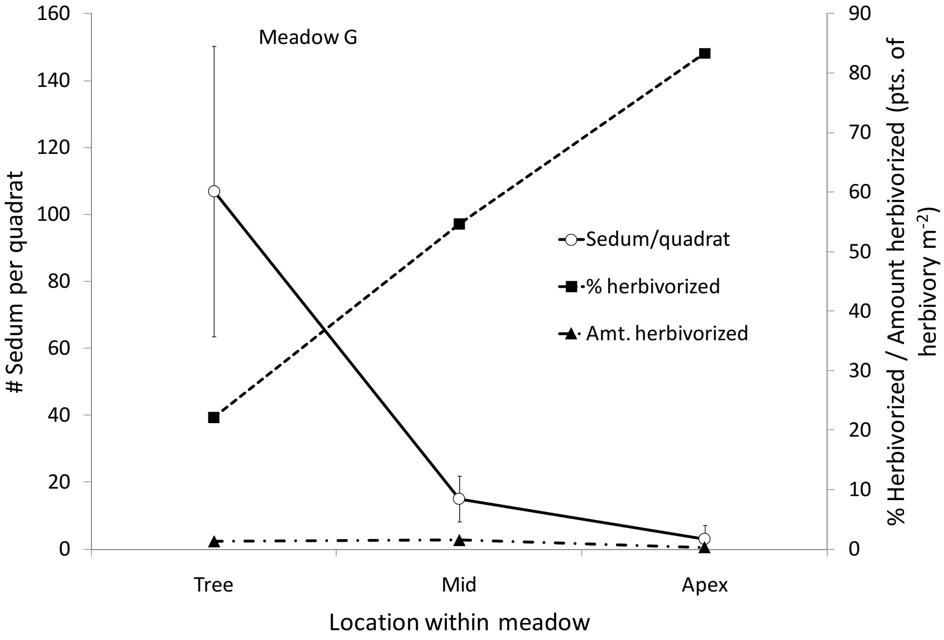

Figure 4.3: Mean *S. lanceolatum* abundance per quadrat, percent herbivory, and amount herbivory m^{-2}from treeline to meadow apex for meadow G only. Open circles (solid line) show mean number of *S. lanceolatum* for all quadrats in each meadow zone, and are represented on the left *y*-axis. Standard deviations are indicated. Closed squares (dashed line) show the percentage of eaten *S. lanceolatum* plants in quadrats from each meadow zone, and are represented on the right *y*-axis. Closed triangles (dash-dot) show the amount eaten in points of herbivory m^{-2}in quadrats from each meadow zone, and are also represented on the right *y*-axis. *Sedum lanceolatum* abundance decreases from treeline to meadow apex, while percent herbivory increases and actual amount eaten remains similar. Compare with Fig. 4.4.

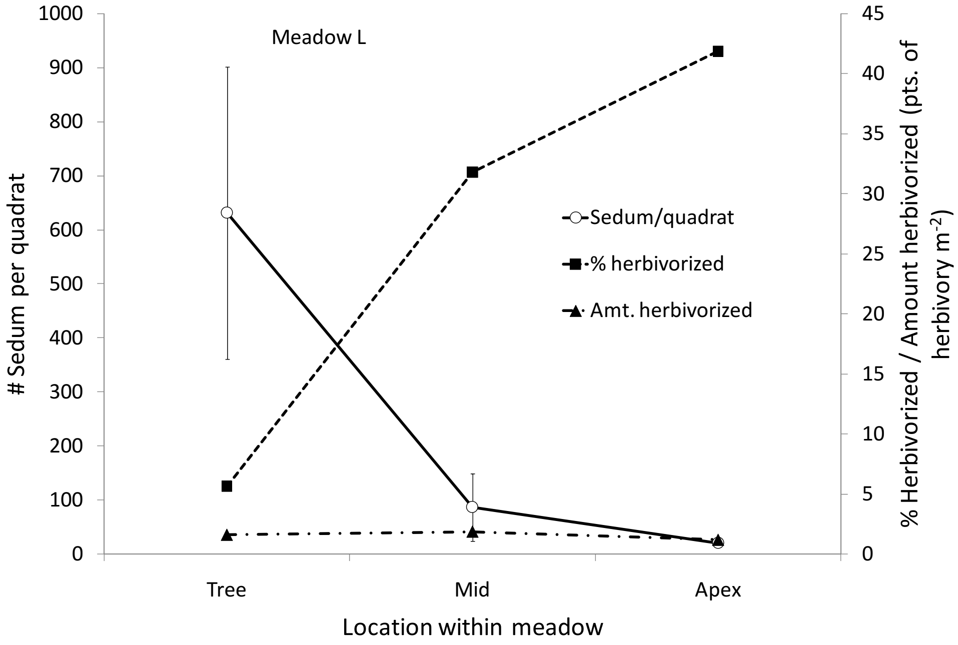

Figure 4.4: Mean *S. lanceolatum* abundance per quadrat, percent herbivory, and amount herbivory $m^{-2}$from treeline to meadow apex for meadow L only. Open circles (solid line) show mean number of *S. lanceolatum* for all quadrats in each meadow zone, and are represented on the left *y*-axis. Standard deviations are indicated. Closed squares (dashed line) show the percentage of eaten *S. lanceolatum* plants in quadrats from each meadow zone, and are represented on the right *y*-axis. Closed triangles (dash-dot) show the amount eaten in points of herbivory m^{-2}in quadrats from each meadow zone, and are also represented on the right *y*-axis. *Sedum lanceolatum* abundance decreases from treeline to meadow apex, while percent herbivory increases and actual amount eaten remains similar. Compare with Fig. 4.3.

There was a significant difference in *S. lanceolatum* abundance from treeline to meadow apex in both meadows ($F_{2,\,22} = 10.74$, $p = 0.001$); specifically, *S. lanceolatum* was more abundant near treeline (631.5±270.10) than in mid-meadow (86.71±62.81; $p = 0.001$) or at the meadow apex (20.25±6.13; $p = 0.002$), and similarly abundant between mid-meadow and apex ($p = 0.886$) (Figs. 4.3 and 4.4, and Table 4.1). Individual plant size was highly variable in both meadows, but generally spanned the same range of sizes: in meadow G mean *S. lanceolatum* plant size was 4.93±4.23 stems/rosette (n=200) near treeline, 5.51±4.13 (n=200) in mid-meadow, and 4.97±3.48 (n=122) near meadow apex. In meadow L it was 5.3±4.15 stems/rosette (n=200) near treeline, 4.72±4.13 (n=200) in mid-meadow, and 4.35±4.28 (n=200) near meadow apex.

The intensity of herbivory generally increased from treeline to meadow apex, and was slightly more intense overall in meadow G than in meadow L. Based on my qualitative index, severity of herbivory increased in meadow G from 1.26 near treeline, to 1.42 in mid-meadow and 1.43 at meadow apex. In meadow L severity decreased from 1.15 near treeline to 1.08 in mid-meadow, before increasing to 1.24 at meadow apex.

4.3.2 Differential patterns of resource use

The spatial patterns of herbivory and total *S. lanceolatum* abundance were unrelated near treeline, but related away from treeline (Fig. 4.5). Away from treeline, the spatial pattern of herbivory by *P. smintheus* closely mirrored the spatial pattern of available host plants—an ideal distribution of herbivory. However, near treeline the pattern of herbivory was significantly more random than that of the host plants. This suggests either that larvae do not feed "ideally" near the treeline, or else that the pattern is still in fact ideal, but based on considerations such as host plant quality or physical accessibility that are unique to near-treeline habitat.

There was a significant interaction between meadow location and D of hosts and herbivory ($F_{2,38} = 5.23$, $p = 0.01$). Orthogonal contrasts between D of hosts and herbivory at different locations showed a difference

Variable	Treeline (a)		Mid-meadow (b)		Meadow apex (c)		Significance		Contrasts
	$\overline{x}$	S.D.	$\overline{x}$	S.D.	$\overline{x}$	S.D.	F	p	
Abundance (both)	631.50	270.10	86.71	62.81	20.25	6.13	10.74	0.001	a-b, a-c
Size (G)	4.93	4.23	5.51	4.13	4.97	3.48	1.22	0.296	-
Size (L)	5.30	4.15	4.72	4.13	4.35	4.28	1.41	0.245	-

Table 4.1: The effect of meadow location on *S. lanceolatum* abundance and size. $\overline{x}$ refers to mean, S.D. refers to standard deviation, and letters (a, b, c) refer to meadow locations used in post-hoc contrasts. For example, “a-b” means that contrasts showed that the variable in question differed between treeline and mid-meadow.

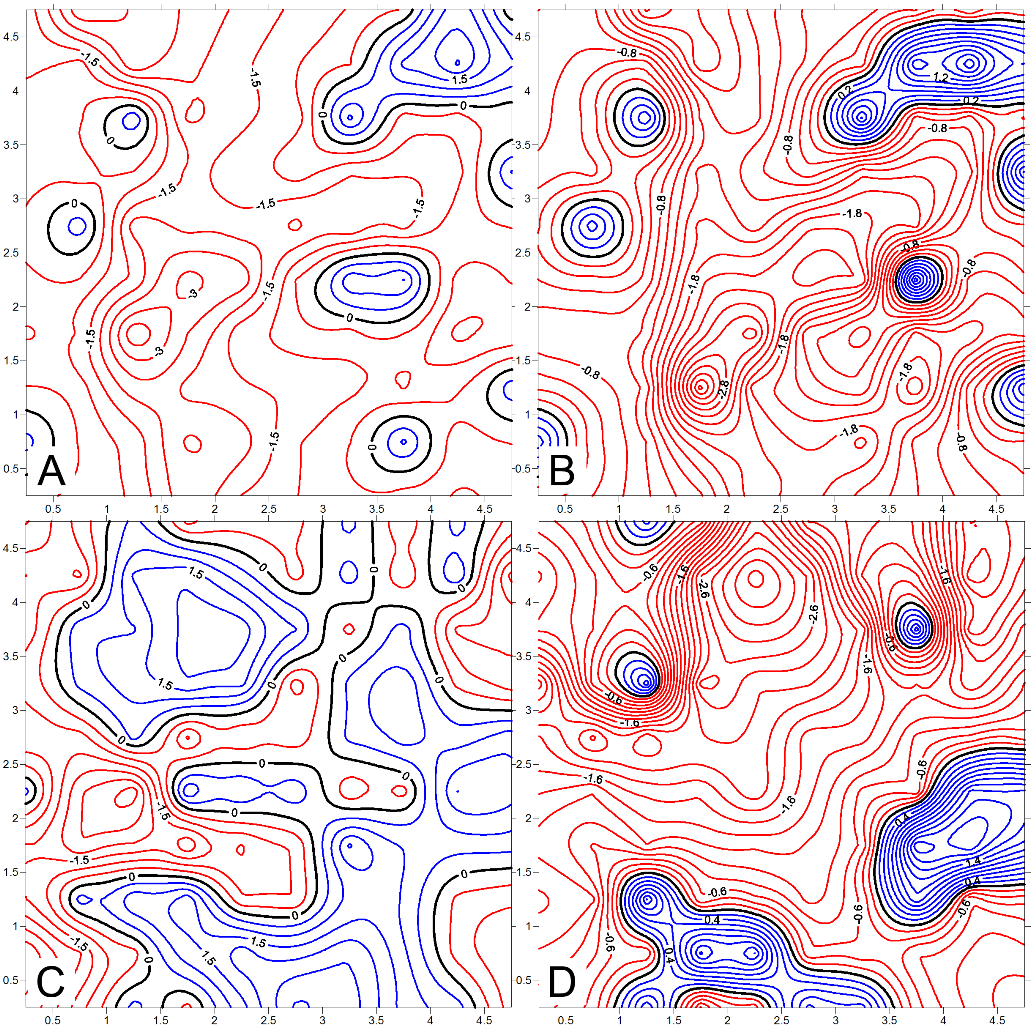

Figure 4.5: Graphical output from SADIE analysis, illustrating the spatial distribution of both *S. lanceolatum* and herbivory by *P. smintheus*. Blue isobars indicate an increasingly clumped distribution, while red isobars indicate a random distribution. (A) illustrates *S. lanceolatum* in an example quadrat located near the meadow apex, while (B) illustrates points of herbivory in the same quadrat. By contrast, (C) illustrates *S. lanceolatum* in a quadrat located near the treeline, while (D) illustrates points of herbivory in the same quadrat. The spatial arrangements of host plants and herbivory are largely congruent near the meadow apex, but unrelated near the treeline (see Fig. 4.6).

(Bonferroni corrected $\alpha = 0.017$) at treeline (host 3363±829 vs. herbivory 1666±595; $F_{1,38} = 24.48$, $p < 0.001$) but not at mid-meadow (1364±634 vs. 866±563; $F_{1,38} = 3.69$, $p = 0.062$) or at meadow apex (1062±402 vs. 808±361; $F_{1,38} = 0.49$, $p = 0.484$), suggesting that the observed interaction is driven solely by the decoupled spatial patterns of hosts and herbivory near treeline. Specifically, near treeline *S. lanceolatum* was clumped ($P_{hosts} = 0.04$) but herbivory randomly distributed ($P_{\text{herbivory}} = 0.21$), while in mid-meadow ($P_{\text{hosts}} = 0.45$ vs. $P_{herbivory} = 0.37$) and at meadow apex ($P_{\text{hosts}} = 0.21$ vs. $P_{herbivory} = 0.22$), distributions of hosts and herbivory were similarly random (Fig. 4.6). Data from two quadrats were classified as outliers (box plot; >1.5x the interquartile range, either above the upper quartile or below the lower quartile) and excluded from the analysis.

