Climate change and invasion impacts
in the sub-Antarctic

by

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Declaration

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Date: 25 January 2012

"A NATURALIST'S PARADISE

For more than 100 years after their discovery, Nature succeeded in protecting the scientific secrets of the Prince Edward Islands by the simple expedient of blowing away the inquisitive naturalists and surveyors who tried to land."

(from 'No pathway here' by John H. Marsh, 1948)

Cloud bank, Marion Island

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Abstract

Climate change and biological invasions are major threats to biodiversity. In particular, these threats are predicted to influence terrestrial systems in the sub-Antarctic, where significant ecosystem responses to both have already been seen. In this thesis, the sub-Antarctic Prince Edward Island group is used as a model system in which to investigate key questions relating to climate change and invasive species impacts. The island group comprises two islands, Marion (MI) and Prince Edward (PEI), both of which are experiencing rapid warming, yet have different invasive assemblages and in consequence are experiencing different impacts.

Variation in the patterns of invasive species richness and abundance and their underlying causes are matters of considerable ecological and conservation significance. While an increase in thermal energy availability typically results in an increase in species richness, the mechanisms underlying these patterns are poorly understood. In Chapter 2 of this thesis, these relationships are explored for springtails, an important component of the soil fauna on Marion Island. Energy explains a large amount of the spatial variation in indigenous and invasive springtail species richness. Disturbance thresholds and stressful temperatures are more important than increased population sizes in determining this variation in species richness. As both indigenous and invasive springtail species richness and abundance are strongly related to temperature, a warming climate could have far-reaching consequences for these organisms. In particular, invasive species are predicted to be at an advantage relative to indigenous species under warming conditions.

One species where this seems especially likely, given its physiological responses to experimental warming and drying, is the large invasive tomocerid, *Pogonognathellus flavescens*. Determining whether this will be the case depends on understanding the factors underlying its range limits and abundance structure. Moreover, few studies have sought to distinguish the causal basis of abundance structure and range limits, particularly for invasive species. Thus, in Chapter 3, local microclimate variables and physiological tolerances of the invasive springtail, *P. flavescens* (a habitat generalist), are examined. The results suggest that the species should be widely distributed across a range of habitats on MI. However, the springtail is restricted to indigenous *Poa cookii* tussock grassland habitats in the southeast. The current range limits are set by dispersal limitation (i.e. contingent

absences) whilst abundance structure is a function of variation in soil substrate quality. However, over time, the widening distribution of *P. cookii*, as a consequence of a major management intervention (the eradication of feral cats), may enable *P. flavescens* to colonise all suitable areas.

In Chapter 4, the focus changes to what has been considered the third major response to climate change, along with range and phenological responses - changing animal body sizes. Body size is one of the most significant and obvious features of animals and is of considerable ecological and physiological importance. A prediction of the temperature-size rule (TSR) is that with warming, body size of the weevil species on both MI and PEI should decline. However, predation by mice of the weevils on MI should fundamentally affect the pattern of such change, causing it to differ from neighbouring PEI, indicating synergistic impacts between climate change and invasions. Analysis of a 24-year data set indicates a decline in the body size of all weevil species on PEI with increasing temperature. However, on MI, a negative relationship between mean annual temperature and body size is found only for *Palirhoeus eatoni*, a species not eaten by mice. A possible explanation for the positive relationships found for the other species could be due to higher metabolic demands imposed on mice in colder years than in warmer ones. Any increase in predation coupled with a preference for larger sizes, which the mice clearly show, would lead to a decline in the mean size of the weevil species. Due to the relationship between body size and metabolic rate and the importance of the weevils in the islands' food webs, changes to the body size of these organisms could have significant consequences for the island ecosystems' functioning.

The thermal environment experienced by organisms also has a direct effect on survival, growth and reproduction. The physiological response of organisms to rapidly changing climates is therefore a primary concern. Organisms may respond to variable environmental conditions through phenotypic plasticity as well as behaviour. Chapter 5 of this thesis shows that of the weevil species and populations investigated on MI, most display phenotypic plasticity, the form of which is in keeping with the 'Hotter is Better' hypothesis. This could be due to rare extreme temperature events and the advantage for the performance curves to incorporate high temperatures experienced in the environment. Mismatches between thermal optima and preferred temperatures displayed by all species could mean that these weevils are well equipped to cope with warming conditions on MI

unless the prediction of an increase of rare extreme events such as extreme temperatures is realised.

Rapidly changing climates and an increase in the introduction of non-indigenous species are issues of major conservation concern. This has increased the significance of studies on the impacts of these threats. However, this thesis shows that to understand such processes, it is essential that an integration of disciplines be undertaken. This thesis thus adopts a multidisciplinary approach and highlights key issues associated with both climate change and biological invasions. The patterns and predictions of species and community responses to these environmental changes are complex. Moreover, predicting such responses is likely to be problematic, especially as multiple factors will change concurrently and how these factors might change is unclear. This highlights the importance of long-term records for understanding organism responses to such changes. Furthermore, impacts on indigenous species are likely to be exacerbated by the predicted increase in the rate of introductions with climate change. This makes the case for preventing the dispersal of invasive species to new areas all the more important.

Opsomming

Klimaatsverandering en indringer spesies is belangrike bedreigings vir biodiversiteit. In besonder word voorspel dat hierdie bedreigings terrestriele sisteme in die sub-Antarktiese sal beïnvloed, waar beduidende ekosisteem reaksie aan beide reeds gesien is. In hierdie tesis word die sub-Antarktiese Prince Edward eiland groep as a model sisteem gebruik om belangrike vrae met betrekking tot klimaatsverandering en die impak van indringer spesies te ondersoek. Die eiland groep bestaan uit twee eilande, Marion (ME) en Prince Edward (PEE), wat beide versnellende verwarming ervaar, maar tog verskillende indringer samestellings het en vervolgens verskillende impakte ervaar.

Variasie in die patrone van indringerspesierykheid en vollopheid en hulle onderliggende oorsake is van aansienlike omgewings en bewarings betekenis. Terwyl 'n toename in die beskikbaarheid van energie tipies lei tot 'n toename in spesierykheid, word die onderliggend meganismes van hierdie patrone swak verstaan. In Hoofstuk 2 van hierdie tesis, word hierdie verhoudings vir springsterte ondersoek, 'n belangrike komponent van die grond fauna op ME. Energie verduidelik 'n groot hoeveelheid van die ruimtelike variasie in inheemse en indringende springstert spesierykheid. Versteuringsdrempels en stressvolle temperature is meer belangrik as die toename in bevolking groottes in die bepaling van hierdie variasie in spesierykheid. Aangesien beide inheemse en indringende springstert spesierykheid en vollopheid sterk verwant is aan temperatuur, kan 'n verwarmende klimaat verreikende gevolge vir hierdie organismes hê. In die besonder word voorspel dat indringerspesies bevoordeeld sal wees relatief tot inheemse spesies onder verwarmende toestande.

Een spesie waar dit veral blyk om geneig te wees, gegewe sy fisiologiese reaksie tot eksperimentele verhitting en uitdroging, is die groot indringer tomocerid, *Pogonognathellus flavescens*. Om te bepaal of dit die geval sal wees, hang af van die begrip van die onderliggende faktore van sy voorkomsgrense en vollopheidstruktuur. Daarbenewens is daar min studies wat gepoog het om te onderskei tussen die veroorsakende basis van vollopheidstruktuur en voorkomsgrense, veral vir indringerspesies. Dus, in Hoofstuk 3, word plaaslike mikroklimaat veranderlikes en fisiologiese toleransies van die indringer springstert, *P. flavescens* ('n habitat generalis), ondersoek. Die resultate stel voor dat die spesie wyd verspreid moet wees oor 'n

verskeidenheid van habitatte op ME. Maar, die springstert is beperk tot inheemse *Poa cookii* polle grasveldhabitatte in die suidooste. Die huidige voorkomsgrense word daar gestel deur verspreidingsbeperking (dws voorwaardelike afwesighede), terwyl vollopheidstruktuur 'n funksie is van die variasie in die grond substraat kwaliteit. Maar, die uitbreidende verspreiding van *P. cookii* as gevolg van 'n groot bestuursingryping (die uitwissing van wilde huiskatte), kan *P. flavescens* in staat stel om alle geskikte gebiede te koloniseer met verloop van tyd.

In Hoofstuk 4 verander die fokus na wat as die derde groot reaksie op klimaatsverandering beskou word, saam met voorkoms en fenologiese reaksies - veranderende diere liggaamsgroottes. Liggaamsgrootte is een van die beduidendste en mees voor die hand liggende eienskappe van diere en is van aansienlike ekologiese en fisiologiese belang. 'n Voorspelling van die temperatuur-grootte-reël (TGR) is dat met verwarming, liggaamsgrootte van die snuitkewerspesies op beide ME en PEE sal afneem. Hoe ookal, predasie deur muise van die snuitkewers op ME moet fundamenteel die patroon van sodanige verandering op PEE beïnvloed, wat sinergistiese impakte tussen klimaatsverandering en indringings aandui. Die ontleding van 'n 24-jarige datastel dui aan op 'n afname in die liggaamsgrootte van alle snuitkewer spesies op PEI met 'n toename in temperatuur. Maar, op ME is 'n negatiewe verhouding tussen die gemiddelde jaarlikse temperatuur en liggaamsgrootte net gevind vir *Palirhoeus eatoni*, 'n spesie wat nie deur die muise geëet word nie. 'n Moontlike verduideliking vir hierdie positiewe verhoudings wat gevind is vir die ander spesies kan wees as gevolg van hoër metaboliese eise op die muise in kouer jaar as in warmer jare. Enige toename in predasie, tesame met 'n voorkeur vir groter mates, wat die muise duidelik wys, sou lei tot 'n afname in die gemiddelde grootte van die snuitkewer spesies. As gevolg van die verhouding tussen liggaamsgrootte en metaboliese tempo, sowel as die belangrikheid van die snuitkewers in die eilande se voedselwebbe, kan veranderinge in die liggaamsgrootte van hierdie organismes beduidende gevolge op die eiland ekosisteme se funksionering hê.

Die termiese omgewing wat deur organismes ervaar word het ook 'n direkte invloed op oorlewing, groei en voortplanting. Die fisiologiese reaksie van organismes op vinnig veranderende klimate is dus 'n primêre bron van kommer. Organismes kan reageer op veranderlike omgewingstoestande deur fenotipiese plastisiteit sowel as gedrag. Hoofstuk 5 van hierdie tesis toon dat van die snuitkewerspesies en bevolkings wat ondersoek is op ME,

die meeste fenotipiese plastisiteit vertoon, die vorm wat in ooreenstemming is met die 'Warmer is Beter' hipotese. Dit kan wees as gevolg van seldsame uiterste temperatuur gebeure en die voordeel vir die prestasie kurwes om hoë temperature wat ervaar word in die omgewing in te sluit. Mismatches tussen termiese optima en voorkeur temperature vertoon deur alle spesies kan beteken dat hierdie snuitkewers goed toegerus is om die verhitting op ME te hanteer, tensy die voorspelling van 'n toename van seldsame uiterste gebeure soos uiterste temperature gerealiseer word.