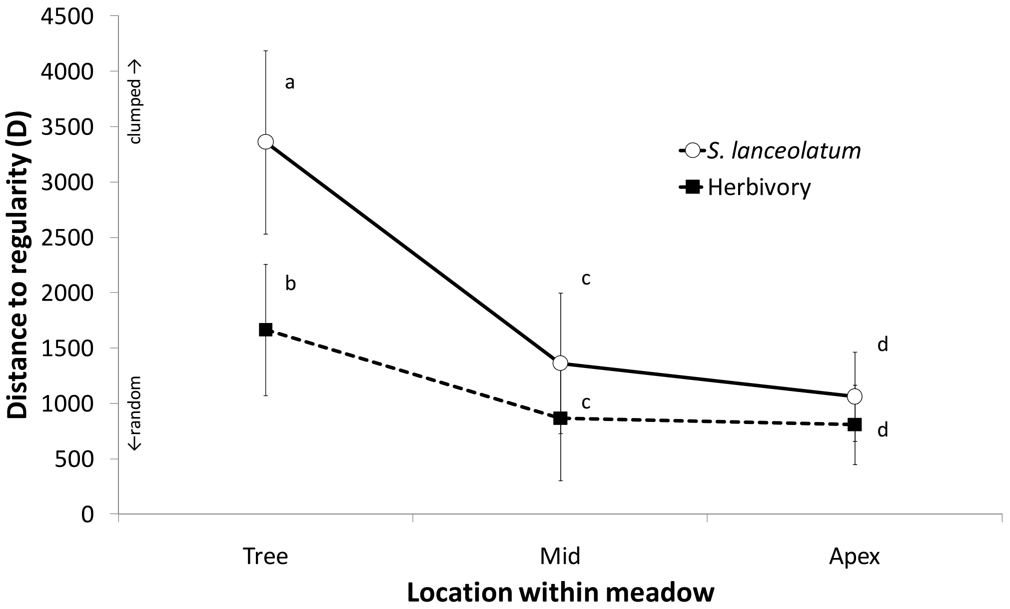

Figure 4.6: Distances to regularity (*D*), or degree of clumping, for the spatial patterns of herbivory and all *S. lanceolatum* from treeline to meadow apex. Higher values of *D* indicate greater aggregation, i.e. non-random clumping of plants. Open circles represent mean *D* for all *S. lanceolatum* in all quadrats, while closed squares represent mean *D* for fed-upon host plants only. Standard errors are indicated. Means with the same letter (within locations) are not significantly different from each other. Spatial patterns of *S. lanceolatum* and larval herbivory are closely related away from treeline, but decoupled near treeline.

4.4 Discussion

My results show a clear trend of altered herbivory on *S. lanceolatum* by *P. smintheus* larvae from treeline to meadow apex, both in terms of the amount of herbivory and the degree to which it is distributed among plants. Two elements of this trend are striking: first, herbivory per unit area—and thus, larvae per unit area—is highly similar both within and between meadows. Second, this similarity amounts to *dis*similarity in herbivory expressed as proportion eaten at each location within the meadows. Near the treeline *P.*

Variable	Treeline (a)		Mid-meadow (b)		Meadow apex (c)		Significance		Contrasts
	$\overline{x}$	S.D.	$\overline{x}$	S.D.	$\overline{x}$	S.D.	F	p	
D hosts (1)	3363	829	1364	634	1062	402	24.22	<0.001	a-b, a-c
D herbivory (2)	1666	595	866	563	808	361	5.05	0.018	a-b, a-c
Herb'd?							19.09	<0.001	-
Location							26.69	<0.001	-
Herb x loc							5.23	0.01	a1-a2

Table 4.2: The effects of location on the spatial distribution pattern of *S. lanceolatum* plants and herbivory. Italicized variables relate to the 2-way ANOVA discussed above, where “Herb'd?” is the main effect of plant category (either all *S. lanceolatum* or only herbivory damaged *S. lanceolatum*), “Location” is the main effect of meadow location, and Herb x loc refers to the interaction between the two. $\overline{x}$ refers to mean, S.D. refers to standard deviation, and letters (a, b, c) and numbers (1, 2) refer to meadow locations and plant classes, respectively, used in post-hoc contrasts. For example, “a1-b2” means that contrasts showed that D of all host plants at treeline differs from D of damaged plants at mid-meadow .

smintheus larvae consume only a small fraction of the host plants available to them, while away from treeline they consume both a higher percentage of all available host plants, and a more significant portion of each plant eaten. These results run counter to established theories of optimal foraging (e.g. Zimmerman, 1981; Bernstein *et al.*, 1991), to evidence of an induced defense by *S. lanceolatum* which should produce broadly consistent patterns of herbivory in relation to available resources (Roslin *et al.*, 2008), and to the expectation of greater larval survival, and therefore abundance, in areas of high host-plant density (Fred and Brommer, 2010). In other words, *P. smintheus* larvae appear to avoid the treeline.

Adult female *P. smintheus* oviposit in response to the presence (Fownes and Roland, 2002) and, as I showed in Chapter 3, the density of *S. lanceolatum*. Adult male *P. smintheus* avoid the treeline—where *S. lanceolatum* is most abundant—up to 20m into the meadow (Ross *et al.*, 2005*a*). If females similarly avoided treeline when ovipositing, the majority of *P. smintheus* larvae would simply emerge some distance from the treeline, offering a potential explanation for my observed patterns of intense herbivory away from treeline. However, my results from Chapter 3 suggest that females have no aversion to ovipositing near the treeline. This, coupled with the high density of *S. lanceolatum* near the treeline, might lead to the prediction that near-treeline habitat should actually be preferred by ovipositing females. Yet my results from Chapter 3 show that this is not the case for females, nor, as my results from the present chapter show, is it the case for larval herbivory expressed as proportion eaten.

Marginal value theory (Zimmerman, 1981) predicts that where travel costs between food sources are higher (in this case mid-meadow and meadow apex, where *S. lanceolatum* is sparser), foragers should exploit each source more completely before moving on. That a higher proportion of each plant is eaten where *S. lanceolatum* is sparsest supports this notion. Then again, late instar *P. smintheus* larvae can move 10m in a day (Roslin *et al.*, 2008), or further (Kurt Illerbrun, *pers. obs.*) when searching for host plants—enough to effectively redistribute themselves in relation to *S. lanceolatum* abundance regardless of initial hatch location. Assessing the host-finding ability of phytophagous insects in general is challenging. Laboratory studies indicate that some insects, including Lepidoptera, locate hosts at distances up to 10s of meters (summarized in Schoonhoven *et al.*, 2005), while many field studies fail to demonstrate meaningful host-finding abilities at any distance (e.g. Dethier, 1959*b*; Jermy *et al.*, 1988; Schoonhoven *et al.*, 2005), in part due to the confounding effects of fine-scale local topography (Jermy *et al.*, 1988). Early instar larvae of the closely related *P. apollo* show extremely limited host-finding ability, orienting more to environmental factors like sunlight than to host presence (Fred and Brommer, 2010). I address the question of whether later instar *P. smintheus* can actively orient towards *S. lanceolatum* in Chapter 5. Regardless, if *P. smintheus* larvae are capable of significant movement, the pattern of their herbivory should, by either chance or intent, correspond to that of host plants in a given area (Zimmerman, 1981; Bernstein *et al.*, 1991). However, my spatial results show that only near the treeline is larval herbivory significantly decoupled from the distribution of hosts. Specifically, even though *S. lanceolatum* is itself spatially clumped, larvae feed randomly among those clumps instead of following the distribution of their hosts as they do elsewhere in the meadow. This runs counter to the pattern that would be expected with an "ideal" distribution of herbivory in relation to host-plant abundance across all meadow regions (Bernstein *et al.*, 1991) (again, recall that the specific patterns of spatial distribution are of less interest to this discussion than the degree to which they are congruent between hosts, and herbivory on those hosts). Further, herbivory damage on *S. lanceolatum* near the treeline is sparser, and less intense per plant, than elsewhere in the meadow, suggesting that caterpillars feed less per plant and move more among them. This rapid switching of plants is consistent with browsing behaviour, which increases in response to lower quality host plants in many insect herbivores (Edwards and Wratten, 1983), including *P. smintheus* (Roslin *et al.*, 2008). That *P. smintheus* larvae do not consistently feed on available host plants in proportion to their abundance implies that not all *S. lanceolatum* is created equal—and that hosts near the treeline are less palatable, and perhaps more variable in quality, than those elsewhere in the meadow.

More intensive herbivory on *S. lanceolatum* comes with increased foraging costs. Herbivory lowers the quality of individual plants (Roslin *et al.*, 2008), while at a broader scale the costs of locating uneaten plants will tend to rise as the percentage of eaten plants increases. Yet *P. smintheus* larvae seem willing to incur these costs rather than forage near the treeline where *S. lanceolatum* is more abundant. I have already shown in Chapter 2 that the nutritional quality of *S. lanceolatum* varies relative to the treeline (Figs. 2.7 and 2.8), and in Chapter 3 that female *P. smintheus* oviposit in response to host plant quantity and quality, and that proximity to the treeline is no deterrent (Tables 3.3 and 3.4). Further, larvae are highly mobile and should be capable of moving to superior habitat if necessary. Given the variation in nutritional content of *S. lanceolatum* (Chapter 2; Figs.

2.7 and 2.8), I propose that *S. lanceolatum* near the treeline is both less palatable and, because of lingering snow cover, initially less accessible to *P. smintheus* larvae.

Snow cover is an important mediator of plant growth in alpine ecosystems (Stinson, 2005; Huelber *et al.*, 2006; Jonas *et al.*, 2008), while the winter dormancy induced by snow is known to increase toxicity in other species (Ralphs *et al.*, 2002), and anecdotally in *S. lanceolatum* (Guppy and Shepard, 2001). Further, prolonged snow cover would serve to physically protect host plants near the treeline from larval herbivory, at least during early larval development. Perhaps still other aspects of near-tree habitat contribute to the deterrent effect—heterogeneity of the plant community (Agrawal *et al.*, 2006), or soil composition (De Bruyn *et al.*, 2002; Cornelissen *et al.*, 2008).

Further research is needed to conclusively determine why *S. lanceolatum* near treeline is underexploited by *P. smintheus* larvae. However, I suggest that in a broader sense the reasons may be less important than the implication of an ecological shadow projected well beyond the physical location of the treeline. My results give strong evidence that *S. lanceolatum* near the treeline is less attractive to *P. smintheus* larvae, and that they alter their feeding behaviour accordingly. Since it comes at the apparent cost of over-inducing host defenses away from treeline and of incurring fitness penalties as a result (Roslin *et al.*, 2008), this behaviour should not be dismissed as trivial. Thus, where Boggs and Inouye (2012) showed that temporal variation in snow cover affects populations of a montane butterfly both directly, by mediating overwinter exposure, and indirectly, through effects on adult nectar flowers, I suggest that *spatial* variation in snow cover

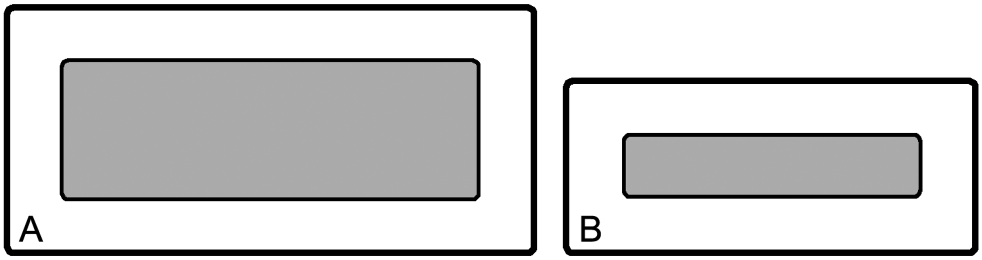

Figure 4.7: Two hypothetical meadows, where meadow B is smaller than meadow A due to treeline encroachment. White regions near the meadow edge contain high densities of *S. lanceolatum*, but are underutilized by *P. smintheus* larvae. Grey, core regions of the meadow contain less *S. lanceolatum*, but may be more suitable for *P. smintheus* larvae. When meadow B is 50% smaller overall than meadow A, the core meadow habitat (grey) of meadow B is in fact 57% smaller than that of meadow A. If treeline continues to rise and result in overall meadow shrinkage, this discrepancy between realized and apparent habitat will increase due to the larger perimeter-to-area ratio found in smaller meadows.

may affect *P. smintheus* larvae both directly, by physically isolating them from potential hosts, and indirectly, through altered host plant palatability.

If treeline continues to rise, *P. smintheus* larvae will incur ever greater fitness costs as the treeline zone grows as a proportion of meadow area. Further, costs will increase faster as available *S. lanceolatum* numbers decline. A simple, hypothetical example illustrates the point (Fig. 4.7): in a meadow of roughly the same dimensions (100*m* x 400*m*) as my study meadows, and with *S. lanceolatum* distributed in relation to the treeline as presented here, a 10% reduction in overall meadow area means a 7% decline in total host plants, but a nearly 12% decline in host plants away from the treeline. With 50% meadow shrinkage—far less than the 78% drop observed in the past 60 years on Jumpingpound Ridge (Roland *et al.*, 2000)—total *S. lanceolatum* abundance would fall by 36%, but non-treeline abundance would decline by nearly 57%.

Although rising treeline alters butterfly population dynamics by reducing adult movement (Roland *et al.*, 2000; Ross *et al.*, 2005*a*), my results suggest an additional mechanism by which treeline rise can exert more insidious pressures on the population. For several species of birds undergoing similar altitudinal range shifts due to climate change, total population size is predicted to decline faster than distribution area under continued climatic warming, and habitat area alone is a poor indicator of potential population size (Shoo *et al.*, 2005). My results support a similar pattern for alpine Lepidoptera. More specifically, they imply that *P. smintheus* larvae can be adversely affected even while adult movement appears sufficient for survival, and total host availability seems ample—the decoupling of realized habitat from apparent habitat. The implication that treeline effects are not limited only to impeding movement is of particular interest to Lepidopteran systems where larvae differ greatly from adults in their hosts, habitat requirements and dispersal ability. My findings are relevant to endangered congeners (*P. apollo*; *P. mnemosyne*), and more generally to Lepidoptera (and perhaps other non-Lepidopteran herbivores) threatened by advancing treeline. I conclude that surreptitious effects of climate change cannot be overlooked when considering conservation strategies for alpine Lepidoptera, and emphasize that the effects of rising treeline may extend beyond the immediately obvious signs of habitat loss or fragmentation.