Vinnig veranderende klimate en 'n toename in die bekendstelling van nie-inheemse spesies is kwessies van groot bewarings kommer. Dit het die betekenis van studies oor die impak van hierdie bedreigings verhoog. Hierdie tesis toon egter dat om sulke prosesse te verstaan, dit noodsaaklik is dat 'n integrasie van die dissiplines onderneem word. Hierdie tesis aanvaar dus 'n multi-dissiplinêre benadering en beklemtoon die belangrike kwessies wat verband hou met beide klimaatverandering en biologiese indringing. Die patrone en voorspellings van spesies en die gemeenskapsreaksies op hierdie omgewingsveranderinge is kompleks. Verder, die voorspelling van sodanige reaksies sal waarskynlik problematies wees, veral omdat verskeie faktore gelyktydig sal verander en hoe hierdie faktore kan verander is onduidelik. Dit beklemtoon die belangrikheid van lang termyn rekords vir die begrip van organisme reaksies op sulke veranderinge. Verder, die impak van inheemse spesies is geneig om te vererger deur die voorspelde toename in die tempo van bekendstellings met klimaatsverandering. Dit maak die taak vir die bestuur van die voorkoming dat indringerspesies nuwe gebiede bereik al hoe meer belangrik.

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Chapter 1 – General introduction

Albatross Lakes, Marion Island

Environmental change

Human-induced impacts are well known as major drivers of biodiversity change. As these impacts are perturbing natural communities at a rapid rate, it is essential to know how species and communities are responding to perturbations now and how this may change in the future (Begon *et al.* 1996). Two key perturbations that are receiving increasing attention and have been recognised as major threats to biodiversity are climate change and the introduction of invasive species (Millennium Ecosystem Assessment 2005).

The biological consequences of climate change have been widely reported in the scientific literature and considerable impacts on species and communities have been recorded (see Parmesan 2006 for a review). These are broadly summarised into four categories: (1) changes to physiology and performance, (2) changes in distribution, (3) changes in phenology, and (4) adaptation to the new climatic conditions (Hughes 2000), where responses in the fourth category depend also on the level of phenotypic plasticity displayed by the organisms (Agrawal 2001). In addition, through the temperature-size rule (Atkinson 1994), warming conditions have also been shown to influence the body size of some taxa, including birds, mammals and insects (Smith *et al.* 1995; Smith & Betancourt 2006; Gienapp *et al.* 2008; Gardner *et al.* 2009; Gardner *et al.* 2011). Due to the relationship between body size and metabolic rate (Chown *et al.* 2002a; Brown *et al.* 2004), declines in the body size of some species could have far reaching consequences for ecosystem functioning.

At the same time, biological invasions pose a significant threat to biodiversity. Humans have served as both accidental and deliberate dispersal agents of invasive species (Mack *et al.* 2000; Mack & Lonsdale 2001) and the frequency and extent of introductions worldwide is alarming (Gaston *et al.* 2003; Strauss *et al.* 2006a). For example in some cases the number of invasive species outnumbers the number of indigenous species (Frenot *et al.* 2005), which is particularly concerning due to the negative consequences that invasive species can have on some indigenous flora and fauna (Mack *et al.* 2000; Frenot *et al.* 2005; Blackburn 2008). The sensitivity of indigenous species to disturbances such as invasions depends on both the direct effects of the perturbation on that species, and the indirect effects caused by changes in abundances of other species (Jonsson *et al.* 2006; Blackburn *et al.* 2009). Direct effects are defined as the positive or negative influence that one species

has on another that does not involve another species (Wootton 2002). These could be instances such as trampling (Convey 2001; Frenot *et al.* 2005), browsing (Strauss 1991), and predation of birds eggs and terrestrial invertebrates (Chown & Smith 1993; Convey 2001). Indirect effects occur when the impact of one species on another requires the presence of a third species (Wootton 1994). For example, one species causing the alteration of habitat structure which could lead to changes in the dominance or behaviour of another species (Convey 2001). Indirect effects could also influence the evolutionary trajectory of a species through the alteration of ecological interactions (Wootton 1994; Strauss *et al.* 2006b; Traveset & Richardson 2006). As invaders and natives interact, networks of new direct and indirect effects that result in novel coevolutionary relationships between native and invasive species are likely to result (Strauss *et al.* 2006b). Thus, Strauss *et al.* (2006b) propose that a likely consequence of invasion is the loss of genetic variation from indigenous populations, which may compromise species ability to respond to shifting selection from other, new invaders or other selective forces such as habitat destruction or climate change. Moreover, climate change is predicted to facilitate the introduction of invasive species (Walther *et al.* 2002; Frenot *et al.* 2005). Interactions between climate change and invasive species are poorly understood, but evidence of them is accumulating. For example, warming conditions can facilitate colonisation success (Lee *et al.* 2007) and give invasive species a physiological advantage over indigenous species (Chown *et al.* 2007; Janion *et al.* 2010). This in turn is predicted to alter patterns of abundance and species richness of both invasive and indigenous species (Smith & Steenkamp 1990; Dukes & Mooney 1999; Bale *et al.* 2002; Stachowicz *et al.* 2002; Chown *et al.* 2005; Theoharides & Dukes 2007). Warming conditions can also facilitate easier dispersal of invasive species (Mainka & Howard 2010). For this reason, understanding spatial variation in abundance, and what drives it, is essential for forecasts of responses to environmental change and development of effective conservation strategies. However, few studies have sought to distinguish the causal basis of abundance structure and range limits, particularly for invasive species.

Effects of climate change and biological invasions have been recorded around the world (Parmesan 2006; Mainka & Howard 2010). In particular, these threats are predicted to influence terrestrial systems in the sub-Antarctic (Chown & Convey 2007) where significant ecosystem responses to both have already been seen (Convey 1997; Bergstrom

& Chown 1999; Convey *et al.* 2003; Lebouvier *et al.* 2011). The isolated systems in the region and their unusual and important biotas make them important to study for conservation purposes. Moreover, these relatively simple and intact systems, rapid climate change and the introduction of invasive species makes the sub-Antarctic islands good models for climate change and invasion studies.

Climate change and invasive species in the sub-Antarctic

Islands across the world are severely threatened by invasive species, the impacts of which have received considerable attention (Lonsdale 1999; Mack *et al.* 2000; Chapuis *et al.* 2004; Frenot *et al.* 2005; Blackburn 2008). In particular, there is substantial interest in the islands of the sub-Antarctic as the region is threatened by an increase in invasive species as well as rapid climate change (Kennedy 1995; Convey 1997; Bergstrom & Chown 1999; Frenot *et al.* 2005; Chown & Lee 2009). These islands thus provide model systems where answers to important ecological questions can be sought.

The significant increase in the rate of introduction of invasive species to the sub-Antarctic over the last two centuries is coincident with human activity in the region (Chown *et al.* 1998; Chown *et al.* 2005). These introductions have had wide ranging ecological impacts (Frenot *et al.* 2005) and there is a growing literature recording these impacts on sub-Antarctic islands due to invasive plants (e.g. Gremmen *et al.* 1998; Frenot *et al.* 2001; Gremmen & Smith 2008), mammals (e.g. Chown & Smith 1993; Smith *et al.* 2002; Frenot *et al.* 2005; Wanless *et al.* 2007; Angel *et al.* 2009; Bergstrom *et al.* 2009), and invertebrates (e.g. Crafford & Scholtz 1986; Chown & Block 1997; Hänel & Chown 1998; Lee *et al.* 2007, 2009; Greenslade *et al.* 2007). Moreover, patterns and the rate of introductions of invasive species are expected to shift with climate change. An increase in air temperature and decrease in precipitation have already been recorded from various sub-Antarctic sites (Bergstrom & Chown 1999; Smith 2002; Convey *et al.* 2003; le Roux & McGeoch 2008a). Ecosystem responses to these changes are predicted to be rapid and visible, such as potentially large population increases of some species (Convey 1997; Bergstrom & Chown 1999; Convey *et al.* 2003). Some species are also predicted to shift their distributions, and an increase in the introduction as well as the success of colonisation of invasive species is likely to be seen (Kennedy 1995; Bergstrom & Chown 1999; Frenot *et*

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al. 2005). Climate change is also likely to have direct and indirect effects on microarthropod community structure (McGeoch *et al.* 2006). However, predicting the outcome of climate change on indigenous community structure is likely to be problematic (Gabriel *et al.* 2001; McGeoch *et al.* 2006), as not only are multiple climatic factors predicted to change concurrently (le Roux & McGeoch 2008a), but the physiologies and life histories of such organisms are already adapted to unpredictable variations in environmental conditions (Convey 1997). Furthermore, species responses to climate change show a range of variation leading to community reorganisation (le Roux & McGeoch 2008b).

Importantly, interactions between climate change and invasion are predicted to significantly influence terrestrial systems in the sub-Antarctic (Chown & Convey 2007). For example, invasive springtail species on Marion Island may be at a physiological advantage relative to indigenous species under conditions of warming and drying (Chown *et al.* 2007). However, it is notable that due to generally wide safety margins and warming tolerances in contrast to indigenous tropical ectotherms, indigenous temperate ectotherms are predicted to be at an advantage under changing climatic conditions in that warming could increase mean fitness (see Deutsch *et al.* 2008; Martin & Huey 2008; Kingsolver 2009).

Study site: The Prince Edward Island group

The sub-Antarctic Prince Edward Island group comprises two close islands, the larger Marion (46°54'S, 37°45'E) and smaller Prince Edward (46°37'S, 37°55'E), and is located in the South Indian Ocean approximately 2300 km southeast of Cape Town, South Africa (Fig. 1). The island group is volcanic in origin and the oldest recorded date for lavas on Marion Island is 450 000 years (McDougall *et al.* 2001). The islands have an oceanic climate with a mean annual coastal temperature recorded on Marion Island of *c.* 6.5°C, total annual precipitation of *c.* 1900 mm (as measured at the meteorological station on the north eastern coast of Marion Island), generally high humidity, and strong winds. The climate has shown substantial change over the last 50 years, including an increase in mean annual temperature of more than 1°C and a decline in precipitation of more than 500 mm per annum (Smith 2002; le Roux & McGeoch 2008a). Furthermore, the frequencies of extreme events such as freeze-thaw cycles and extreme temperatures are predicted to increase into the future (le Roux & McGeoch 2008a).
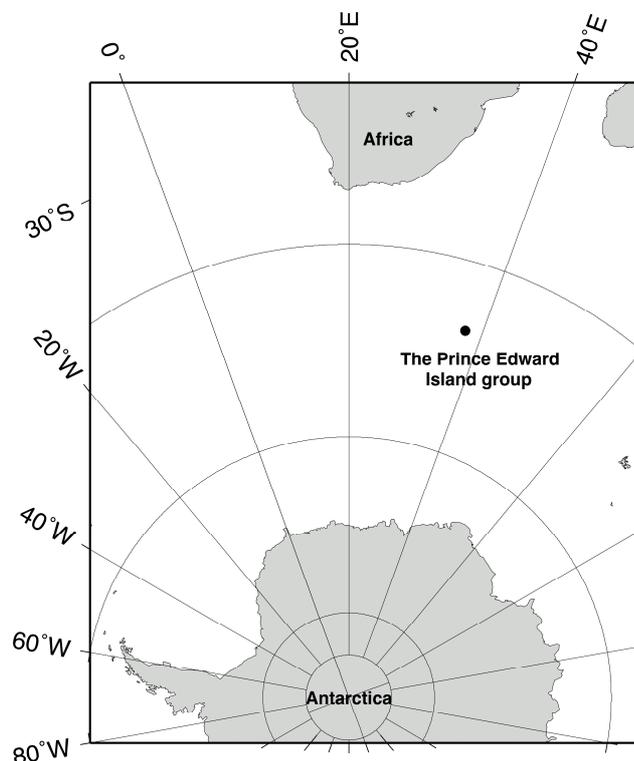
Figure 1. Location of The Prince Edward Island group.

The islands have two major biomes: barren polar desert at higher elevations (*c.* > 650 m), and tundra which predominates in lowland areas (Smith & Mucina 2006; Gremmen & Smith 2008). Six habitat complexes have been identified on the islands, namely mire, slope, fellfield, polar desert, saltspray and biotic (Gremmen & Smith 2008) (Fig. 2). Species richness of plants and invertebrates is low and no indigenous terrestrial vertebrates are found except for the Lesser Sheathbill (*Chionis minor marionensis* Reichenow) (see Chown & Froneman 2008 for an overview). The islands share most of the indigenous species that have been found in the archipelago; however, Prince Edward Island has far fewer invasive species than Marion Island (Crafford *et al.* 1986; Crafford & Scholtz 1987; Gremmen & Smith 1999; Chown *et al.* 2002b). This is due to the long history of human habitation on Marion Island and the strict controls in place for human visitation to Prince Edward Island. Most notably, Marion Island has suffered extensive impacts due to

the now exterminated Domestic Cat (*Felis catus* L.) by removal of thousands of seabirds (van Aarde 1980; Bester *et al.* 2000) and it still supports the House Mouse (*Mus musculus* L.). Neither of these invasive vertebrates have ever been present on Prince Edward Island.

Diverse communities of both macro- (including weevils (Curculionidae)) and micro-arthropods (including springtails (Collembola)) are found in the sub-Antarctic, including the Prince Edward Island group. Both weevils and springtails are major components of sub-Antarctic terrestrial communities and food webs (Burger 1985) and expected impacts to them with climate change and biological invasions are therefore a major concern. Evidence of the negative effects of invasive species and potentially of warming has already been recorded for weevils. Declines in body size of those weevil species preferred by invasive mice on Marion Island have been found, and declines in size of weevils on both islands indicate a potential effect of warming (Chown & Smith 1993) because of the temperature-size rule (Atkinson 1994). However, as only two time periods were compared in Chown & Smith (1993), these issues require further investigation. Furthermore, both an increase in temperature and a reduction in precipitation are likely to have a dramatic impact on springtail communities on Marion Island (McGeoch *et al.* 2006). Under combined experimental warming and drying treatments, McGeoch *et al.* (2006) found a decline in springtail abundance while the identities of dominant springtail species in the community changed. Gabriel *et al.* (2001) investigated the distribution and abundance of indigenous and introduced springtails across 13 habitats on Marion Island and reported contrasting responses to the abiotic environment. Indigenous springtail abundance and species richness tends to be highest in cooler, high altitude habitats whereas invasive springtails prefer moist, warm lowland habitats (Gabriel *et al.* 2001). While these results suggest that predictions of species and community responses to climate change may be difficult (Gabriel *et al.* 2001; McGeoch *et al.* 2006), physiological evidence indicates that invasive springtails should cope better with the warming and drying climate (Chown *et al.* 2007; Slabber *et al.* 2007).
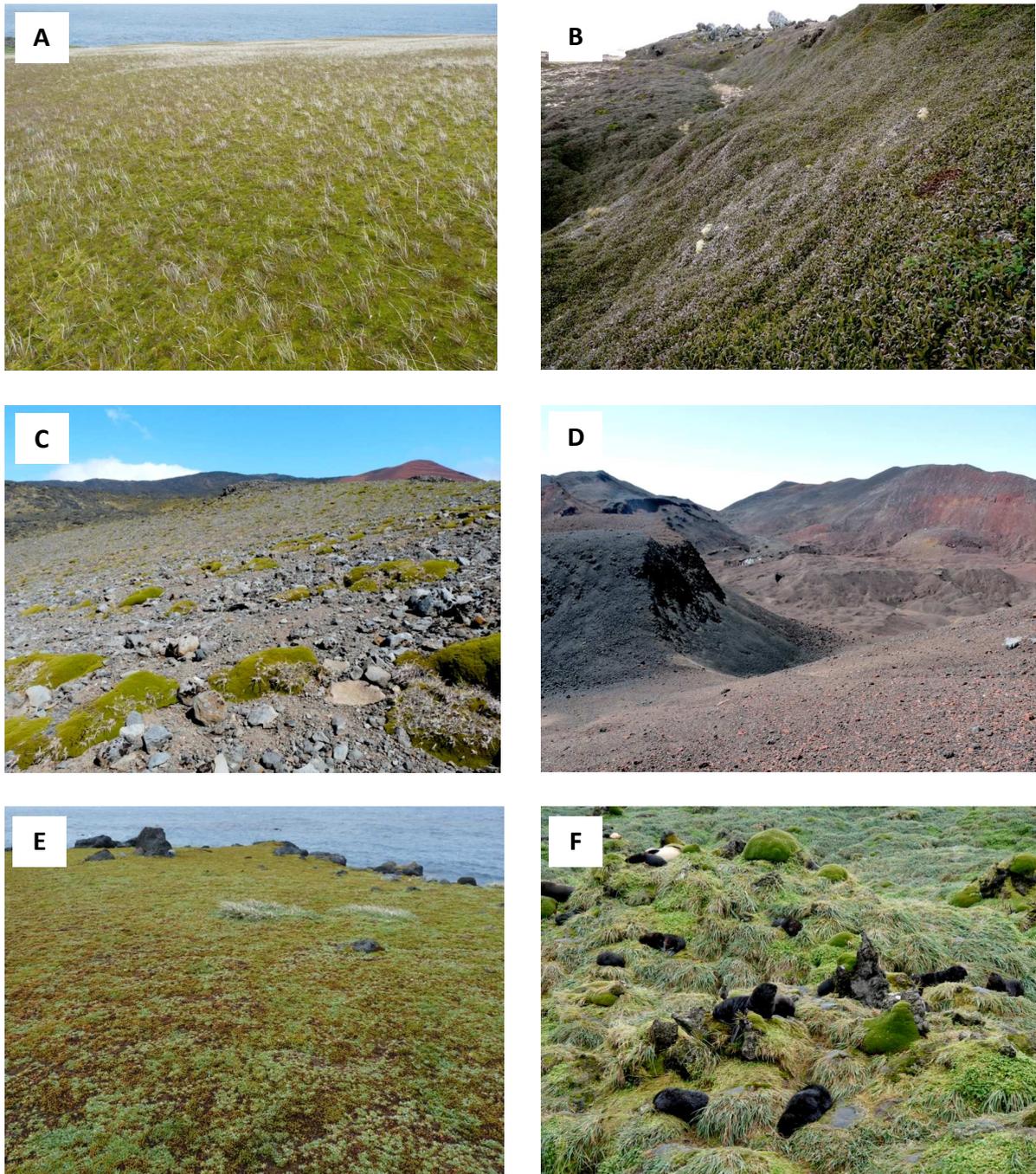
Figure 2. The six habitat complexes that have been identified on the Prince Edward Islands, namely (A) mire, (B) slope, (C) fellfield, (D) polar desert, (E) saltspray and (F) biotic (Gremmen & Smith 2008).

Weevils and springtails form important components of the terrestrial ecosystems on the Prince Edward Island Group (Smith 1977; Burger 1985; Smith & Steenkamp 1992a, b; Smith 2008). Weevils are crucial to nutrient cycling on the islands (Smith & Steenkamp 1992b), while springtails are ubiquitous in terrestrial communities with recognised importance in soil ecosystem functioning (Hopkin 1997; Wardle *et al.* 2004). Impacts to these two groups of organisms could therefore have significant consequences for the islands' functioning. As these groups of organisms are threatened by both climate change and biological invasions, they are thus exemplar taxa on which to base studies of these impacts.

Thesis outline

This thesis explores climate change and invasive species impacts on the sub-Antarctic Prince Edward Islands. To understand such processes, it is essential that an integration of disciplines be undertaken. Thus, focusing on exemplar invertebrate taxa (springtails and weevils), the work adopts a multidisciplinary approach and integrates experimental, modelling and field approaches to investigate key questions relating to both climate change and biological invasions. Each chapter is written and formatted as a stand-alone study with its own aims, methods, results and discussion (Chapter 2-5), whilst Chapter 6 provides a synthesis of the previous chapters and an assessment of the broader topics dealt with in this thesis.

- Positive relationships between indigenous and invasive species richness have frequently been recorded (e.g. Chown *et al.* 2005; Evans *et al.* 2005a; Borges *et al.* 2006; Terauds *et al.* 2011). Where positive relationships occur, it has been suggested that both groups of species are responding favourably to environmental factors (Levine & D'Antonio 1999; Stohlgren *et al.* 1999) such that an increase in thermal energy availability typically results in an increase in species richness and abundance (Evans *et al.* 2005b). This suggests that the same mechanisms that give rise to positive richness relationships in indigenous species should operate for alien or invasive species as well. However, the mechanisms underlying this are not well understood. **Chapter 2** therefore tests empirically whether indigenous and invasive springtail richness patterns are similar, whether similar species-energy mechanisms underlie

these patterns and, if so, what these mechanisms are. Specifically, the nine mechanisms identified by Evans *et al.* (2005b) are explored. The extent to which the mid-domain effect (MDE) (Rahbek 1995, 2005) explains the data is also investigated and whether interactions between indigenous and invasive species groups contribute to observed patterns is also assessed. As rising temperatures are likely to facilitate both the establishment and subsequent spread of invasive species, understanding how this might influence patterns of indigenous and invasive species richness and abundance is of considerable ecological significance.