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Chapter 5

Age-dependent host recognition by larvae of the Rocky Mountain apollo butterfly, *Parnassius smintheus*

5.1 Introduction

Most natural habitats contain a diversity of plant species. From the perspective of host-plant specialist insects, this means that palatable host-plants are typically interspersed throughout a matrix of unpalatable vegetation. The ability to differentiate and move toward host-plants in such habitats is vitally important to host-plant specialist insects, and has been studied extensively via both lab- and field-based research. Schoonhoven *et al.* (2005) provide an idealized overview of the process by which an insect may locate and select its host-plant: first, the insect moves randomly; second, having perceived either visual or olfactory cues from the host-plant, the insect begins moving toward the plant; third, the putative host-plant is encountered and tested; and finally, the insect either accepts (e.g. begins eating or ovipositing) or rejects (leaves to begin the process anew) the host-plant. While theoretically simple, this process is constrained by the insect's ability to move, and it further assumes that the insect is capable of perceiving cues from its host-plant in the first place—yet the distance over which such perception is possible varies widely between species and environmental conditions. For example, some specialist insects show host-plant orientation in response to olfactory or visual cues from distances of up to 100 m (though distances of less than 3 meters are much more common; summarized in Schoonhoven *et al.* 2005), while others are apparently unable to discern host-plants at distances of a few millimeters and instead find them simply by chance (Dethier, 1959*b*; Kennedy *et al.*, 1961; Jermy *et al.*, 1988; Wan and Harris, 1996; Bierzychudek *et al.*, 2009). Still other insects which show host orientation in response to host-plant cues in the lab show no response under field conditions (Visser, 1976; Ma and Visser, 1978; Thiery and Visser, 1986; Nottingham *et al.*, 1991).

The picture that emerges from these studies is that, while many host-plant specialist insects are capable of responding to cues from their host-plant—albeit over relatively short distances, and dependent on the degree of their mobility—host-finding in the field is contingent on a number of factors. The effects of weather and microclimate, risk from natural enemies, local vegetation/topography (Jermy *et al.*, 1988), fine-scale air turbulence (Schoonhoven *et al.*, 2005), or conflicting olfactory cues from non-host plant species (Hamback *et al.*, 2000; Agrawal *et al.*, 2006) may all confound an insect's ability to perceive its host. The ability to perceive and move toward a host may also vary with age or life stage; for example, neonate Lepidopteran larvae are usually much less mobile than their older counterparts (Zalucki *et al.*, 2002), while older larvae may abandon any interest in host location in the days prior to pupation (Riemann *et al.*, 1986; Dominick and Truman, 1984). Despite these intrinsic and extrinsic challenges, specialist phytophagous insects can and do locate their host plants from a distance, and the proficiency with which they do so has important implications for how they interact with available host resources.

Here, I assess the host-recognition ability of larvae of the Rocky Mountain apollo butterfly, *Parnassius smintheus* Doubleday (Lepidoptera: Papilionidae), which is endemic to alpine meadows throughout the Rockies, and whose populations are increasingly threatened by rising treeline (Roland *et al.*, 2000). *Parnassius smintheus'* host plant, *Sedum lanceolatum* Torr. (Crassulaceae), is low growing and, from a larva's perspective, would often be visually obscured behind other vegetation. For this reason I focus only on host-recognition via olfactory cues. *Parnassius smintheus* lays its eggs off the host plant, requiring neonate larvae to locate a suitable host before feeding can commence, yet early instar larvae of *P. apollo*, a congener that also feeds on *Sedum* spp., show no host-location ability (Fred and Brommer, 2010). However, it is unknown if later instar larvae would show a similar inability to locate and orient towards their hosts.

In Chapter 4 I showed that late instar *P. smintheus* larvae underutilize host-plant resources near the treeline-delimited edges of their alpine meadow habitat, counter to predictions of herbivory in proportion to host abundance (Zimmerman, 1981). Because of this pattern, realized larval host-plant resources may be considerably less than apparent resources, with significant implications for conservation if the treeline continues to rise. Understanding *P. smintheus*' larval host-finding ability is important to understanding the mechanisms behind the underutilization of host-plant resources near the treeline: if larvae can detect and locate their host-plant then underutilization of hosts near the treeline may be a product of active avoidance, suggesting that *S. lanceolatum* near the treeline may be less palatable, or otherwise of lower quality, than that growing elsewhere in the meadow. Conversely, if larvae move effectively at random then the patterns of host-plant use are probably due to another factor, such as the physical unavailability of near-treeline habitat by snow cover that persists during *P. smintheus*' early growth period.

Here, I address two specific questions. First, are late-instar *P. smintheus* larvae capable of host-recognition from a distance, and if so, what are the implications for the patterns of host-plant use I described in Chapter 4? If larvae are capable of host-recognition and orientation, then the feeding patterns I observed likely reflect deliberate choices; on the other hand, if larvae cannot detect and orient towards their hosts, then the feeding patterns I observed may simply be an artifact of larvae remaining near where they hatched (i.e., where females oviposited). Lab-based examinations of host-recognition may overestimate host-finding ability because they involve highly simplified conditions and/or host-plant volatile cues that are unrealistically isolated from natural "background noise". Conversely, field assays of host-finding necessitate their own compromises in the interests of tractability. To address these issues, I used a novel olfactometer intended to strike a reasonable balance between the variability of field trials and the control afforded by lab-based assays.

Second, how does host-recognition and orientation by *P. smintheus* change as larvae age and pupation approaches? Because I used late instar *P. smintheus* larvae for this experiment, I was serendipitously able to observe changes in larval host-location behaviour associated with the preamble to pupation, when larvae cease eating and begin to wander in search of a suitable place to pupate. While the onset of wandering and associated physiological and behavioural changes are well documented in Lepidoptera, few studies explicitly quantify changes in larval host-orientation behaviour in the days immediately before pupation, as I do here.

Overall, I aim first to determine the extent of host-recognition and orientation behaviour in *P. smintheus* larvae, and secondarily to relate changes in that behaviour to age relative to pupation. My findings will aid in understanding observed patterns of host plant use relative to the alpine treeline (see Chapters 4 and 6), aiding habitat assessment and conservation decisions for both *P. smintheus* and endangered congeners elsewhere (e.g., *P. apollo; P. mnemosyne*). They will also provide a unique perspective on the behavioural changes that occur in Lepidopteran larvae before pupation.

5.2 Methods

5.2.1 Study species

Research took place in 2010 at the Canadian Rockies & Foothills Biogeoscience Institute using larvae collected from the alpine meadows of Jumpingpound Ridge, in Kananaskis Country, Alberta, Canada (50°57'N, 114°55'W), a series of meadows used for the long-term study of *P. smintheus* population dynamics (e.g. Roland *et al.*, 2000; Fownes and Roland, 2002; Roland and Matter, 2007). *Parnassius smintheus* is common in alpine meadows throughout the Rockies, but is limited to areas with a sufficient supply of its host plant, *S. lanceolatum* (Fownes and Roland, 2002), and nectar flowers like *Arnica* spp., *Senecio* spp., *Aster* spp., and other large yellow or yellow-centered composites. *Parnassius smintheus'* life cycle varies along the species' north-south range; on Jumpingpound Ridge adults fly from mid-July until late August and occasionally into September (Guppy and Shepard, 2001), during which time eggs are laid near, but not directly on, *S. lanceolatum* (Fownes and Roland, 2002). Eggs hatch after snow melt, usually in April, with larvae locating the host-plant and feeding continuously before pupating in mid- to late June. Larvae, especially in later instars, may be quite mobile, moving 10s of meters in a single day in search of food (Roslin *et al.*, 2008). Although *P. smintheus* larvae feed on a small number of related host species throughout their range (Guppy and Shepard, 2001), on Jumpingpound Ridge they feed almost exclusively on *S. lanceolatum*, and only rarely on ledge stonecrop, *Sedum integrifolium* (Roslin *et al.*, 2008). Due to the general lack of *S. integrifolium* at our study site, *P. smintheus* larvae in this study can be considered monophagous on *S. lanceolatum*. Total meadow area along Jumpingpound Ridge has declined more than 75% over the past 50 years due to treeline rise (Roland *et al.*, 2000), and forest stands and *krüppelholz* (Holtmeier, 1981) now intervene between some previously connected meadows. In Chapter 4 I showed that larvae underexploit dense *S. lanceolatum* growing near the treeline and overexploit *S. lanceolatum* growing far from the treeline, counter to predictions of herbivory in proportion to host-plant abundance, and despite incurring fitness penalties associated with the plant's inducible defenses (Roslin *et al.*, 2008). I further predicted that, if the treeline continues to rise, realized larval habitat may decline more rapidly than apparent habitat, leading to overestimation of available larval resources. These issues form the context for the experiment described in this chapter.

5.2.2 A "naturalistic" olfactometer

Olfactometers are commonly used to create highly controlled conditions in which to test insect recognition of, and preference for, olfactory cues. As such, they are well suited to answering simple questions, such as whether a given insect species *can* recognize and respond to olfactory cues from its host-plant. But they are often highly idealized systems, unrepresentative of natural conditions, and therefore less suited to addressing the extent to which the insect *does* respond to those cues in the field. I constructed an olfactometer (Fig. 5.1) that replicated aspects of *P. smintheus'* natural habitat while still allowing experimental control, as a compromise between field and laboratory methods.

My olfactometer was based on the commonly used Y-tube olfactometer, with several modifications. Larvae were allowed to wander freely in a central, 15 cm diameter circular chamber containing natural substrate and alpine meadow vegetation, to simulate larval habitat. There were three 15 cm long, 2 cm diameter tubes spaced equidistantly around the perimeter of the central chamber, leading to 1) a chamber containing a whole, potted specimen, grown from field-collected seed, of the host-plant, *Sedum lanceolatum*; 2) a chamber containing a selection of live, carefully transplanted alpine meadow vegetation but no host-plant; and 3) a chamber containing a small air pump. Unfiltered air was pumped out of the central chamber, in turn drawing air into the central chamber through both of the plant chambers, picking up only whatever scent was naturally released. Tubes opened onto the central chamber at a height of 1 cm above the substrate, and airflow was measured to be a modest 10 cm^3s^{-1} from each plant chamber. This design ensured that influent scents from the plant chambers entered the central chamber gently and mingled above the larvae, preventing larvae from “accidentally” encountering direct airflow from either plant chamber during their perambulations; it also crudely simulated the boundary layer inside which larvae would typically move. Tube placement also ensured that larvae could still investigate and enter each tube, but only after a deliberate choice to do so, and only with some difficulty. I inserted a fine mesh stopper 2 cm into each tube from the central chamber so that larvae could fully commit to a tube without getting irretrievably stuck. Overall, my intention was to allow scents from each of the plant choices to mingle freely and unpredictably in an environment designed to simulate real larval habitat—in short, to remove much of the certainty of laboratory olfactometer studies, while maintaining a clear, binary choice between stimuli.

5.2.3 Olfactometer trials

I used 15 5th (final) instar *P. smintheus* larvae for the trials. Larvae were field collected in their 5th instar and kept in individual plastic cups in climate controlled growth chambers set to mimic field temperature and photoperiod. Larvae were fed *ad libidum* after trials were completed for the day, but then starved until the next day's trials (approximately 10 hours). Although I initially intended to assess larval feeding behaviour only, some larvae began exhibiting pre-pupal behaviour such as restless wandering and web-spinning after the first day of trials. I amended my protocol, recording the days-to-pupation of each larva, carefully watching for signs of pre-pupal behaviour, and continuing the trials until all larvae had pupated. This resulted in a total of 40 trials over 6 days, with each larva tested between 1 and 3 times.

Trials lasted for 10 minutes. Because larvae often curled up defensively when placed into the central chamber, the clock started only when larvae began to explore their surroundings. I recorded larval preference for the plant options—host-plant or meadow vegetation—in two ways: first, I recorded the total number of seconds spent actively investigating each choice. Active investigation was considered to be when a larva actively raised its head into, or physically entered, one of the tubes. I did not record seconds spent investigating the effluent tube; however, this was generally negligible. Second, I considered a larva to have *chosen* one of the two plant options if it spent 15 or more *consecutive* seconds actively investigating a tube. Thus it was possible for a larva to spend a disproportionate amount of time actively investigating one tube, but still not have made a choice if the time was not consecutive. I also recorded the date that larvae first began to exhibit pre-pupal behaviour, with the outcome that 21 of the 40 trials were run on larvae that had yet to show signs of pre-pupal behaviour, while the remaining 19 trials were run on larvae that displayed pre-pupal behaviour during the trials. Most larvae began to exhibit obvious pre-pupal behaviour approximately 2 days before pupation.

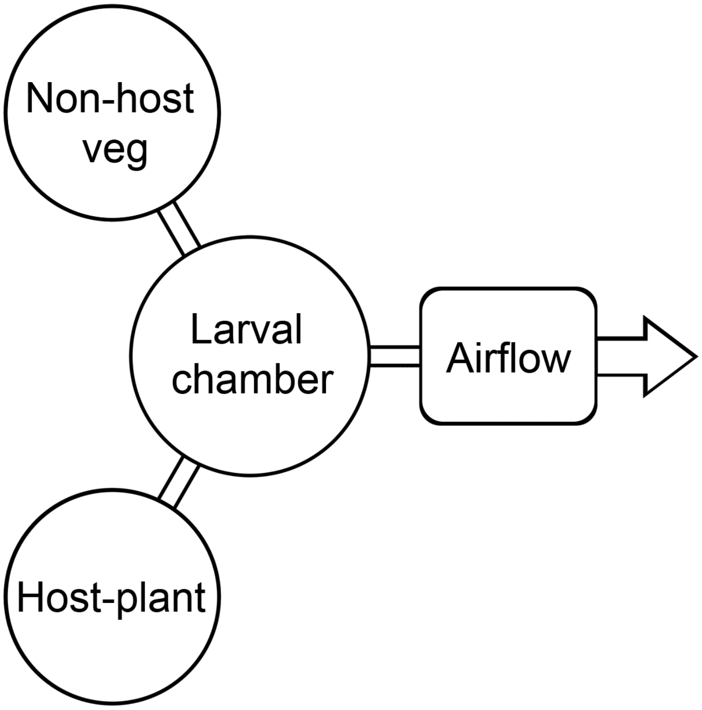

Figure 5.1: Schematic of the naturalistic olfactometer

The contents of the plant chambers—host-plant or meadow vegetation—were randomized between trials, but the chamber and tubes were not cleaned or sterilized: my intention was to simulate the natural mingling of plant scents, since in the field the scent of one plant is likely to cross and mingle with that of another before reaching a larva.

5.2.4 Statistical analysis

I compared time spent actively investigating *S. lanceolatum* vs. meadow vegetation for each of the non-pupal and pre-pupal larval groups, using paired *t*-tests. I assessed the relationship between days-to-pupation and the probability of a larva choosing *S. lanceolatum* using a logistic regression. Analyses were performed using IBM SPSS 17.0 (IBM corp.) for the *t*-tests and R (R development core team, 2012) for the logistic regression.

5.3 Results

Larvae appeared to behave normally in the larval chamber, exploring their surroundings and occasionally stopping to raise and sway the anterior portion of their bodies in a behaviour associated with the search for food (Jones, 1977). Larvae often moved haltingly towards a scent tube of interest via a series of stops and head-raises and, having located the tube opening, climbed into it.

Significantly more time was spent actively investigating the scent of the host-plant, *S. lanceolatum*, among larvae that had yet to exhibit pre-pupal behaviour (98.1 ± 24.3 seconds vs. 4.5 ± 1.4 seconds; $p < 0.001$; Fig. 5.2). This apparent preference was often accompanied by what appeared to be increasing agitation: larvae would move slowly and indecisively towards the tube leading to the host-plant chamber, enter it with more resolve, encounter the mesh barrier, and spend the ensuing seconds forcibly searching for a way through. Reaction to the tube leading to the non-host vegetation was more tepid: though a minority of larvae did enter the tube, they typically did so as part of their exploration and generally left soon after entering. Conversely, there was no significant preference for either plant option in larvae that had already begun to show pre-pupal behaviour (4.6 ± 2.8 vs. 6.7 ± 2.0; $p = 0.570$; Fig. 5.2). Likewise, larvae in the pre-pupal state were often indifferent to exploring their surroundings, preferring instead to wander continuously around the larval chamber's perimeter, or spin silken webbing in preparation for pupation.

The probability of a larva choosing the host-plant, *S. lanceolatum*, declined dramatically as the larvae neared pupation ($p < 0.001$; Fig. 5.3). Larvae almost always preferred *S. lanceolatum* 5-6 days prior to pupation,

Figure 5.2: Mean time investigating the host plant, *S. lanceolatum* (grey bars) and meadow grass (clear bars) by non-pupal larvae that showed no signs of imminent pupation, and pre-pupal larvae that did (± S.E.).

but gradually lost interest in the host-plant 3-4 days prior to pupation, and were essentially unresponsive by 1-2 days prior to pupation.

5.4 Discussion

Late instar *P. smintheus* larvae are capable of recognizing and orienting toward olfactory cues from their host-plant, *S. lanceolatum*. This result runs counter to that of Fred and Brommer (2010), who found no evidence for host-finding abilities in early instar *P. apollo*. This apparent contradiction, however, is probably explained by the differences in larval age between the studies. Although *P. smintheus* larvae *can* respond to host-plant cues in an olfactometer, my experiment, like other studies of specialist insect host-

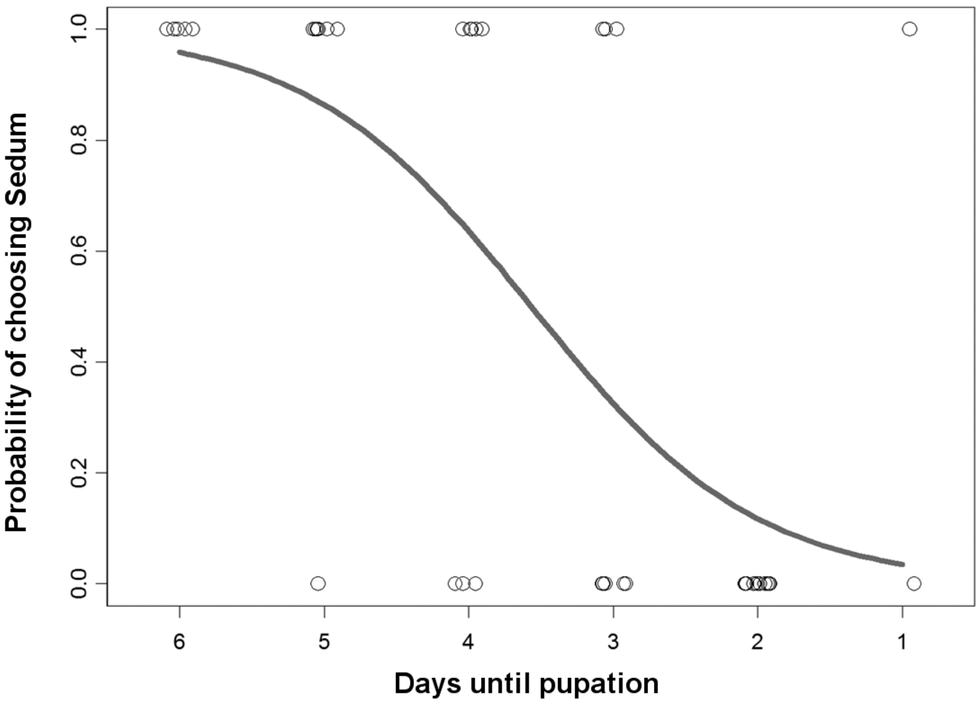

Figure 5.3: Probability of a larva choosing to continuously investigate the host plant, *S. lanceolatum*, as a function of days prior to pupation.

orientation, falls short of showing unequivocally that they *do* respond under fully natural conditions. Nevertheless, it seems reasonable to extrapolate many of my findings beyond the lab. First, pre-pupal *P. smintheus* larvae showed a strong response to the scent of whole, undamaged host-plants such as they would routinely encounter in nature. Second, this response came despite the free and varied mixing of scents within the olfactometer's larval chamber, which would be expected to confound host-finding responses, and despite the heterogeneous substrate on which the trials occurred. Finally, the cues to which larvae responded came from plants that were physically separated from, and not visible to, larvae, and they consisted of only those scents which could be picked up by a passing breeze. Taken together, my results strongly suggest that, though doubtless confounded by topography, distance, and wind patterns, *P. smintheus* larvae in their natural habitat do not find host-plants purely by chance. To this end, the naturalistic olfactometer appeared successful in its goal of making a laboratory study more relevant to field conditions.

This study was conducted on late instar larvae, with the unintended consequence that larvae began to exhibit pre-pupal behaviour over the course of my work. While this reduced the sample size of trials conducted on non-pupal larvae, it provided an interesting chance to observe and quantify the effects of pupation onset on larval host-finding. In the days preceding pupation, Lepidopteran insect larvae show characteristic changes in their hormonal environment: juvenile hormone (JH) is no longer produced and is reduced to undetectable levels (Plantevin *et al.*, 1987; Rembold and Sehnal, 1987; Grossniklaus-Burgin and Lanzrein, 1990). This allows the release of prothoracicotropic hormone (PTTH) (Rountree and Bollenbacher, 1986; Sakurai *et al.*, 1989), which in turn allows ecdysteroid levels to increase; without JH, ecdysteroid stimulates the shift from feeding to wandering behaviour aimed at avoiding predators and finding a suitable site for pupation (Dominick and Truman, 1985). My results provide a striking demonstration of these behavioural changes, as *P. smintheus* larvae switch from showing a strong attraction to their host-plant 5-6 days before pupation, to showing no interest by 1-2 days before pupation. Based on my results it appears that pre-pupal behaviour, as manifested through altered appetite and host-finding behaviour, begins in earnest 3-4 days prior to actual pupation—somewhat before it manifests itself more obviously as wandering or web-spinning behaviour. Although it is unclear if this is the result of a loss of sensory ability or simply the increasing precedence of other impulses, to my knowledge this is one of the only studies to explicitly show a decline in Lepidopteran larval host-orientation as a function of days before pupation.

More generally, my results show that *P. smintheus* larvae are capable of locating their host-plants from at least a short distance and that they are unlikely to move and find host-plants at random. Given this fact, their feeding might be expected to correspond to the distribution of palatable host plants in a given area (Zimmerman, 1981; Bernstein *et al.*, 1991). In turn, the fact that larvae can host-orient suggests that the pattern of host-plant exploitation I observed in Chapter 4, where larvae avoid host-plants growing near the treeline, is the result of feeding choices made by larvae—not simply due to chance, oviposition choices by adult female butterflies, or the effects of snow accumulation around trees. If this is so then *P. smintheus* larvae may actively avoid host-plants near the treeline, implying that these plants and/or the habitat in which they grow are in some way unattractive to *P. smintheus* larvae. Since *S. lanceolatum* grows most densely near the treeline, and since treeline continues to encroach upon *P. smintheus*' alpine meadow habitat (Roland *et al.*, 2000), it is possible that as meadow area declines, realized larval habitat will decline faster than apparent habitat, and near-treeline plants will represent an increasing percentage of remaining *S. lanceolatum* resources. This information is useful in understanding likely *P. smintheus* larval response to treeline encroachment, and is potentially applicable to other systems threatened by habitat loss, fragmentation, and local extinction in small habitat patches.

My use of a naturalistic olfactometer provides another tool for the study of host-plant finding in other insects, while the demonstration of age-dependent changes in host-finding behaviour gives insight into the timing and behavioural consequences of impending pupation. Whereas many studies focus on insect host-finding abilities from the perspective of pest-management (e.g. Cortesero *et al.*, 2000; Finch and Collier, 2000; Sarfraz *et al.*, 2006), this study considers the issue for its relevance to conservation. My findings add to the natural historical knowledge of a well-studied butterfly and improve our understanding of *P. smintheus* host-plant use in relation to the alpine treeline, clarifying habitat assessment and conservation implications for *P. smintheus* and its endangered congeners.

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Chapter 6

Between-year effects of *Parnassius smintheus* herbivory on *Sedum lanceolatum* growth and reproductive output

6.1 Introduction

Spurred by Ehrlich and Raven's (1964*b*) seminal examination of plant-butterfly coevolution, the relationship between insect herbivores and their hosts, and its role in producing the diversity of extant plants and insect herbivores, has long been a topic of interest. Because many insect herbivores specialize on groups of taxonomically related plant species (Schoonhoven *et al.*, 2005), much research has focussed on the microevolution of hosts and their insect herbivores—reciprocal evolution coupled with episodic speciation—and, more recently, on long-term macroevolution, including the roles of community associations (Futuyma and Agrawal, 2009). Many studies have considered the effects on insect herbivores of host plant quality (e.g. Feeny, 1970; Haukioja, 1980; Awmack and Leather, 2002) and spatial distribution (e.g. Bach, 1980; Kareiva, 1982; Bach, 1984; Turchin, 1991*b*), while others have examined tri-trophic effects on plant-herbivore interactions (e.g. Price *et al.*, 1980; Clay, 1988; Turlings *et al.*, 1990; De Moraes *et al.*, 1998; Pare and Tumlinson, 1999; Kessler and Baldwin, 2001). Overall, the diverse responses of plants to herbivory are often considered in light of their effects, in turn, on herbivores (e.g. Karban and Myers, 1989; Walling, 2000; Kessler and Baldwin, 2001, 2002).

Although the induction or pre-emptive production of plant defenses is a well known plant response to herbivory and such defenses come with potentially serious costs (e.g. review by Herms and Mattson, 1992; Strauss and Agrawal, 1999), there are also direct impacts of herbivory on the growth, survival, and reproduction of the damaged plant. Damage by herbivores impairs or eliminates the function of affected plant organs, and requires the diversion of resources for regrowth that might otherwise be used elsewhere. A large body of literature addresses these effects, which, for individual plants, may include loss of reproductive potential (i.e., outright consumption of or damage to flowers, fruits, seeds and so on), arrested growth, lowered competitive ability, and increased mortality (reviewed e.g. by Crawley, 1990; more recently e.g. Ancheta and Heard, 2011; Suwa and Louda, 2012; Underwood and Halpern, 2012). At higher levels of organization herbivory may affect plant population dynamics (Crawley, 1990) and alter community structure (Huntly, 1991; Olff and Ritchie, 1998). And, although herbivory has negative consequences for many plants, moderate levels of herbivory may instead increase plant growth in some species (e.g. Paige and Whitham, 1987; Belsky *et al.*, 1993).

Meanwhile, variation in habitat characteristics has its own effects on plant-herbivore interactions. Nutrient and water availability, light, microclimate, and other variables may affect herbivory both directly (e.g. local wind patterns confounding an insect herbivore's ability to find its host by scent, or shade locally lowering temperature below the preferred range) and indirectly (e.g. nutrient and water availability altering plant chemistry, and thus palatability to herbivores). Likewise, episodes of growth or reproduction are costly, and may alter the host plant's ability to produce both new tissues and defenses, making a plant's history an important determinant of its palatability to herbivores, its potential for future growth and reproduction (summarized e.g. in Obeso, 2002), and even its survival (Aragon *et al.*, 2009). Moreover, interactions within the plant community may alter plant apparency and palatability. Huffaker (1958) and Elton (1958) first showed that heterogeneous plant communities are generally more resistant to specialist herbivores than simple or uniform communities, and therefore more stable. Feeny (1976) highlighted the role of plant apparency, proposing that apparent plants need more costly defenses against a broader range of herbivores than unapparent plants. Recent theories continue to postulate mechanisms by which plants in a heterogeneous community may experience altered herbivory. Hamback *et al.* (2000) proposed that plants derive associational resistance from neighbouring plants, while Agrawal (2004) proposed the reverse case of associational susceptibility. Others suggested that diverse plant communities complicate and confound herbivore foraging decisions (Bernays, 2001), or that competition with nearby plants may change the costs and benefits of defending against herbivory (Tiffin, 2002). Thus, herbivores influence plant survival and reproduction in highly diverse ways, plants influence the performance and distribution of herbivores—a “chicken and egg” scenario—and variability in habitat and community interactions mediates it all.