- One of the invasive springtail species on Marion Island, *Pogonognathellus flavescens* (Tullberg 1871), is habitat specific and restricted to the southeast of the island. It was therefore not detected in the investigation of springtail distribution and abundance patterns in Chapter 2. As understanding spatial variation in abundance is essential for forecasts of and conservation responses to environmental change impacts on diversity, the causal basis of the abundance structure and range limits of this species were investigated in **Chapter 3**. Specifically, the BAM framework developed by Soberón (2007) to explain such patterns is tested explicitly.

- In **Chapter 4**, the potential synergistic impacts of climate change and invasive species are further explored. A prediction of the temperature-size rule (see Atkinson 1994) is that with warming, body size of the weevil species on both islands should decline. However, predation by mice of the weevils on Marion Island should fundamentally affect the pattern of such change, causing it to differ from neighbouring Prince Edward Island. To test these predictions, a 24-year time series is used to explore the relative effects of warming and mouse predation on body size distributions of the six indigenous weevil (Curculionidae) species that occur on the Prince Edward Islands. Body size is one of the most obvious features of animals and is of considerable ecological and physiological significance (Peters 1983; Brown *et al.* 2004). Both temperature (through the temperature-size rule (Atkinson 1994)) and invasive species (by influencing mortality and production rates (Chown & Smith 1993; Phillips & Shine 2004; Fisk *et al.* 2007)) can influence body size. The potential synergistic impacts of these threats are tested here, as both islands are experiencing rapid warming, and weevils are predated on by mice on Marion Island, which are not present on Prince Edward Island. The indigenous weevil species on the Prince Edward

Islands form an important component of the islands relatively simple food webs (Smith & Steenkamp 1992b). As body size relates directly to metabolic function (Brown *et al.* 2004), body size changes of these weevils could have serious impacts on the islands' functioning.

- The thermal environment experienced by organisms has a direct effect on survival, growth and reproduction (Chown & Nicolson 2004; Angilletta 2009). The physiological response of organisms to rapidly changing climates is therefore a primary concern. One way that organisms may respond to variable environmental conditions is by altering their phenotypes (Kingsolver & Huey 1998). **Chapter 5** therefore tests proposed theoretical models of environmental adaptation of weevils along an altitudinal gradient on Marion Island by assessing acclimation responses of performance of different weevil populations. In addition, the thermal preference of the populations is also determined to explore the extent to which the preferred temperatures of the organisms match the thermal optima for performance. In particular the 'Hotter is Better' (Huey & Kingsolver 1989; Angilletta *et al.* 2010) hypothesis is tested within a strong inference framework, and Asbury & Angilletta's (2010) thermodynamic effect hypothesis is also examined.

- Finally, **Chapter 6** draws together the outcomes of this work and considers the broader implications of climate change and invasive species impacts.
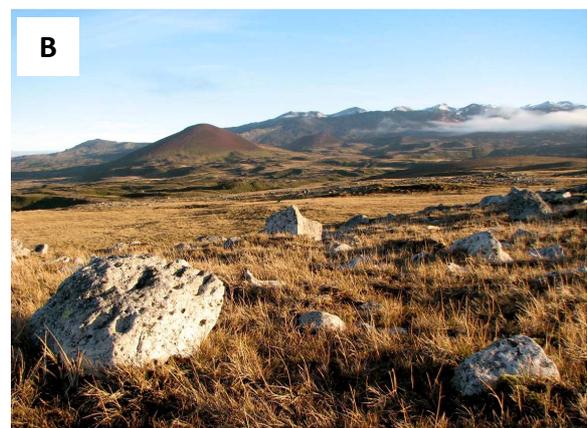
(A) Looking down on the lowlands of Prince Edward Island, and (B) view towards the highlands of Marion Island.

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Chapter 2 – A test of the mechanisms underlying species-energy relationships in indigenous and invasive springtails[1]

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Invasive springtails on Marion Island, *Isotomurus cf. palustris* (Müller 1876)

Introduction

Variation in the patterns of alien and invasive species richness and abundance, and their underlying causes are matters of considerable ecological and conservation significance. From an ecological perspective, variation in alien species diversity and investigations of the mechanisms underlying it can, with the necessary caution, provide much fundamental insight (Sax *et al.* 2007; Blackburn *et al.* 2009). Given the impacts invasive species have on species and ecosystems (Vitousek *et al.* 1997; Mack *et al.* 2000; Blackburn *et al.* 2004; Butchart *et al.* 2010), and indications that the scale of invasion will continue to increase (Hulme *et al.* 2008; Walther *et al.* 2009; Pyšek & Richardson 2010), understanding the factors underlying variation in invasive species diversity is also important for conservation management.

While early views held that more diverse sites should be less prone to biological invasions (Elton 1958), a positive relationship between indigenous and alien species richness has been recorded at relatively large spatial scales for a variety of taxa, such as plants (e.g. Stohlgren *et al.* 1999, 2008; Fridley *et al.* 2004; Herben *et al.* 2004; Stark *et al.* 2006; Fridley *et al.* 2007; Souza *et al.* 2011), invertebrates (e.g. Chown *et al.* 2005; Borges *et al.* 2006; Lilleskov *et al.* 2008; Terauds *et al.* 2011) and birds (e.g. Evans *et al.* 2005a). At these larger spatial scales, the positive relationship is a consequence of variation in thermal energy availability, spatial heterogeneity and/or metacommunity dynamics (Levine & D'Antonio 1999; Stohlgren *et al.* 1999; Evans *et al.* 2005b; Melbourne *et al.* 2007; Souza *et al.* 2011). At smaller spatial scales the form of the relationship is more variable and reflects a variety of processes including interactions among indigenous and invasive species, and the effects of particular species (Davies *et al.* 2007; Diez *et al.* 2008; Terauds *et al.* 2011. The influences of thermal energy availability and community dynamics suggest that the same mechanisms responsible for positive species-energy relationships in indigenous species at the macro-scale, result in positive relationships in alien species.

Much information now exists on the form of the relationship between species richness and thermal energy availability (Gaston 2000; Hawkins *et al.* 2003; Kalmar & Currie 2007; Mittelbach 2010; Whittaker 2010), and several studies have explored the extent to which these relationships are mirrored among indigenous and alien species in the same general geographic area (Chown *et al.* 1998, 2005; Marini *et al.* 2009). However,

examination of the mechanisms underlying these relationships remains uncommon. Positive species-energy relationships at the macro-scale can be generated by one or more of nine principal mechanisms: sampling, increased population size (or the more individuals hypothesis, see Wright 1983; Srivastava & Lawton 1998; Currie *et al.* 2004; Scheiner & Willig 2005), dynamic equilibrium, niche position, niche breadth, more trophic levels, consumer pressure, range limitation and diversification rate (Evans *et al.* 2005b). Although studies of the diversification rate and more individuals hypotheses are growing in frequency (Currie *et al.* 2004; Hurlbert 2004; Evans *et al.* 2006; Mönkkönen *et al.* 2006; Sanders *et al.* 2007; McCain & Sanders 2010), especially given increasing interest in metabolic ecology (Brown *et al.* 2004; Hawkins *et al.* 2007), strong inference approaches (Platt 1964) exploring the alternative mechanisms simultaneously are less common. Moreover, these mechanisms are rarely examined for alien species (Marini *et al.* 2009), and where investigations of positive alien species-energy relationships or relationships between alien and indigenous richness are undertaken these typically focus on plants or birds (Herben *et al.* 2004; Hurlbert 2004; Evans *et al.* 2005c; Pyšek & Richardson 2006; Lambdon *et al.* 2008; Marini *et al.* 2009), and less commonly on arthropods (Terauds *et al.* 2011). Indeed, Fridley *et al.* (2007) have argued that empirical evidence for indigenous-invasive richness relationships still lags behind theory.

Here, I therefore examine the form of the species-energy relationship and the mechanisms underlying the patterns for indigenous and invasive alien springtails along two altitudinal gradients on sub-Antarctic Marion Island. In doing so the relationship between indigenous and alien richness is also examined. Altitudinal gradients provide powerful 'natural experiments' to investigate macroecological hypotheses due to large environmental variation over short spatial distances (Körner 2000, 2007; McCain 2007; Nogués-Bravo *et al.* 2008), reflected by a rapidly growing literature on altitudinal variation in species richness, its patterns and causes, focused predominantly on indigenous species (e.g. Rahbek 1995, 1997, 2005; Grytnes 2003; Sanders *et al.* 2003, 2007; McCain 2004, 2007; but see Pauchard *et al.* 2009). Two significant factors that influence richness gradients should be considered when undertaking such studies. First, area typically declines with altitude and has significant effects on the richness of both indigenous and alien species richness (Rosenzweig 1995; Hulme 2008; but see also McCain 2007, 2009). Second, spatial variation in richness might also arise as a consequence of sampling of species ranges

across a geographic domain (Colwell & Hurtt 1994; Colwell & Lees 2000). Although support for this mid-domain effect (MDE) is not extensive (Zapata *et al.* 2003; McCain 2009, 2010) and especially not for relatively constrained spatial extents (Brehm *et al.* 2007; Dunn *et al.* 2007), it has been argued that the MDE should first be examined as a null explanation for spatial variation in richness (Colwell *et al.* 2004). Thus, I examine the extent to which geometric constraints can account for richness variation as well as the effects of area.

The study organisms and study site were selected for several reasons. First, both indigenous and invasive springtail species are well documented on the island, likely play significant roles in terrestrial system functioning (Hopkin 1997; Smith 2008), and previous sampling indicates that richness and abundance vary with altitude (Barendse & Chown 2001; Gabriel *et al.* 2001). Moreover, all of the alien species examined here have been present on the island for at least 50 years (Deharveng 1981), are widely distributed, and considered invasive (Hugo *et al.* 2004; Janion *et al.* 2010). In consequence, sufficient time has elapsed for the species to disperse to all habitats that would sustain populations thereof (i.e. occupancy and therefore richness variation is unlikely to be a consequence of dispersal limitation - see Soberón 2007). Second, a pronounced altitudinal gradient in temperature exists on the island, which rises to 1230 m above sea level (Deere *et al.* 2006; le Roux & Mcgeoch 2008). Although precipitation also varies with elevation (Blake 1996), the island is not a water-limited system, receiving more than 1500 mm per annum (and greater amounts at higher elevations) in a relatively cool environment. In consequence, species-energy dynamics are more likely to be a consequence of temperature variation *per se* than variation in water-energy dynamics (see Hawkins *et al.* 2003; Clarke & Gaston 2006). Third, several studies have shown that the eastern and western sides of the island differ in terms of species abundances with these tending to be higher in the west (Hugo *et al.* 2004), thus lending some independence to the two elevational gradients examined. Finally, evolutionary dynamics, which play an important role elsewhere in determining the identity and number of species (Ricklefs 2004), are likely to be less significant on the island (see also Terauds *et al.* 2011). This is because none of the species examined are endemic (with the possible exception of the species currently known as *Cryptopygus antarcticus travei* (Deharveng 1981), see Stevens *et al.* 2006), and because Marion Island and its neighbour Prince Edward Island share the same indigenous fauna and the islands are isolated (900 km from the closest other island group, Chown & Froneman 2008).

Materials and methods

Study area and species

Sub-Antarctic Marion Island (MI) (46°54'S, 37°45'E) is part of the Prince Edward Island group, which lies approximately 2300 km southeast of Cape Town, South Africa (see Chown & Froneman 2008 for review). The island has a cool, wet, windy climate, which varies considerably with elevation. Mean annual temperature has increased and precipitation declined in step with global climate change (Smith 2002; le Roux & McGeoch 2008). The island has two major biomes: tundra, which predominates in lowland areas, and polar desert, restricted to high elevations (Smith & Mucina 2006; Gremmen & Smith 2008). Springtails (Collembola) form an important component of the soil fauna (Gabriel *et al.* 2001). Sixteen species of springtails have been recorded on Marion Island, of which six are introduced and invasive (Deharveng 1981; Gabriel *et al.* 2001; Chown *et al.* 2007).

Thermal energy availability

Soil temperature was used as a measure of thermal energy availability given that the Marion Island system is not water limited (see Hawkins *et al.* 2003; Clarke & Gaston 2006 for further discussion of temperature as a measure of thermal energy availability). Thermochron I-button temperature dataloggers (accurate to ± 0.5°C, Dallas Semiconductors, Dallas, TX, USA) were placed 5 cm below the ground surface at each site. Hourly temperatures were recorded for three winter months (mid May 2008 to mid August 2008) and three summer months (mid November 2008 to mid February 2009). Temperature data were processed to obtain mean daily minimum, maximum, mean and range for each site in R2.12.0 (R Development Core Team 2010). These are referred to as the short-term data throughout.

To confirm high water availability at areas other than the meteorological station where precipitation has typically been measured (Chown & Froneman 2008; but see also Blake 1996), a rain gauge was installed at each site. The gauges consisted of 300 mm^3 PVC pipes surmounted by standard graduated funnels. They were checked and emptied at least once a month. However, the higher altitude gauges were damaged by ice and after several unsuccessful attempts to repair them, were discontinued.

A longer-term data series (hereafter long-term dataset) was obtained by logging soil temperatures on an hourly basis at *c.* 100 m intervals from sea level to 750 m (the island is 1230 m high), across the eastern slope of Marion Island between 2002 and 2009 using I-button Thermochron dataloggers (Deere *et al.* 2006). Missing data (due to datalogger loss, exposure, or damage) were interpolated using a sinusoidal function (i.e. to approximate daily temperature cycles), with the initial and final interpolated values (as well as the amplitude of the sinusoidal curve) calculated from the temperature records of the 48 hours before and after the missing data.

Sampling protocol: altitudinal distribution and density

Species composition and abundance were investigated along two coast to inland altitudinal transects, one on the eastern side of the island and one on the west (Appendix A). As some springtail species on Marion Island show a pronounced seasonality in abundance (Barendse & Chown 2001), both transects were sampled twice: once in winter (June-July 2008) and once in summer (February 2009). The habitat complexes (see Chown & Froneman 2008) sampled were representative of those found at the specific altitudes: biotically influenced vegetation (10 m.a.s.l.), mires (50 m.a.s.l. and 200 m.a.s.l.), fellfield (400 m.a.s.l. and 600 m.a.s.l.) and high altitude polar desert (750 m.a.s.l., 850 m.a.s.l. and 1000 m.a.s.l.). Thus, each elevation is represented by a single site, giving a total of eight sites along each transect.

For each of the low elevation (10 m.a.s.l., 50 m.a.s.l., 200 m.a.s.l.) sites, four quadrats of 2 m x 2 m each were randomly placed on average 100 m apart. Global Positioning System (GPS) coordinates of and plant species present in quadrats were recorded. Each quadrat was divided into 10 cm x 10 cm blocks and a core sample was collected in the centre of each of five randomly selected blocks for each quadrat using a 34 mm (diameter) stainless steel soil corer. The mid-altitude sites at 400 m.a.s.l. and 600 m.a.s.l. consisted of fellfield habitat, which is characterised by individuals of the cushion-forming *Azorella selago* Hook. f. separated by rocky areas (Gremmen 1981). At each mid-altitude site, an 80 m x 80 m quadrat was established and one core sample collected from each of 20 randomly selected *A. selago* cushions using a 34 mm stainless steel corer. Cores were taken from the southern sides of the plants (< 20 cm diameter) as

higher abundances of microarthropods have been found on these sides as opposed to other areas of *A. selago* cushions (Hugo *et al.* 2004). Cores were taken midway between the centre of the cushion and the cushion edge. For all of these sites each core sample was placed in an individually labelled plastic bag and processed on the same day of collection.

Springtails were extracted from the top 10 cm of cores using a Macfadyen high-gradient extractor into 40% ethanol (extraction protocol followed that of previous studies on MI: 2 days at 25°C followed by 2 days at 30°C (Barendse & Chown 2001) for the east transect. Samples from the west transect were extracted using Tullgren funnels in a field hut (see Appendix B). The difference in sampling method is not considered problematic because densities obtained were well within those found by Hugo *et al.* (2004) for comparable west coast sites after high gradient extraction. Samples were then sorted and Collembola species identified using a Leica MZ75 microscope. Springtails were stored in 99% ethanol at Stellenbosch University.

For each of the high altitude polar desert sites (750 m.a.s.l., 850 m.a.s.l., 1000 m.a.s.l.), soil samples at each site were collected along four 25 m transects, which were demarcated horizontally across the slope at least 50 m apart. Samples were collected at 5 m intervals along each transect, resulting in a total of 20 samples per site. The same area was never sampled twice, with samples from the second sampling period being interspersed between those from the first sampling period. For each sample, the top 5 cm of soil and stones was removed from a 10 cm x 10 cm area because core sampling is not effective and the two methods used this way yield comparable results (Gabriel *et al.* 2001). This material was washed and sieved through 125 μm mesh three times. Large stones (diameter > 2 cm) from the samples were rinsed with water into the mesh and discarded. The water used was obtained from the field huts, strained through 125 μm mesh to prevent possible contamination and transported to the field sites in large plastic bottles. A separate piece of mesh was used for each sample and after sample washing, each mesh was placed in a separate 35 ml plastic container with 40% ethanol. Samples were then returned to the laboratory where they were sorted and Collembola species identified using a Leica MZ75 microscope. Springtails were stored in 99% ethanol at Stellenbosch University.

Sampling adequacy, species richness and abundance

Raw abundance data for indigenous and invasive species were converted to density (individuals.m^{-2}; ± standard error) to enable comparison of data from different sampling methods. To determine sampling adequacy, sample-based (i.e. per core or 10 cm x 10 cm sample) rarefaction curves (see Gotelli & Colwell 2001) were calculated using the Mao Tau moment-based interpolation method in EstimateS (Colwell 2005). Sampling is considered to be adequate if the observed rarefaction curve approaches an asymptote. Indigenous and invasive species richness was calculated for each of the sites using the non-parametric Jacknife2 estimator (Magurran 2004) using the vegan package in R2.12.0. The Jacknife2 richness estimator was used because, unlike most other estimators, it does not require sample points to be compositionally similar, species to be independent or data to be normally distributed (Magurran 2004).

Geometric constraints and area effects

To test for the strength of the mid-domain effect, the species richness data were compared with null model predictions using a Monte Carlo simulation procedure, Mid-Domain Null, in Visual Basic for Excel (McCain 2004). This program simulates species richness curves based on empirical range sizes or range midpoints within a bounded domain (McCain 2004). Randomisation techniques were used, based on 50 000 simulations sampled without replacement from empirical range sizes, and a regression of the empirical values on predicted values gave r^2, slope (*a*) and intercept (*b*) estimates of the fit of the null model (McCain 2004, 2007; Zapata *et al.* 2003, 2005).

Species area effects were investigated using generalised linear models with species richness as the response variable, and log surface area and mean thermal energy availability as the predictor variables because this approach gave the best fit. For surface area, sites were allocated to altitudinal bands and the surface area of each of these bands as per Meiklejohn & Smith (2008) was used in the models. Mean thermal energy availability was calculated for each site from the short-term data series. Full models included energy and area as variables, and single term models included either energy or area individually as variables. However, it should be noted that area and mean thermal energy availability are collinear (r^2 = 0.63, p < 0.001), making estimates of their independent combination to

richness difficult to distinguish. Nonetheless, because area was not significant in any of the full models (see Results), energy only was included in the models (see also Storch *et al.* 2005; Kalmar & Currie 2007).

Species richness and abundance patterns

Generalised linear models (in R2.12.0, each assuming a Poisson distribution with a log link function) were used to investigate the relationships between indigenous and invasive species richness and $\log_{10}(x+1)$ transformed abundance. First, invasive abundance was used as the response variable with the predictor variables including indigenous abundance and indigenous richness. Second, the same predictor variables were used with invasive richness as the response variable. Squared terms of the predictor variables were also included in the models to identify any curvilinear relationships (Terauds *et al.* 2011). Similar models were run for indigenous abundance and species richness. Optimal models were sought by sequentially removing non-significant terms until only significant terms remained. Full and optimal models were then ranked in accordance with Akaike's Information Criterion values (AIC_c) and weights (AIC_w).

Tests of the mechanisms

Sampling mechanism and increased population size mechanism

The sampling and increased population size mechanisms predict that increased thermal energy availability elevates the number of individuals an area can support, and thus generates positive decelerating relationships between species richness, abundance and energy (Evans *et al.* 2005b). The sampling and increased population size mechanisms can be distinguished from each other by controlling for sampling effect for the latter (i.e. by using an estimator of species richness). If the relationships are not positive and decelerating for the second assessment using the estimator, but are for the first, then support for the sampling mechanism is provided (Evans *et al.* 2005b).