Since they typically contain taxa from both sides of the transitional zone, ecotones are often home to greater species diversity than the ecosystems they bisect (Schilthuizen, 2000), and for this reason ecotones present an interesting opportunity to examine spatial variation in the interactions between herbivore and host. The alpine treeline is one such ecotone. Although it is the border between subalpine and alpine ecosystems, the alpine treeline ecotone may vary from a gradual transition zone to a highly compressed boundary (Allen and Walsh, 1996), and must be understood in the context of potentially complex variation in microclimate, moisture and nutrient availability, biotic processes, and edaphic and geomorphological heterogeneity (e.g. Stevens and Fox, 1991; Körner, 1998). Where it is relatively compressed, alpine treeline may show larger, longer lasting snow accumulation (Walsh *et al.*, 1994), and increased self-limiting edge effects (Stevens and Fox, 1991)—i.e. shade, altered local wind patterns, cross-ecotone root competition, and so on. Consequently, the alpine tree-line ecotone may also display increased plant species richness and diversity, and greater heterogeneity of spatial pattern, including abrupt local-scale changes in plant community (Batllori *et al.*, 2009*a*).

Numerous studies report changes in the growth and distribution of high-altitude forest due to global warming (e.g. Luckman and Kavanagh, 2000; Lloyd and Fastie, 2003; Moen *et al.*, 2004; Danby and Hik, 2007*a*;

Wieser *et al.*, 2009; Harsch *et al.*, 2009). Although tree distribution may be governed by a suite of factors, elevated temperatures contribute to raising treeline—the ecotone where trees give way to shrubs and shorter vegetation—by allowing seedling establishment and persistence in previously inhospitable areas (Körner, 1998; Grace *et al.*, 2002; Wieser *et al.*, 2009). Higher temperatures may also coincide with disruption of natural fire regimes (Grabherr *et al.*, 1994; Luckman and Kavanagh, 2000), changing the frequency with which treeline location is reset. Rising treeline is particularly important to alpine meadow ecosystems, which are usually surrounded by trees and, as the treeline advances, both size and connectedness of meadows decline (Grabherr *et al.*, 1994; Fagre *et al.*, 2003; Millar *et al.*, 2004; Parmesan, 2006). As the alpine treeline ecotone rises and, potentially, critical aspects of habitat and community are altered (Gottfried *et al.*, 1998), one unexplored question is how interactions between phytophagous insects and their host plants will be affected. Although the reciprocal effects of herbivores and their hosts have been extensively studied, fewer studies have considered the impact of herbivory in one year on herbivory and plant growth in a subsequent year, and fewer still have examined how these impacts vary across a climate-mediated habitat gradient. Working with a host-specialist phytophagous insect, these are my objectives here.

Larvae of the alpine apollo butterfly *Parnassius smintheus* (Lepidoptera: Papilionidae) are specialists on the perennial succulent *Sedum lanceolatum*, and both species are found in alpine meadows throughout the Rocky Mountains. Several studies have examined the direct effects of treeline rise on adult *P. smintheus* movement and population dynamics, showing that forest encroachment—causing meadow shrinkage of more than 75%—impedes butterfly movement, reducing connectivity between neighbouring populations, and isolating previously contiguous populations (Roland *et al.*, 2000; Roland and Matter, 2007). But as the treeline ecotone encroaches further into alpine meadows, as I showed in Chapter 4, near-treeline habitat will represent an ever greater proportion of the remaining meadow, decoupling *P. smintheus*-*S. lanceolatum* interactions near the treeline. By extension, *P. smintheus* herbivory might accelerate this process by more intensively targeting hosts away from the treeline. *Parnassius smintheus* herbivory may be locally intense but, as I showed in Chapters 4 and 2, respectively, its intensity and spatial distribution, as well as the abundance, spatial distribution, and nutritional content of *S. lanceolatum,* do vary with distance from the meadow edge. In particular, I showed that individual plants are fed upon more intensively away from the treeline than near it and, if herbivory reduces *S. lanceolatum* growth and reproductive output, this might compound the trend of most *S. lanceolatum* occurring near the treeline. Further, *Parnassius smintheus* herbivory results in physical damage, and may induce a chemical defense by *S. lanceolatum* (Roslin *et al.*, 2008) that reduces the plant's quality for subsequent consumption. Because the distribution and abundance of both the host plant and damage by its insect herbivore vary spatially, this system thus provides a good opportunity to observe how the effects of damage by an insect herbivore—altered growth, reproductive potential, and palatability for future generations of the herbivore—vary across an alpine meadow gradient.

With the goal of elucidating these effects, I ask the following questions: First, to what extent does herbivory change the rate at which *S. lanceolatum* produces new green (growth) and inflorescing (reproductive) stems?

Second, what effect does herbivory damage have on the likelihood and intensity of subsequent herbivory? Third, what other factors, such as past flowering history, affect the growth of new stems? And fourth, how do these effects vary across an alpine meadow gradient, from treeline-delimited edge to meadow apex? I anticipate that herbivory will reduce the growth of new stems and that this effect will be most pronounced near the drier, more exposed meadow apex. Finally, I consider the likely consequences of continued treeline rise for interactions between *P. smintheus* larvae and their hosts and, by extension, for the long term population health of both. My results will provide a unique, between-year perspective on the consequences of herbivory in a harsh alpine environment, as mediated by habitat variation across the alpine treeline ecotone, and add to our understanding of the great complexity of plant-herbivore interactions.

6.2 Methods

6.2.1 Study site and species

Research took place over 2 years (2009-10) in the alpine meadows of Jumpingpound Ridge, in Kananaskis Country, Alberta, Canada (50°57'N, 114°55'W), a series of meadows used for the long-term study of *P. smintheus* dynamics (e.g. Roland *et al.*, 2000; Fownes and Roland, 2002; Roland and Matter, 2007). Data were collected in four separate but ecologically similar meadows (J, L, M, N) near the southern end of Jumpingpound Ridge (Fig. 6.1). Meadows J and L face west, with a gentle incline of 5-10° from the treeline to the meadow apex, and a sharp drop-off on the eastern side, while meadows M and N face southwest and south, respectively, and show a steeper incline of 10-15°. Vegetation is essentially the same among meadows, consisting of *Dryas* spp., *Salix* spp., grasses and wildflowers, interspersed with *S. lanceolatum*. Vegetation gives way to increased amounts of exposed rock towards meadow apex and, although plant community composition remains broadly similar throughout the meadows used (Kurt Illerbrun, *pers. obs.*), the meadow apex tends to be drier and less lush than mid-meadow or, especially, near-treeline meadow. Plants at the meadow apex are more likely to be stressed by drought and wind exposure but, conversely, to have more access to light and be freer from competition with surrounding vegetation. Plants growing near the treeline likely have abundant moisture and potentially greater access to water borne nutrients that trickle down from the upper meadow, but at the cost of a shorter growing season due to lingering snow cover, increased competition, and less access to light, both because of surrounding vegetation and proximity to the treeline. Meanwhile, the mid-meadow represents a transitional zone between these regions, with generally intermediate conditions. All 4 meadows are surrounded by subalpine forest dominated by a mix of Engelmann spruce (*Picea engelmannii*), lodgepole pine (*Pinus contorta*) and subalpine fir (*Abies lasiocarpa*). Total meadow area along Jumpingpound Ridge has declined more than 75% over the past 50 years (Roland *et al.*, 2000); forest stands and climatically stunted *krüppelholz* (Holtmeier, 1981) now intervene between some previously connected meadows, and encroach upon others. Treeline is well defined in both meadows, with little transitional area between trees, *krüppelholz* and lower shrubs/meadow flora. Due to the western aspect, shade from treeline is generally negligible until early evening.

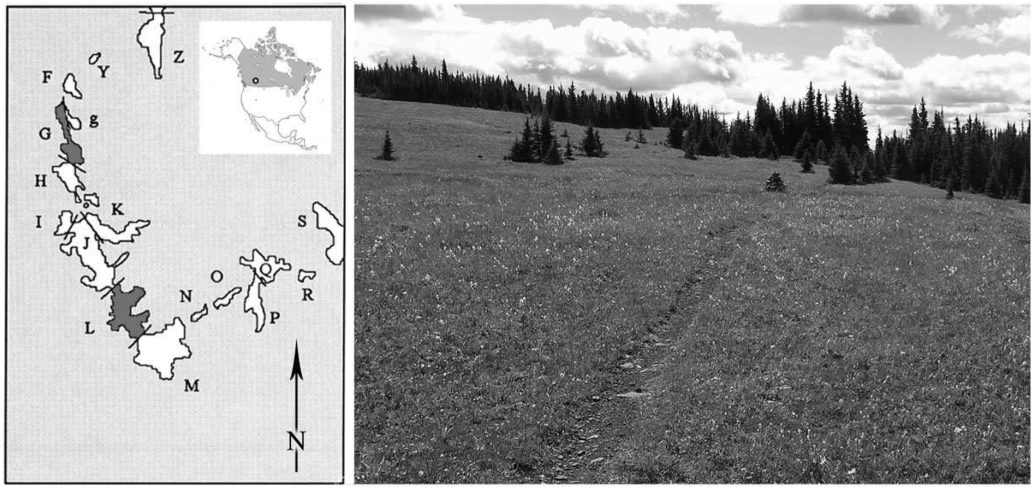


Figure 6.1: Jumpingpound Ridge, showing locations of meadows J, L, M, and N, and detailed view of meadow L looking south.

Parnassius smintheus is common in alpine meadows throughout the Rockies, but is limited to areas with a sufficient supply of its host plant, *S. lanceolatum* (Fownes and Roland, 2002), and nectar flowers like *Arnica* spp., *Senecio* spp., *Aster* spp., and other large yellow or yellow-centered composites. *Parnassius smintheus*' life cycle varies along the species' north-south range, and many of its details remain unknown. On Jumpingpound Ridge adults fly from mid-July until late August and occasionally into September (Guppy and Shepard, 2001), during which time eggs are laid near, but not on, *S. lanceolatum* (Fownes and Roland, 2002). Eggs hatch after snow melt, usually in April, with larvae feeding continuously before pupating in mid- to late June. Larvae, especially later instars, may be quite mobile, moving 10s of meters in a single day in search of food (Roslin *et al.*, 2008), and show no antagonism toward each other.

Although *P. smintheus* larvae feed on several related host species throughout their range (Guppy and Shepard, 2001), on Jumpingpound Ridge they feed almost exclusively on *S. lanceolatum*, and only rarely on ledge stonecrop, *Sedum integrifolium* (Roslin *et al.*, 2008). Due to the general lack of *S. integrifolium* at our study site, *P. smintheus* larvae in this study may be considered effectively monophagous on *S. lanceolatum*. Since *S. lanceolatum* is relatively long-lived, its spatial arrangement within meadows remains largely constant between years.

Herbivory by all but very early instar *P. smintheus* larvae is visible as physical damage to the host plant's leaves, and can be distinguished from other damage (e.g. trampling or desiccation) by its characteristically clean scars, and by its orientation perpendicular to each leaf's axis (Fig. 6.2). Although some generalist insect or mammalian herbivores may occasionally feed on *S. lanceolatum*, the damage caused by these is negligible compared to herbivory by *P. smintheus*. Recent herbivory produces fresh, green scars, which gradually harden to grey by mid-summer; in this way it is possible to estimate the age of feeding damage—for instance, a hardened, grey scar in spring or early summer indicates herbivory in the previous growing season. Typical herbivory can range from small nibbles to near complete defoliation; stems are rarely, if ever, eaten (Kurt Illerbrun, *pers. obs.*).

Larvae underexploit dense *S. lanceolatum* growing near the treeline and overexploit *S. lanceolatum* growing far from the treeline (Illerbrun and Roland 2011), counter to predictions of herbivory in proportion to host-plant abundance, and despite incurring fitness penalties associated with the plant's inducible defenses (Roslin *et al.*, 2008). This has prompted the prediction that, as treeline continues to rise, realized larval habitat may decline more rapidly than apparent habitat, leading to overestimation of available larval resources (Illerbrun and Roland 2011).

6.2.2 Tagged *S. lanceolatum* plants

Figure 6.2: Late instar *Parnassius smintheus* larva feeding on *Sedum lanceolatum*. Telltale signs of herbivory are clearly visible as blunt scars directly beneath the larva's head. (*photo*: Dave Roth)

In the summer of 2009, after *P. smintheus* larval pupation, I uniquely labelled more than 1200 *S. lanceolatum* plants by inserting a nail with a metal plant tag into the ground next to each plant. I recorded the number of stems (i.e., plant size), the number of inflorescing stems, and the degree of herbivory by larval *P. smintheus* using a 3-point scale where no herbivory = 0, minor herbivory (e.g. to the tips of leaves) = 1, and major herbivory (e.g. defoliation of >25% of leaves and/or severe damage to the meristem) = 2. Because my objective was to observe year-to-year changes in the state of individual *S. lanceolatum* plants given their state and location in the first year, I randomly selected plants from 3 locations relative to the treeline (near the treeline, mid-meadow, and meadow apex), 2 flowering states (flowering or not flowering), and the 3 levels of herbivory discussed above, in an effort to provide similar sample sizes for all combinations of location, flowering, and herbivory. Thus the distribution of the 1200+ plants among these categories did not reflect actual relative abundance in the field, but was intended to provide comparable sample sizes among categories.

I additionally recorded several variables related to *S. lanceolatum* and its community. To determine if the surrounding plant community influences year-to-year growth of *S. lanceolatum*, I measured percent-cover of vegetation in a 5cm radius around each tagged plant. I chose the 5cm scale to provide a reasonable estimate of cover that might directly influence individual *S. lanceolatum* plants, rather than using a larger scale that might average out, and therefore obfuscate, effects for individual plants. I also characterized the community on a scale of 1 (dry and/or sparse vegetation) to 3 (moist and/or lush vegetation). Next, I recorded the colour of all tagged plants. *Sedum lanceolatum* ranges from deep rust-red to bright green in colour, and plants growing in drier, more exposed meadow regions are more likely to be at least partially red (Kurt Illerbrun, *pers. obs.*; see also Bachereau *et al.*, 1998 for an analogous example concerning the congener, *S. album*). Colour may therefore be a proxy for stress, and I recorded the colour of each plant as "percent greenness", from completely red (0%) to completely green (100%). Finally, I recorded subjective "healthiness" of tagged *S. lanceolatum* on a scale of 1 (shrivelled, stressed-looking plants; "health (low)") to 3 (turgid, vigorous plants; "health (high)"). These latter two variables were intended, in part, to test whether subjective measures of the plants' appearance, such as might develop after years of working with the species, are relevant to predicting growth or flowering.

In the summer of 2010 I returned and again recorded the number of stems and inflorescing stems, and the herbivory level for all plants whose labels remained. Many labels were lost due to the effects of melt water, soil upheaval and well-intentioned hikers, leaving a final total of 761 plants with complete data for both years. This total was spread unevenly among meadow locations, herbivory levels, and flowering statuses, as shown in Table 6.1. Although every effort was made to equalize the sample sizes of

	Location					
	Treeline		Mid-meadow		Meadow apex	
Flowered in 2009?	No	Yes	No	Yes	No	Yes
Herbivory 0	103	88	88	108	40	76
Herbivory 1	25	28	48	40	20	30
Herbivory 2	4	12	12	13	5	21
Total	132	128	148	161	65	127

Table 6.1: Distribution of sampled *S. lanceolatum* plants among meadow locations, herbivory levels, and flowering statuses.

each of these groups, they reflect the scarcity of herbivory, especially among plants growing near the treeline.

Although a major goal of this study was to examine the effect of previous herbivory damage on the likelihood of future *P. smintheus* herbivory, the second year of the study (2010) saw a significant drop in the *P. smintheus* population. Consequently, only 17 plants, spread among the various meadow locations, herbivory levels, and flowering statuses, received herbivory in both 2009 and 2010.

6.2.3 Analyses

6.2.3.1 Year-to-year increases in *S. lanceolatum* vegetative and reproductive stem number

I used mixed effects logistic regression with meadow as a random effect, with a combination of backwards and forwards model selection, validated with Akaike's Information Criterion (AIC), to estimate the probability that a given plant showed new growth, vs. not showing new growth, between 2009 and 2010. I chose this method in part because of problems meeting the assumptions of data normality and variance homogeneity for other analyses (see below). I performed 3 similar logistic regressions for increase in flowering stem number, increase in green stem number, and increase in number of all stems combined, vs. no increase. A change in the number of infloresscing stems might be taken as a crude proxy for reproductive effort of plants within a single season, while change in the number of green stems is a proxy for non-reproductive growth (and, though not directly addressed in this study, implicitly also future reproductive potential, since inflorescences develop from pre-existing stems). Meanwhile, change in the total number of stems may be viewed in the context of a tradeoff between reproductive and non-reproductive effort within a season: if, for example, a plant produces many inflorescences yet the total number of stems remains the same or even declines, this would suggest the the flowering came at the expense of future growth.

Predictor variables included *S. lanceolatum* colour ("colour"), percent-cover of plants in a 5cm radius around each tagged *S. lanceolatum* ("cover"), total number of inflorescing stems ("inflorescence number"), subjective healthiness on a scale of 1-3 ("healthiness"), amount of herbivory damage ("herbivory"), location within each meadow (treeline, mid-meadow, and apex; "location"), plant community type ("community type"), and meadow as a random effect ("meadow (random effect)"). Since the number of inflorescing, green, and total stems in 2009 were highly correlated with each other (i.e., a large plant with many stems is likely to have more inflorescing stems in absolute terms than a smaller plant), I chose to use only number of inflorescing stems as a predictor variable in each model, although I considered inflorescing, green, and total stems as response variables.

6.2.3.2 Magnitude of year-to-year changes in *S. lanceolatum* vegetative and reproductive stem number

The previous analysis considered increases in number of stems vs. no increase, but it did not consider the magnitude of any change in stem number. To explore this issue further, I quantified changes in the number of inflorescing stems, non-inflorescing (green) stems, and all stems combined as the change in stem number from 2009 to 2010, and considered them relative to herbivory and meadow location. For example, a plant that had 3 inflorescing stems in 2009 and 1 in 2010 would be represented as -2 with respect to inflorescences, while a plant that had 4 green stems in 2009 and 5 in 2010 would be represented as +1 with respect to green stems.

Because flowering may take place at the expense of future growth, I performed 1-way ANOVAs to test for differences in the magnitude of change for all 3 stem types (inflorescing stems, green stems, and all stems combined) between plants that did and did not flower in 2009. Results justified considering separately plants that did, and did not, flower in 2009. Further, although it would be preferable to include 2009 flowering status as a factor in the analysis, doing so made meeting the requirements for data normality and homogeneity of variances impossible.

Because sample sizes for the various combinations of meadow location, herbivory level, and flowering status were highly uneven, achieving data normality and homogeneity of variances for multi-way comparisons continued to prove challenging and ultimately required that plants with level 1 and level 2 herbivory damage be combined into a single “damaged by herbivory” category. For plants that flowered in 2009, I used 2-way ANOVAs to test for interactions between herbivory damage and meadow location on change in number of green stems and on change in number of all stems combined. For plants that did not flower in 2009, I likewise performed 2-way ANOVAs to test for interactions between herbivory damage and meadow location with respect to changes in the number of green stems and all stems combined. For all 2-way ANOVAs with significant interactions, I used planned orthogonal contrasts to test for differences between meadow locations in the effects of herbivory on changes in the variable of interest (inflorescing stems; green stems; all stems combined). Because of problems with data normality even after repeated transformation, I was unable to perform a 2-way ANOVA for changes in the number of inflorescing stems of plants that did not flower in 2009, and instead performed a non-parametric Brunner-Dette-Munk (BDM) test (Brunner *et al.*, 1997) with interaction. Means are reported ± S.E. throughout, unless otherwise indicated.

6.3 Results

As indicated above, I considered *S. lanceolatum* growth in two ways, and the results are organized accordingly. First, I considered whether or not there was any <u>increase</u> in inflorescing, green, or total stems between years. These analyses therefore use a binary response variable: increase in stem number, or no increase. Second, I considered the actual <u>magnitude</u> of any change in inflorescing, green, or total stem number, whether positive or negative.

6.3.1 Year-to-year increases in *S. lanceolatum* vegetative and reproductive stem number

Initial and final models used for these results are summarized in Table 6.2, while coefficients and p-values for the variables retained in each model are given in Table 6.3. The descriptions below relate to these tables.

6.3.1.1 Effect of previous herbivory

Herbivory had no overall effect on the likelihood of *S. lanceolatum* growing new, additional stems of any kind, whether vegetative or reproductive.

6.3.1.2 Effect of location in meadow

Growing at mid-meadow marginally reduced the likelihood of a plant increasing its number of inflorescing stems and total stems, while growing at the meadow apex increased the likelihood. Although meadow (as opposed to location within the meadow) was included as a random effect in the initial models, it was not retained.

6.3.1.3 Effects of flowering history and plant condition

Plants that had many inflorescences in 2009 were much less likely to produce further reproductive stems in 2010, but flower number in 2009 did not affect the growth of green, vegetative stems in 2010. The subjectively healthiest looking plants were more likely to develop new stems of all types, but the effect was only marginal for vegetative stems. Likewise, physically greener *S. lanceolatum* were much more likely to produce new reproductive

Model	AIC
(a) *Year-to-year increase in flowering stems*	
Initial: colour + cover + inflorescence number + healthiness + herbivory + location + community type + meadow (random effect)	307.6
Final: colour + cover + healthiness + inflorescence number + location	305.2
(b) *Year-to-year increase in green stems*	
Initial: colour + cover + inflorescence number + healthiness + herbivory + location + community type + meadow (random effect)	667.0
Final: cover + healthiness	659.9
(c) *Year-to-year increase in all stem types combined*	
Initial: colour + cover + inflorescence number + healthiness + herbivory + location + community type + meadow (random effect)	609.6
Final: cover + healthiness + inflorescence number + location	602.8

Table 6.2: AIC values for initial and final models for year-to-year increase in *S. lanceolatum* stem growth. Underlined text indicates a continuous variable.

stems than were redder plants, but colour did not affect growth of new vegetative stems, or total stems.

6.3.1.4 Effect of community attributes

Percent-cover of surrounding vegetation had a significant negative effect on the likelihood of increasing inflorescing stems, green stems, and all stems combined. In other words, more surrounding vegetation resulted in fewer stems of all types being added between years. However, community type had no effect on growth of any stem type.

6.3.2 Magnitude of year-to-year changes in *S. lanceolatum* vegetative and reproductive stem number

6.3.2.1 Effects of flowering history

Plants that flowered in 2009 saw their reproductive output in 2010 decline by more than 1.1 inflorescing stems, on average, while plants that did not flower in 2009 saw an increase of more than 0.4 inflorescing stems between years ($F_{1,\,759} = 294.837$, $p < 0.001$). Plants that flowered in 2009 did show a net increase of more than 0.7 vegetative stems between years, but plants that did not flower in 2009 showed an even greater net increase of more than 1.6 stems ($F_{1,\,759} = 11.824$, $p = 0.001$). Overall, plants that flowered in 2009 suffered a net decrease of more than 0.42 stems between years, whereas plants that did not flower enjoyed an increase of more than 2 stems ($F_{1,\,759} = 77.738$, $p < 0.001$; Table 6.4). On the strength of these results, I describe plants that did and did not flower in 2009 separately below.

Variable	Coefficient	S.E.	*z*	*p*
Number of flowers	-1.13	0.24	-4.64	<0.001
Health (medium)	1.43	1.09	1.31	0.191
Health (high)	2.51	1.19	2.11	0.035
Percent cover	-0.54	0.16	-3.31	<0.001
Colour	0.90	0.23	3.90	<0.001
Location (Mid)	-0.40	0.40	-0.99	0.321
Location (Apex)	0.69	0.44	1.59	0.111

(a) Final model for increase in number of inflorescing stems

Variable	Coefficient	S.E.	*z*	*p*
Health (medium)	-0.04	0.31	-0.13	0.900
Health (high)	0.59	0.37	1.59	0.113
Percent cover	-0.32	0.09	-3.52	<0.001

(b) Final model for increase in number of green stems.

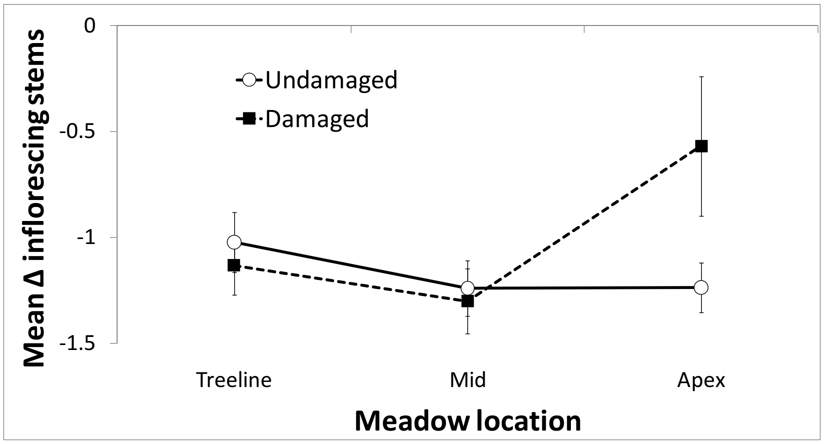
Variable	Coefficient	S.E.	*z*	*p*
Number of flowers	-0.92	0.14	-6.58	<0.001
Health (medium)	<0.01	0.33	<0.01	0.99
Health (high)	0.87	0.41	2.13	0.033
Percent cover	-0.20	0.10	-2.03	0.042
Location (Mid)	-0.14	0.25	-0.54	0.587
Location (Apex)	0.91	0.31	2.93	0.003

(c) Final model for increase in number of all stems combined.

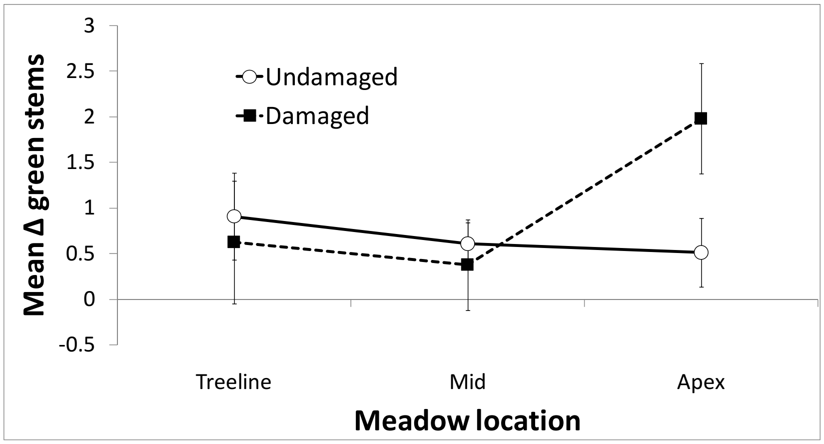
Table 6.3: AIC values for initial and final models for increase of each stem type (a); variables retained in the final model for increase of inflorescing stems (b), increase of green stems (c), and increase of all stems combined (d).

Variable	Flowered ('09)		No flower ('09)		Significance	
	$\overline{x}$	S.D.	$\overline{x}$	S.D.	F	*p*
Δ Inflor. stems	-1.13	0.07	0.41	0.05	294.84	<0.001
Δ Green stems	0.74	0.18	1.62	0.19	11.82	0.001
Δ Total stems	-0.42	0.19	2.04	0.20	77.74	<0.001

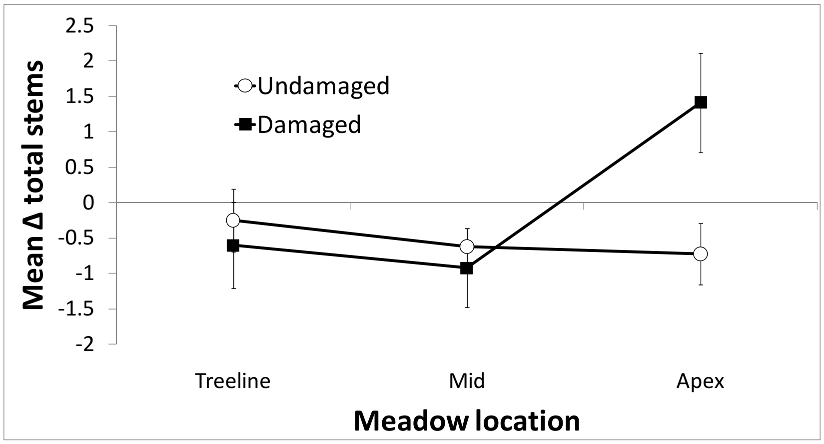
Table 6.4: The effect of flowering history (i.e., plants that did or did not flower in 2009) on *S. lanceolatum* growth (positive or negative) of new inflorescing, green, and total stems. $\overline{x}$ refers to mean, and S.D. refers to standard deviation.