To test for these mechanisms, relationships between species richness and abundance/energy were first investigated using S_{obs}. Second, relationships between an estimate of species richness (here Jacknife2) and abundance/energy were investigated to

remove any influence of sampling effect. The relationships between species richness (using S_{obs} for the sampling mechanism and Jacknife2 estimators for the increased population size mechanism) and $\log_{10}(x+1)$ transformed abundance, species richness and mean thermal energy availability, and between $\log_{10}(x+1)$ transformed abundance and mean thermal energy availability were investigated for both indigenous and invasive species as well as combined species using generalised linear models (GLZs) (each assuming a Poisson distribution with a log link function and corrected for overdispersion), with the glm function in R2.12.0. To test for decelerating relationships, the GLZs were rerun using a squared term for the predictors. A significant outcome indicates a decelerating relationship. In all cases the units were sites on each transect, resulting in $n = 16$.

Dynamic equilibrium mechanism

The dynamic equilibrium mechanism posits that elevated energy enables populations to recover faster from disturbance (Evans *et al.* 2005b). The mechanism thus requires demographic information about populations such as growth rates or abundance, which can be compared to disturbance events. On MI, springtails are found island-wide and large scale disturbances are mostly in the form of occasional low temperature events (Lee *et al.* 2009). Landslides are uncommon, the island is volcanically inactive, and indigenous land mammals and birds (apart from the Lesser Sheathbill (*Chionis minor marionensis* Reichenow)) are absent (see Chown & Froneman 2008 for a review). Therefore, in this study, I hypothesised that if this mechanism were operating, the abundance of the springtail species would be correlated with disturbances that occur when temperatures cross three different thresholds, namely 0°C (the freezing point of water), the mean freezing or supercooling point (SCP) of the springtails, and the mean lower development threshold (LDT) of the springtails, a temperature at which development ceases (Honěk 1996). The rationale is that if a springtail population is not resilient to the crossing of such thresholds, its abundance would be lower in areas where more of these disturbances occur compared with those areas with fewer disturbances. It was hypothesised that both the number of times the thresholds were crossed and the maximum amount of time spent below the thresholds at any one time (hereafter called longest duration) are important disturbance events. Substantial information exists on the thermal biology of the springtail

populations on MI, which I used to obtain SCP and LDT values (Janion *et al.* 2009; Janion *et al.* 2010; C. Janion unpublished data). The number of times that temperatures dropped below mean SCP and LDT, and the longest duration that temperatures remained below mean SCP and LDT were calculated for the indigenous, invasive and combined groups of springtails present at each site. Mean values were calculated for these springtail groups (see Janion *et al.* 2009). Temperatures did not drop below springtail SCP values and therefore SCP was excluded from the analyses.

The short-term and long-term temperature datasets were used for calculating the number of times that temperature dropped below, or the longest duration below, these thresholds. To account for differences in the elevation of the dataloggers in the short- and long-term soil temperature data, the adiabatic lapse rate in soil temperature was calculated from the long-term soil temperature data (-5.83°C per 1000 m). Temperature data at the nearest comparable (long-term) location were then adjusted by adding (or subtracting) a constant value, calculated as the product of the lapse rate coefficient and the altitudinal difference between the long- and short-term data logging sites. Analysis of the short-term eastern versus western temperature data indicated only slight differences between the two sides of the island at the elevations sampled (see below) and therefore the long-term data from the eastern side of the island was considered sufficient to use for the western side of the island.

The effects of thresholds (number of events and longest duration of sub-zero events and those below the LDT) on the $\log_{10}(x+1)$ transformed abundance of indigenous, invasive and combined species were investigated using path analysis (in IBM SPSS AMOS, Student Version, 5.0.1). Path analysis was developed as a technique to analyse systems with correlated variables and allows the evaluation of the structure of causal relationships among a group of variables (Sánchez-Piñero & Polis 2000; Vázquez & Simberloff 2004). Although these models are useful, they have been the subject of some discussion (see Petraitis *et al.* 1996). Structural equation modelling (SEM) procedures are used for the estimation of standardised path coefficients and the assessment of model fit (Vázquez & Simberloff 2004). The standardised path estimates (regression weights) represent the amount of change (positive or negative) in the dependent variable that is attributable to a single standard deviation unit's worth of change in the predictor variable.

Range limitation

This mechanism predicts that species occur in areas where they can meet their physiological requirements (Evans *et al.* 2005b), and assumes that more species can tolerate climatic conditions in high energy than in low energy areas (Kerr *et al.* 1998). To test this mechanism and to distinguish possible outcomes from the dynamic equilibrium mechanism, abundance of indigenous, invasive and combined species groups were compared to the minimum and maximum temperatures recorded at each site (i.e. winter mean daily minimum and summer mean daily maximum), as well as the number of generations possible at each elevation. Much evidence exists that ectotherm distributions can be limited either directly by minimum or maximum temperatures, or indirectly as a consequence of inadequate growing season length (Krushelnycky *et al.* 2005; Chown & Gaston 2008). Both the short-term and long-term temperature datasets were used (see above). The number of generations possible was calculated using the sum of effective temperatures (SET), which is the number of day degrees (dd) heat units that are required above the LDT for an individual to complete development (Honěk 1996).

Mean SET values were calculated for the indigenous, invasive and combined groups of species present at each site. When soil temperature exceeded LDT thresholds the difference was calculated and divided by the time interval between subsequent records (usually 1 hour) to estimate the degree hours accumulated by individuals experiencing those conditions. Mean developmental rate was then calculated from the mean proportion of the SET that each temperature recording contributed. Multiplying this rate by the number of days within each time period estimated the number of generations possible. This was calculated for the indigenous, invasive and combined groups of species at each site.

The effects of minimum and maximum temperatures and generations possible on the $\log_{10}(x+1)$ transformed abundance of indigenous, invasive and combined species were investigated using path analysis (in IBM SPSS AMOS, Student Version, 5.0.1). As above, this provided estimations of standardised path coefficients and the assessment of model fit.

Niche position and niche breadth

The niche position mechanism predicts that positive relationships between energy, abundance and species richness will be stronger for niche specialists, as increased energy increases abundance of rare resources (Evans *et al.* 2005b). The niche breadth mechanism predicts that in high energy areas, narrower niche breadths and reduced interspecific competition promotes the coexistence of more species than in low energy areas (Evans *et al.* 2005b). However, most springtails are generalist feeders with catholic diets (Hopkin 1997). This generalist feeding behaviour is evident for both the indigenous and invasive species on Marion Island due to the ease with which they can be maintained in laboratory conditions elsewhere (i.e. not on MI) (e.g. Janion *et al.* 2010). Furthermore, the springtails have fairly wide distributions on MI and, for the invasive species, original distributions in Europe are broad (Fjellberg 1998; Potapov 2001). Thus the niche position and niche breadth mechanisms were not considered.

More trophic levels

The more trophic levels mechanism predicts a positive relationship between thermal energy availability and the number of trophic levels in an area (Evans *et al.* 2005b). The springtails on MI are all at the same trophic level (with the possible exception of *Friesea tilbrooki* (Wise 1970), which is rare on MI, and was not found in the samples). The addition of trophic levels with increasing energy therefore does not apply.

Consumer pressure

The consumer pressure mechanism predicts that due to the other mechanisms, consumers are more abundant in high energy areas thus elevating consumer pressure (Evans *et al.* 2005b). This mechanism is unlikely to be significant for springtails on MI as it assumes that predation contributes to assemblage structure of prey populations. The only likely predators of springtails on MI are spiders. While the density of spiders is higher in areas with greater thermal energy availability (Burger 1978; Lee *et al.* 2011), these are also the areas where springtail abundance and species richness are higher (Gabriel *et al.* 2001). The mechanism assumes that increasing thermal energy availability leads to elevated consumer

pressure, so reducing prey populations. However, it is more likely that this same increasing thermal energy availability also elevates the springtail populations.

Diversification rate

The diversification rate mechanism implies that species richness is the sum of speciation minus extinction events and that thermal energy availability elevates diversification rate (Evans *et al.* 2005b). However, as I have noted previously, only a single indigenous species of springtail is thought to be endemic to MI, and the invasive species were introduced by humans, ruling out a speciation-based mechanism. In consequence, this mechanism was also rejected as implausible.

Results

Thermal energy availability

Microclimate measurements indicate a decline in temperature with altitude during both winter and summer months, on both the east and west sides of the island (Appendix C). The east side of the island is generally slightly warmer. Variation in temperature also tends to be slightly higher at higher elevations than lower ones.

Substantial rainfall was recorded with little monthly variation indicating that the system on MI is not water limited. Rainfall increases with altitude, although at the highest elevations, sub-zero temperatures might limit the availability of water. Contrary to previous assumptions (e.g. Rouault *et al.* 2005), the east side of the island received a much larger amount of rainfall than the west during our measurements (Appendix D).

Sampling adequacy

A total of eight indigenous and four invasive springtail species were sampled on the western side of the island, and seven indigenous and four invasive species were found on the east. Sample-based rarefaction curves for all sites for summer and winter data separately did not show signs of flattening off. However, when summer and winter data were pooled, rarefaction curves indicated that sampling had or mostly reached an asymptote (Appendix E). Thus, sampling was adequate for a combination of summer and

winter data and all subsequent analyses were therefore conducted with this total pooled data. The Jacknife2 estimator provided estimates that were close to observed species richness values (S_{obs}) (Appendix F) and were therefore considered adequate to use in analyses.

Species richness and abundance of both indigenous and invasive springtails declines with altitude (Appendix F). Springtail abundance and species richness was higher on the western side of the island than the east (Appendix F). At low altitudes, the abundance of the invasive species was much higher than that of the indigenous species and this difference was larger on the east side of the island than on the west. However, indigenous species dominate from mid to high altitudes (Appendix F).

Positive species-energy relationships were found (Table 1). Moreover, while both surface area and mean energy were significant predictor variables for indigenous, invasive and combined species richness in the single term generalised linear models, surface area was not significant in the full models (Table 1) and was therefore not considered any further.

Species richness and abundance patterns

Positive relationships were found between invasive and indigenous species richness, and between invasive abundance and indigenous species richness (Table 2). The optimal models for both invasive abundance and species richness included indigenous species richness as a highly significant term. Indigenous abundance and species richness were related to invasive species richness in a curvilinear fashion (Table 2).

Table 1. Results of the generalised linear models (each assuming a Poisson distribution, using a log link function and corrected for overdispersion) looking at the relationships between indigenous, invasive and combined springtail species richness, and mean thermal energy availability and log transformed surface area of altitudinal bands. Full models (A) included both energy and log transformed surface area as variables, and single term models (B) included either energy or log transformed surface area as a variable.