(a) Infloresecing stems



(b) Green stems



(c) Total stems (infloresecing + green)

Figure 6.3: Effects of herbivory on changes in the number of infloresecing (a), green (b), and total (c) stem numbers between 2009 and 2010, for plants that flowered in 2009. Means are shown ± SE.

Variable		Treeline (a)		Mid-meadow (b)		Meadow apex (c)		Significance		Contrasts
	Effects	$\overline{x}$	S.D.	$\overline{x}$	S.D.	$\overline{x}$	S.D.	F	p	
Δ Inflor. stems	Not herbivorized (1)	-1.02	0.14	-1.24	0.13	-1.24	0.12			
	Herbivorized (2)	-1.13	0.14	-1.30	0.15	-0.57	0.33			
	Herbivory							0.35	0.555	-
	Location							2.45	0.088	-
	Herb x loc							4.21	0.015	c1-c2
Δ Green stems	Not herbivorized (1)	0.91	0.48	0.61	0.23	0.51	0.38			
	Herbivorized (2)	0.63	0.67	0.38	0.50	1.98	0.61			
	Herbivory							0.07	0.787	-
	Location							1.92	0.148	-
	Herb x loc							3.35	0.036	c1-c2
Δ All stems	Not herbivorized (1)	-0.25	0.44	-0.62	0.26	-0.72	0.43			
	Herbivorized (2)	-0.60	0.61	-0.92	0.56	1.41	0.70			
	Herbivory							0.33	0.565	-
	Location							3.65	0.027	a-c, b-c
	Herb x loc							5.75	0.003	c1-c2

Table 6.5: The effects of *P. smintheus* herbivory and meadow location on the growth of *S. lanceolatum* that flowered in 2009. $\overline{x}$ refers to mean, S.D. refers to standard deviation, and letters (a, b, c) and numbers (1, 2) refer to meadow locations and herbivory classes, respectively, used in post-hoc contrasts. For example, “a1-b2” for inflorescing stems means that contrasts showed the growth of new inflorescences among undamaged plants at treeline differs from the growth of new inflorescences among damaged plants at mid-meadow.

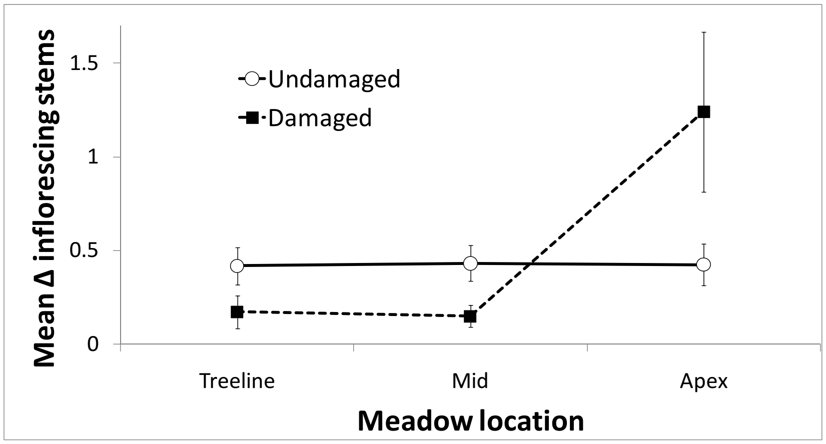
6.3.2.2 Effects of previous herbivory

Herbivory damage had no overall effect on the growth of new stem types of any kind between years, regardless of whether a plant flowered in 2009 (Tables 6.5 and 6.6; for plants that flowered in 2009, all analyses for herbivory are $F_{1,\,409}$, while all for location and all interactions are $F_{2,\,409}$; for plants that did not flower in 2009, all analyses for herbivory are $F_{1,\,339}$, while all for location and all interactions are $F_{2,\,339}$). However, as outlined below, the effects of herbivory did differ depending on meadow location (significant interaction).

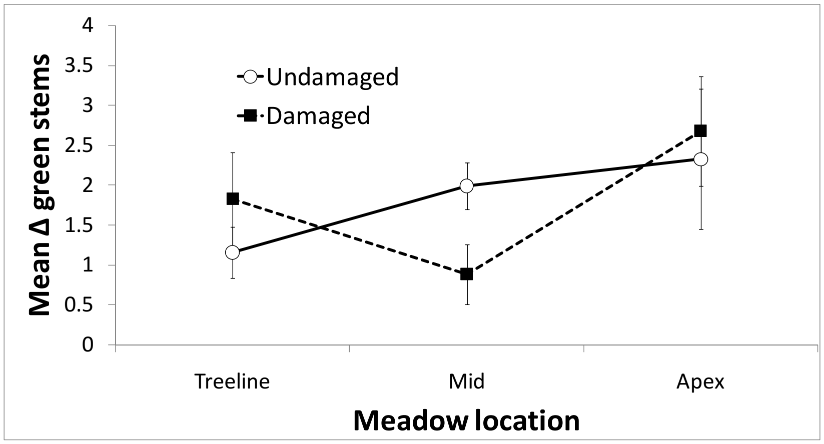
6.3.2.3 Effects of location in meadow

Meadow location had variable effects on *S. lanceolatum* growth, depending on herbivory and flowering history (Tables 6.5 and 6.6). Among plants near the treeline that flowered in 2009, growth of all stem types was enhanced by herbivory (either by reducing net loss of stems, or increasing net gain), compared with plants that were not damaged (Fig. 6.3, and Table 6.5). This effect was not seen elsewhere in the meadow.

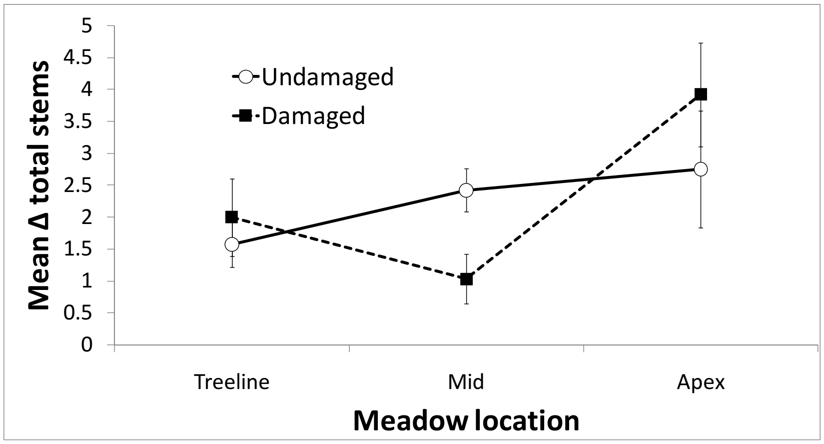
Among plants near the treeline that did not flower in 2009, herbivory again improved reproductive stem growth, but it had no effect on other stem types. Inflorescing stems showed the highest growth near the meadow apex regardless of herbivory. By contrast, for green, vegetative stems there was no effect of meadow location regardless of herbivory, while for total stems mid-meadow showed the lowest growth, also irrespective of herbivory (Fig. 6.4 and Table 6.6).



(a) Inflorescing stems



(b) Green stems



(c) Total stems (inflorescing + green)

Figure 6.4: Effects of herbivory on changes in the number of inflorescing (a), green (b), and total (c) stem numbers between 2009 and 2010, for plants that did not flower in 2009. Means are shown ± SE.

Variable	*Effects*	Treeline (a)		Mid-meadow (b)		Meadow apex (c)		Significance		Contrasts
		$\overline{x}$	S.D.	$\overline{x}$	S.D.	$\overline{x}$	S.D.	F	p	
Δ Inflor. stems	Not herbivorized (1)	0.42	0.10	0.43	0.10	0.43	0.11			
	Herbivorized (2)	0.17	0.09	0.15	0.06	1.24	0.43			
	Herbivory							$0.03^{\dagger}$	$0.854^{\dagger}$	-
	Location							$7.14^{\dagger}$	$0.002^{\dagger}$	a-c, b-c
	Herb x loc							$4.05^{\dagger}$	$0.027^{\dagger}$	b2-c2
Δ Green stems	Not herbivorized	1.16	0.32	1.99	0.29	2.33	0.88			
	Herbivorized	1.83	0.59	0.88	0.38	2.68	0.69			
	Herbivory							0.004	0.951	-
	Location							2.265	0.105	-
	Herb x loc							2.17	0.116	-
Δ All stems	Not herbivorized	1.57	0.35	2.42	0.34	2.75	0.92			
	Herbivorized	2.00	0.61	1.03	0.39	3.42	0.81			
	Herbivory							0.02	0.88	-
	Location							4.41	0.013	a-c
	Herb x loc							3.15	0.044	b1-b2

Table 6.6: 2-way ANOVA summary table for effects of *P. smintheus* herbivory and meadow location on the growth of *S. lanceolatum* that did NOT flower in 2009. $\overline{x}$ refers to mean, S.D. refers to standard deviation, and letters (a, b, c) and numbers (1, 2) refer to meadow locations and herbivory classes, respectively, used in post-hoc contrasts. For example, “a1-b2” for inflorescing stems means that contrasts showed the growth of new inflorescences among undamaged plants at treeline differs from the growth of new inflorescences among damaged plants at mid-meadow.†These values refer to the results of the BDM test.

6.4 Discussion

Overall, my results show that *S. lanceolatum* growth and reproductive output between years may be influenced by a variety of factors, some of which are more intuitive than others. A primary goal of this study was to assess the effect of herbivory on the growth and reproductive potential of *S. lanceolatum*, as well as on future herbivory, but the relative lack of herbivory in the second year of the study forced me to modify my first and second research objectives and focus instead on other determinants of *S. lanceolatum* growth and reproduction.

First, for plants that flowered in 2009, the first year of the study, new growth of all stem types lagged behind that of plants that did not flower (Table 6.4). Plants that flowered in 2009 showed a small increase in the number of green stems, but this was offset by a decrease in the number of inflorescing stems, resulting in an overall net decrease in total stem number (and therefore overall plant size). This is in line with established theories that producing reproductive tissues in a given season comes at the expense of potential growth and reproduction in subsequent seasons (reviewed e.g. in Obeso, 2002). In *S. lanceolatum*, stems usually die and desiccate after inflorescing, so a decline in stem number—particularly of flowering and total stems—is to be expected. Likewise, the subjective measures of *S. lanceolatum* "healthiness"—both colour and holistic appearance of health—were somewhat successful in predicting growth, as subjectively healthier plants were more likely than others to flower.

Second, herbivory had no overall effect on stem growth of any kind, suggesting that damage caused by *P. smintheus* herbivory represents a considerably lesser stress for *S. lanceolatum* than does reproduction (Tables

6.3, 6.5, and 6.6). Herbivore damage can have many consequences for plant growth, ranging from negative (reviewed e.g. by Crawley, 1990; also Ancheta and Heard, 2011; Suwa and Louda, 2012; Underwood and Halpern, 2012), through tolerance (e.g. Rosenthal and Kotanen, 1994), to outright positive (e.g. McNaughton, 1983; Paige and Whitham, 1987; Belsky *et al.*, 1993). However, there are few consistent trends in the impact of herbivory, for example based on plant life history or herbivore feeding mode (Maron and Crone, 2006), and there is no *a priori* reason to assume the *S. lanceolatum*-*P. smintheus* interaction would fall into one category or another in this respect. Further, although herbivory damage from *P. smintheus* can be extreme (Kurt Illerbrun, *pers. obs*.), the *P. smintheus* population was relatively low during the years of this study and extensive herbivory on single plants was uncommon. Thus, my result may reflect the fact that the supply of *S. lanceolatum* simply outstripped demand, resulting in less complete exploitation of individual plants. In any case, there is no evidence for consistent harm to, or compensatory growth by, *S. lanceolatum* as a consequence of herbivory.

Although there was no overall effect of either meadow location or herbivory on the growth of new stems of any kind, there was, surprisingly, a positive effect of herbivory damage for *some* combinations of herbivory, flowering history, and meadow location (Figs. 6.3 and 6.4, and Tables 6.5, and 6.6). In particular, herbivory-damaged plants growing near the meadow apex that had previously flowered produced significantly more stems of all kinds than their undamaged counterparts, while damaged plants at the meadow apex that had *not* previously flowered showed the same trend with respect to development of inflorescing stems only. This result is unexpected, and challenging to explain. Since the meadow apex is generally more exposed, rockier, and therefore drier than other meadow regions of the meadow, one might expect the costs of flowering would be compounded, resulting in reduced growth.