(A) Full models	Estimate	s.e.	x^2	p
Indigenous sp. rich. vs. mean energy + area				
(DE = 63.52%)				
mean energy	0.056	0.032	2.986	0.084
log(area)	0.309	0.219	2.015	0.156
Invasive species richness vs. mean energy + area				
(DE = 51.74%)				
mean energy	0.228	0.110	4.136	0.042
log(area)	0.454	0.718	0.405	0.525
Combined species richness v. mean energy + area				
(DE = 68.45%)				
mean energy	0.103	0.045	5.216	0.022
log(area)	0.341	0.301	1.295	0.255

(B) Single term models		Estimate	s.e.	x^2	p
Indigenous sp. rich. vs. mean energy / area					
mean energy	(DE = 57.93%)	0.092	0.021	19.138	< 0.0001
log(area)	(DE = 55.23%)	0.607	0.145	17.537	< 0.0001
Invasive species richness vs. mean energy / area					
mean energy	(DE = 50.37%)	0.277	0.074	15.666	< 0.0001
log(area)	(DE = 37.66%)	1.578	0.516	9.875	0.002
Combined species richness v. mean energy / area					
mean energy	(DE = 65.13%)	0.142	0.029	24.759	< 0.0001
log(area)	(DE = 55.07%)	0.881	0.217	16.731	< 0.0001

s.e. = standard error, DE = deviance explained

Table 2. Results of the generalised linear models looking at the relationships between springtail species richness and log transformed abundance of indigenous and invasive springtails on Marion Island. Only terms from the optimal models sought by sequential deletion of non-significant terms are shown. AIC_c values and weights (AIC_w) for the optimal models are given, as well as for full models (indicated in brackets). Deviance explained (DE) values for optimal models are also indicated.

	Estimate	s.e.	x^2	p
Invasive abundance				
AIC_c = 47.372 (51.118), AIC_w = 0.87 (0.13)				
(DE = 53.76%)				
Intercept	-3.0586	1.2008		
IndigRich	0.6118	0.1881	12.960	0.0003
Invasive species richness				
AIC_c = 52.287 (56.709), AIC_w = 0.90 (0.10)				
(DE = 53.94%)				
Intercept	-1.7345	0.9416		
IndigRich	0.4376	0.1517	9.2815	0.0023
Indigenous abundance				
AIC_c = 51.506 (57.484), AIC_w = 0.95 (0.05)				
(DE = 67.03%)				
Intercept	0.67823	0.26847		
InvasRich^2	0.03260	0.02732	1.4146	0.2343*
Indigenous species richness				
AIC_c = 64.202 (69.779), AIC_w = 0.94 (0.06)				
(DE = 67.81%)				
Intercept	1.46766	0.18105		
InvasRich^2	0.03204	0.01845	2.9948	0.0835*

IndigRich = indigenous species richness, InvasRich = invasive species richness, s.e. = standard error, * = remaining term although not significant

Mid-domain effect (MDE)

The 95% prediction curves based on 50 000 simulations of spatial constraints are shown in Appendix G. Fit to the null model was poor for both transects (east: r^2 = 0.003; west: r^2 = 0.064) and the relationships were not significant (east: p = 0.66, a = 0.11, b = 6.92; west: p = 0.05, a = 0.42, b = 5.36). Similar outcomes were found for estimated richness (east: r^2 = 0.466, p = 0.06, a = 0.93, b = -0.18; west: r^2 = 0.237, p = 0.22, a = 1.20, b = -3.22).

Species-energy mechanisms

Sampling mechanism and increased population size mechanism

All relationships between species richness, abundance and mean thermal energy availability were positive and generally significant (Table 3; Figs 1, 2 & 3). However, while the GLZs indicated a significant squared term for the predictors in some instances, these were not significant after tabulated data were subjected to sequential Bonferroni correction using the p.adjust function in R2.12.0 (Table 3). Thus, neither the sampling nor the increased population size mechanisms are supported.

Dynamic equilibrium mechanism

Significant relationships were found between species abundance and disturbance thresholds (Fig. 4). Different amounts of variation in abundance were explained over the short-term for indigenous (r^2 = 0.47), invasive (r^2 = 0.84) and combined (r^2 = 0.40) species, as well as over the long-term for indigenous (r^2 = 0.72), invasive (r^2 = 0.92) and combined (r^2 = 0.62) species (Fig. 4). For the short-term data set, the number of sub-zero events (standardised path estimates = -0.75, p < 0.001) and the longest duration below LDT (-1.15, p < 0.001) had significant negative effects on invasive abundance. For the long-term dataset, indigenous abundance was significantly affected by the number of both sub-zero events (-1.68, p < 0.001) and those below LDT (0.92, p < 0.01) (Fig. 4). The number of events below LDT also had significant effects on invasive abundance (-0.26, p < 0.05), and number of sub-zero events significantly affected the combined assemblage (-0.76, p < 0.05) (Fig. 4).

Table 3. Results of the generalised linear models (each assuming a Poisson distribution, using a log link function and corrected for overdispersion) looking at the relationships between species richness (using S_{obs} and the estimator Jacknife2), log transformed abundance, and mean thermal energy availability of indigenous, invasive and combined springtail species. Deviance explained (DE), chi squared (X^2) and p values are shown for models with single term predictor variables. The estimate, standard error (s.e.) and p values are shown for the squared terms from models with these included to investigate the form of the relationships. All squared term's p values were non-significant after applying a Bonferroni correction.