That the abundance of surrounding vegetation negatively affected *S. lanceolatum* growth of all stem types may shed some light on the matter. The net outcome of interactions between plants is generally seen as a balance between positive (facilitation; e.g. shelter from wind or excessive light, amelioration of edaphic attributes, concealment from herbivores, and so on) and negative (competition; e.g. for light, nutrients, space, and so on) effects (Casper and Jackson, 1997; Holmgren *et al.*, 1997; Olofsson *et al.*, 1999; Brooker *et al.*, 2008). In harsh alpine environments, interactions between plants may likewise vary from positive (e.g. Callaway *et al.*, 2002) to negative (e.g. Olofsson *et al.*, 1999), but positive interactions may increase with increasing environmental stress (Callaway *et al.*, 2002): competition is common where conditions are less physically stressful, whereas facilitation prevails at the most exposed sites (though see Klanderud and Totland, 2005*b*,*a*; Klanderud, 2005; Klanderud and Totland, 2007 for a discussion of how alpine inter-plant interactions may be altered by climate change). Moreover, the relative importance of facilitation and competition may vary with a plant's age—for example, from a "nurse" effect when young, to strong resource competition when older—and the prevailing environmental conditions (Holmgren *et al.*, 1997). In this view, *S. lanceolatum* might receive shelter or amelioration of moisture stress from the lush near-treeline plant community, resulting in improved survival and potentially explaining the high densities found in this meadow region (see Chapter 2), yet, when established, be subjected to intense competition for light or nutrients that limits growth between years. Conversely, at the meadow apex, those *S. lanceolatum* plants that do survive might be comparatively free of competitive interactions with their neighbours, leaving them better able to meet the energetic demands of flowering, and recovery after herbivory. Further supporting this view, one meta-analysis suggests that, unlike monocots, which overcompensate after herbivory when resource stress is low, dicots like *S. lanceolatum* are more likely to overcompensate in stressful, resource-poor habitats (Hawkes and Sullivan, 2001).

If this were so, however, we might also expect the trend to be true of all plants, not just of those that flowered in 2009, and not just of those that were damaged by herbivory. Yet this is not the case. As discussed, flowering comes at a significant cost to plants, in terms of the physical production of both the inflorescence, and its associated secretions (e.g. water and sugars required for nectar) (Pyke, 1991; Obeso, 2002). Indeed, even in perennials like *S. lanceolatum*, flowering may be associated with increased mortality (Aragon *et al.*, 2009). If *S. lanceolatum* does not generally overcompensate in response to herbivory, and herbivory is at best neutral and at worst a significant stress, and, further, if any reprieve from competition near the meadow apex is more than offset by the physical stress of exposure and drought, a new picture emerges. In this view, herbivory-damaged plants that have recently incurred the costs of flowering and are growing at the meadow apex would be the single most stressed group of plants in this survey. Where resources are finite, one prediction of optimal reproduction theory is that allocation to current reproduction will trump “saving” for future reproduction when stress and mortality are high (Gadgil and Bossert, 1970; Schaffer, 1974; Kozlowski and Wiegert, 1986; Kozlowski, 1992). While it is clear that the short term growth and reproductive output of these plants is increased, it is unclear whether this investment in current reproduction comes at a significant cost of future reproduction. However, at least among those that flowered in 2009, herbivory damaged plants at the meadow apex produced even more green stems than they did inflorescing stems following herbivory. This is not the hallmark of a stressed plant putting all its resources into a last ditch reproductive effort. Thus for plants that have recently flowered and grow at the meadow apex, it appears that herbivory may actually lead to a kind of compensatory growth after all, comprising an increase in both overall plant size and potential reproductive output, and perhaps even helping to maintain the *S. lanceolatum* population at the meadow apex. The reasons for this remain unclear.

One interesting, but speculative, possibility lies with the fact that many *Sedum* species represent an intermediate step in the evolution from C_3 to CAM (crassulacean acid metabolism) photosynthesis, and display inducible CAM photosynthesis (Martin *et al.*, 1988*b,a*; Borland and Griffiths, 1990, 1992; Gravatt and Martin, 1992; Conti and Smirnoff, 1994). C_3 photosynthesis, while efficient with respect to carbohydrate yield, results in extremely high transpirative water loss (Raven and Edwards, 2001), and is therefore less suited to hot or moisture-limited environments. In CAM photosynthesis the leaf stomata open at night, allowing CO_2 to enter and be fixed as malic acid, which is then stored until daytime (Cushman, 2001). During the day this stored source of carbon is released to the light-requiring Calvin-Benson-Bassham cycle, and photosynthesis proceeds with the stomata closed, thereby conserving water. Inducible CAM plants, although they may be incapable of full-efficiency CAM photosynthesis, can facultatively switch between C_3 (where moisture is abundant) and CAM (under moisture stress) photosynthesis (Cushman, 2001; Black and Osmond, 2003; Herrera, 2009). A plant's photosynthetic pathway results in unique internal chemistry, and may alter the costs and benefits of responding to stress, including herbivore attack (Caswell *et al.*, 1973; Ehleringer and Monson, 1993). For example, in several species inducible CAM is associated with elevated reproductive output under drought stress (summarized in Herrera, 2009). In any case, it is possible that *S. lanceolatum* growing near the meadow apex is undergoing CAM photosynthesis, while plants growing near the treeline are instead undergoing C_3 photosynthesis. Further, as in other plants (Tevini *et al.*, 1991; Veit *et al.*, 1996), the congener *S. album* produces phenolic compounds in response to excessive light as a kind of "sunscreen" (Reuber *et al.*, 1996), altering the plant's chemistry both directly, and indirectly through its effects on the CAM pathway (Bachereau *et al.*, 1998). If this is also true of *S. lanceolatum*, then in a very real sense the responses of plants near the treeline and at the meadow apex to herbivory may be because the plants are themselves fundamentally different.

In Chapter 4 I proposed that, if the treeline continues to encroach upon alpine meadow habitat, near-treeline habitat will grow as a proportion of the remaining meadow. Since *S. lanceolatum* near the treeline is consumed relatively less than its counterparts in mid-meadow and at the meadow apex, this suggests that the amount of available habitat for *P. smintheus* larvae will decline faster than will apparent habitat. A major objective of this chapter was to examine whether *P. smintheus* herbivory might accelerate this process by more intensively targeting hosts away from the treeline. My results show that this is unlikely: herbivory at the meadow apex produced overcompensatory growth of both reproductive and non-reproductive stems in the host, suggesting that *S. lanceolatum* populations in these regions retain their capacity for both current and future reproduction. In fact, these results may provide part of the answer to the question of how *S. lanceolatum* remains at the meadow apex, posed in Chapter 2: perhaps *P. smintheus* herbivory plays its own small part..

Overall, I conclude that herbivory represents a relatively minor stress with little effect overall for *S. lanceolatum*, but that it may lead to compensatory growth via complex interactions with meadow location, flowering history, and potentially plant chemistry. Otherwise, flowering represents a far greater stress for *S. lanceolatum*, and this study provides one of the few between-year demonstrations of the tradeoff between growth and reproduction for a perennial species.

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Chapter 7

Conclusions

7.1 Summary

Throughout this thesis I have used *P. smintheus* and *S. lanceolatum* as a model for exploring variation in a plant herbivore interaction relative to the alpine treeline, and the questions I have asked have varied from the specific to the general. Although I have tried to keep the overall narrative in mind in each chapter, I summarize my key findings here as well.

In Chapter 2, I showed that the distribution and quality of host plant resources for *P. smintheus* vary across an alpine meadow gradient from treeline to meadow apex. In particular, the majority of *S. lanceolatum* grows relatively near the treeline-delimited meadow edge. The precise reasons for this pattern are unclear, but it is likely due to a combination of reduced nutrient and moisture stresses near the treeline, in part because of the effects of lingering snow in these meadow regions. In alpine systems nutrients (especially N and P) are often limited (Bowman *et al.*, 1993), and may be especially limited where spring snow melt exports nutrients to lower elevations (Brooks *et al.*, 1998; Hood *et al.*, 2003). Areas with deeper snow, such as those near the alpine treeline (Walsh *et al.*, 1994), may show reduced N loss because N-retaining soil microbes are better insulated against freezing (Brooks *et al.*, 1997, 1998; Brooks and Williams, 1999; Schimel *et al.*, 2004). Regardless, since snow drifts more deeply, and thus melts later, near physical barriers like the alpine treeline, the regions of greatest *S. lanceolatum* abundance would also be inaccessible to *P. smintheus* for the longest. The key implications of my results are that, as theory predicts that insect herbivores should follow an ideal free distribution based on the spatial arrangement of palatable host plants (e.g. Cates, 1980; Loxdale and Lushai, 1999), and as *P. smintheus* females will not oviposit where the host plant is absent (Fownes and Roland, 2002), *P. smintheus* larval abundance would also be expected to be higher near the treeline. Yet the effects of snow cover, both in terms of accessibility and effects on *S. lanceolatum* quality, work against these expectations.

Chapter 3 extended this idea, exploring whether oviposition, like the underlying distribution of *S. lanceolatum*, varies with distance from the meadow edge. Although male *P. smintheus* avoid the meadow edge (Ross *et al.*, 2005*a*), I showed that females do not, and are effectively neutral to meadow location when ovipositing, provided *S. lanceolatum* is present and of sufficient quality. Females do not oviposit strictly in response to *S. lanceolatum* abundance; if they did, they would have shown a stronger pattern of ovipositing near the treeline. Rather, it appears that host plant quality is paramount in the decision to oviposit. Although I examined only damage from previous herbivory, it is clear that females can and do assess host plants qualitatively, and it is possible that their relative indifference to the meadow edge—in spite of the abundance of host plants there—reflects a decision based on still unknown aspects of *S. lanceolatum* quality. Either way, my results suggest that larval *P. smintheus* begin their lives distributed randomly relative to the treeline.

Extending this idea further, in Chapter 4, I explored the relationship between the distribution of host plants and the distribution of *P. smintheus* larval herbivory upon those host plants. Similar to the pattern for oviposition, herbivory damage was distributed relatively evenly throughout the meadow, though the underlying host plants were not. Digging deeper, the spatial patterns of host plants and herbivory were closely associated away from the meadow edge, yet decoupled near it—in other words, while larvae may follow an ideal distribution (Cates, 1980; Loxdale and Lushai, 1999) at the apex sites, this pattern deteriorates near treeline. Assuming the pattern of herbivory near the treeline is in fact still ideal, but based on new and unique considerations, then *S. lanceolatum* near the treeline may be either inaccessible or less palatable to *P. smintheus* larvae. Further, individual plants were more heavily damaged by herbivory away from treeline. The implication of these findings, which is reflected in both oviposition and larval feeding behaviour, is that although *S. lanceolatum* may be abundant near the meadow edge, it may not be available to *P. smintheus* larvae.

This interpretation assumes that both females when ovipositing, and larvae when feeding, exhibit deliberate, guided movement, and that their behaviour and distribution are thus a result of choice. Female *P. smintheus* are clearly able to discern and respond to meadow suitability for oviposition (Fownes and Roland, 2002), and butterflies in general have well-developed eyesight (Scherer and Kolb, 1987; Bernard and Remington, 1991; Kinoshita *et al.*, 1999). However, larvae of many Lepidoptera apparently find their hosts simply by chance (Dethier, 1959*b*; Kennedy *et al.*, 1961; Jermy *et al.*, 1988; Wan and Harris, 1996; Bierzychudek *et al.*, 2009), and it would theoretically be possible for *P. smintheus* larvae to simply remain near where they hatch, oblivious to the abundant *S. lanceolatum* near the meadow edge. On the other hand, if *P. smintheus* larvae can actively orient towards *S. lanceolatum*, then the pattern I observed in Chapter 4, where larvae ate less relative to the abundance of *S. lanceolatum* near treeline, would more likely be because larvae actually do avoid the meadow edge. Thus, Chapter 5 addressed this issue by examining *P. smintheus* larval host orientation, and showed that larvae can locate and actively orient towards *S. lanceolatum*. This, coupled with my observations that larvae can move 10s of meters in a single day, suggests that near-treeline *S. lanceolatum* is indeed less available—whether physically or nutritionally—to *P. smintheus*.

Finally, having shown that larval herbivory is more intense away from the treeline, I examined the effects of herbivory on *S. lanceolatum* growth and reproductive output relative to the treeline. If, as might be expected, intense herbivory results in lowered growth or reproductive potential, then the pattern I identified in Chapters 2 and 4 should be magnified over time, as already scarce *S. lanceolatum* away from treeline is most heavily eaten. However, herbivory had no negative impact on *S. lanceolatum* growth anywhere in the meadow, and actually improved growth at the meadow apex. Thus, larval *P. smintheus* herbivory may actually help, however slightly, to increase the low abundance of *S. lanceolatum* at the meadow apex. Previous flowering history affected growth of both vegetative and flowering stems, which is in line with theory that predicts that flowering should come at the cost of growth and future flowering, but its effects were not dependent on meadow location. Although the study was hampered by low levels of *P. smintheus* herbivory, my results suggest that *P. smintheus* herbivory is not a major stress for *S. lanceolatum* anywhere in the meadow and, therefore, that it is unlikely to significantly influence *S. lanceolatum* distribution relative to the treeline.

Thus, the thesis forms something of a narrative circle—from the identification of an underlying pattern in *S. lanceolatum* distribution, to that pattern's relationship with patterns of *P. smintheus* oviposition and larval herbivory, through validation of my explanation of that relationship, and finally to the effects of *P. smintheus* herbivory on the underlying pattern of *S. lanceolatum* distribution.

7.2 Final thoughts

Parnassius smintheus and *S. lanceolatum* serve as excellent models for understanding how edge effects, such as those projected into meadows from the alpine treeline, can spatially alter interactions between herbivore and host plant. While some have shown that herbivores can benefit from altered interactions with plants near forest edges (Wirth *et al.*, 2008), I describe an example of the opposite, where a herbivore seemingly avoids outwardly suitable host plants near the forested meadow edge. From a conservation standpoint, there is a danger that such edge effects would confound accurate estimates of available habitat, potentially leading to overestimation. Moreover, I highlight how encroachment of forest, or expansion of edge relative to non-edge habitat for any other reason, would compound this effect. Thus, while altered plant-herbivore interactions near the treeline are interesting and, in this case, contribute to the natural historic understanding of a butterfly with direct relevance to endangered congeners (*P. apollo*; *P. mnemosyne*), it is the broader implications for the potentially large difference between *apparently* available habitat and *actually* available habitat that are truly striking. Further, while most assessments of butterfly habitat consider adults only, and thus arrive at an estimate of usable habitat by drawing a binary distinction between “habitat” and “not habitat”, I show that there are subtler effects extending some distance from the habitat edge that may affect larvae. This is an important point, since it implies that the carrying capacity of a given habitat may be quite different for adults and for larvae. I began by outlining a theoretical case for considering larval habitat requirements along with adult requirements in any assessment of butterfly habitat, and my results, I think, only bolster the argument.

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