		Single term models		Squared term		
		X^2	p	Estimate	s.e.	p
Species richness vs. abundance						
S_{obs}						
Indigenous	(DE = 64.54%)	25.515	< 0.0001	0.000	0.000	0.009
Invasive	(DE = 57.29%)	23.929	< 0.0001	0.000	0.000	0.167
Combined	(DE = 71.18%)	34.076	< 0.0001	0.000	0.000	0.151
Jacknife2						
Indigenous	(DE = 35.79%)	7.651	0.006	0.000	0.000	0.715
Invasive	(DE = 22.27%)	4.276	0.039	0.000	0.000	0.447
Combined	(DE = 46.97%)	13.143	0.000	0.000	0.000	0.135
Species richness vs. mean energy availability						
S_{obs}						
Indigenous	(DE = 57.93%)	19.138	< 0.0001	-0.021	0.015	0.178
Invasive	(DE = 50.37%)	15.666	< 0.0001	-0.076	0.055	0.189
Combined	(DE = 65.13%)	24.759	< 0.0001	-0.031	0.020	0.152
Jacknife2						
Indigenous	(DE = 5.56%)	0.833	0.362	-0.042	0.027	0.139
Invasive	(DE = 24.26%)	4.306	0.038	-0.231	0.079	0.012
Combined	(DE = 24.47%)	4.139	0.042	-0.088	0.029	0.009
Species abundance vs. mean energy availability						
Indigenous	(DE = 22.87%)	3.987	0.046	-0.028	0.021	0.212
Invasive	(DE = 76.41%)	51.601	< 0.0001	-0.069	0.054	0.230
Combined	(DE = 49.52%)	13.461	0.000	-0.004	0.016	0.778
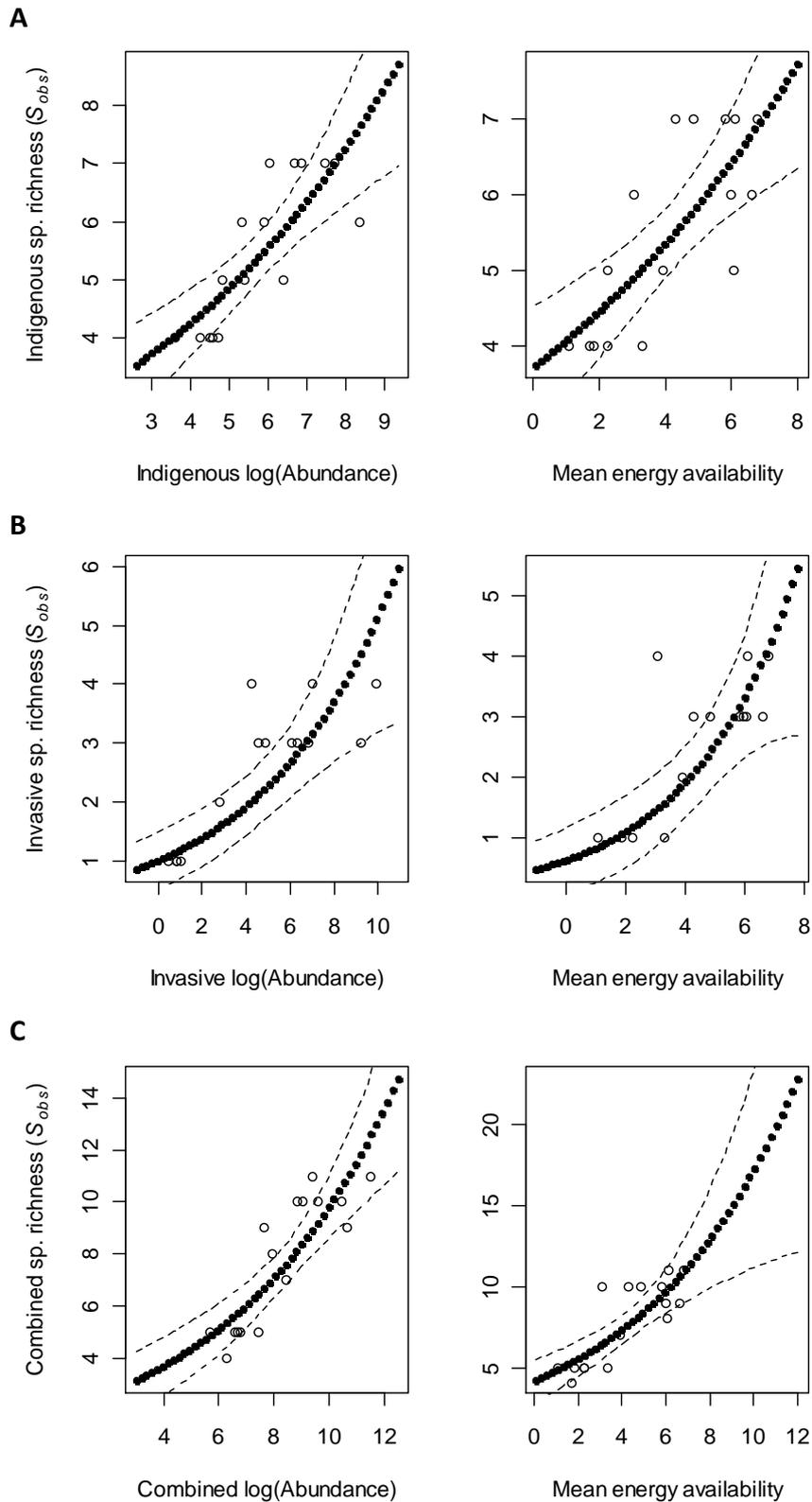
Figure 1. Predictions under the Poisson GLZ models for the sampling mechanism, of species richness (S_{obs}) versus abundance, and mean thermal energy availability for (A) indigenous, (B) invasive and (C) combined springtail species on Marion Island. 95% confidence intervals are shown using dashed lines. Open circles are observed values.
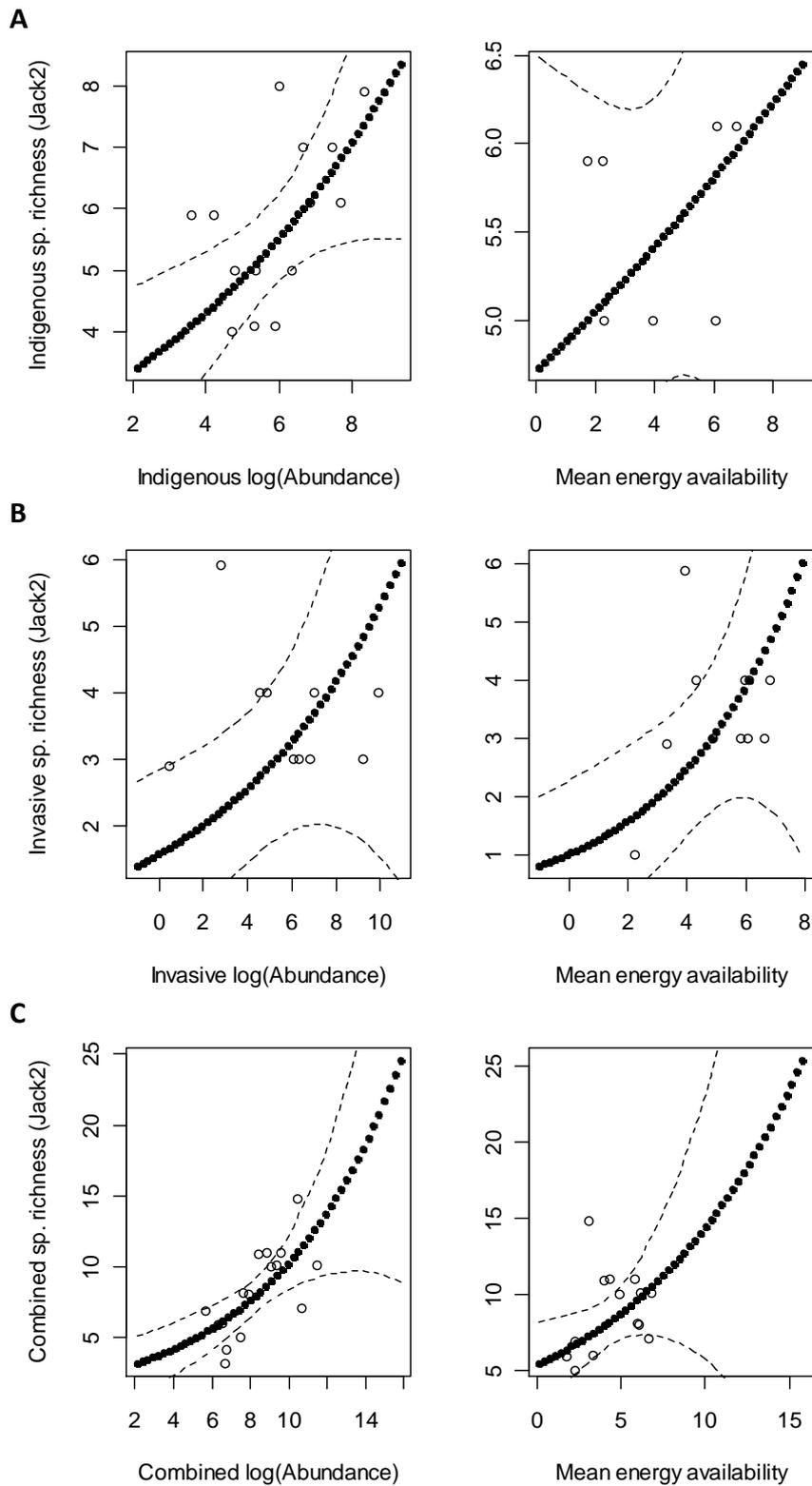
Figure 2. Predictions under the Poisson GLZ models for the increased population size mechanism, of species richness (Jacknife2 estimator) versus abundance, and mean thermal energy availability for (A) indigenous, (B) invasive and (C) combined springtail species on Marion Island. 95% confidence intervals are shown using dashed lines. Open circles are observed values.
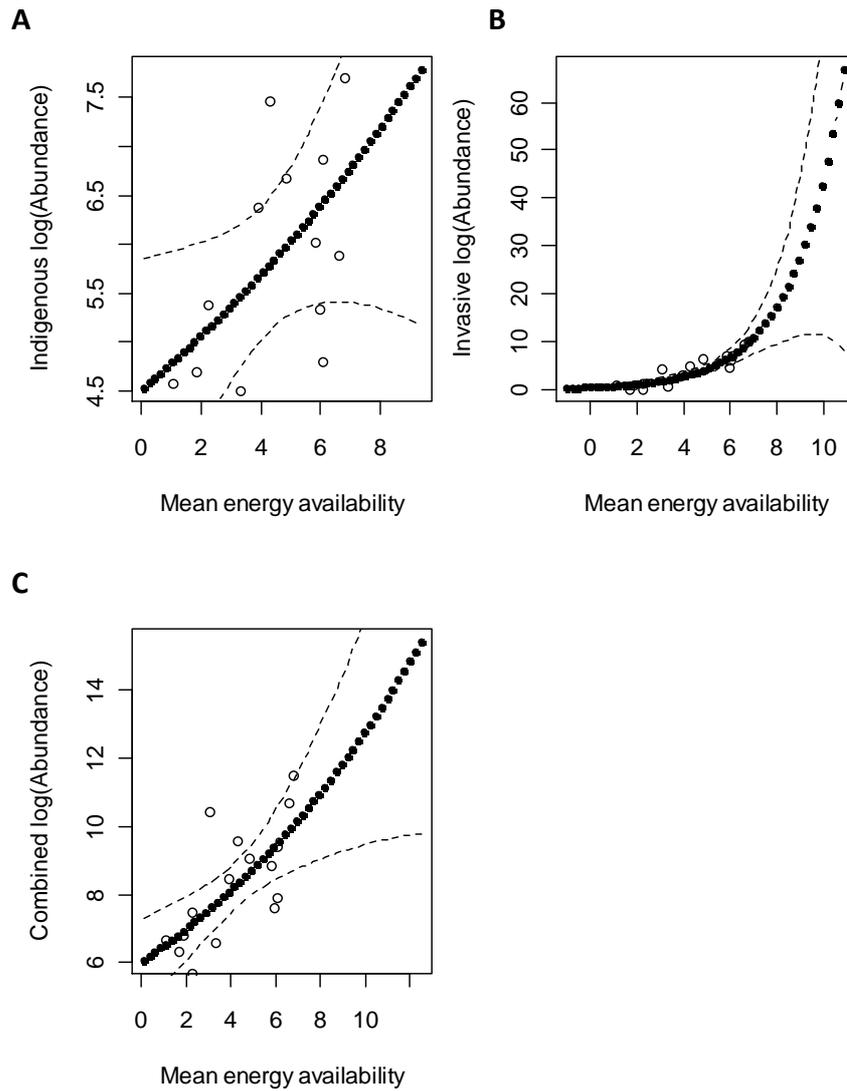
Figure 3. Predictions under the Poisson GLZ models for abundance versus mean thermal energy availability for (A) indigenous, (B) invasive and (C) combined springtail species on Marion Island. 95% confidence intervals are shown using dashed lines. Open circles are observed values.

A. Indigenous species
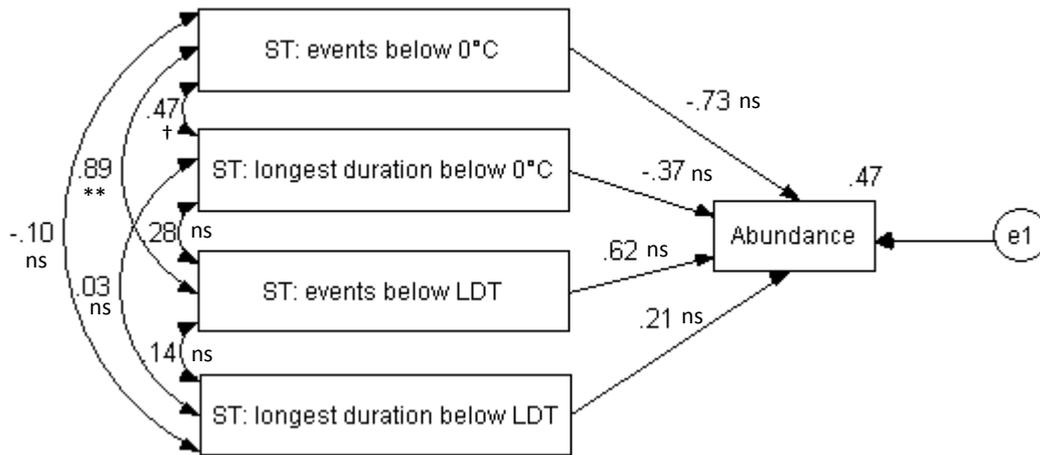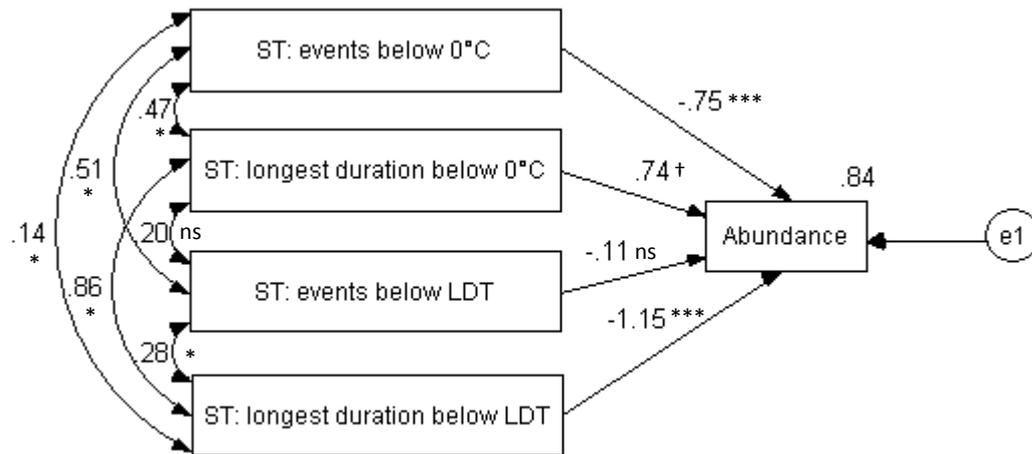
B. Invasive species
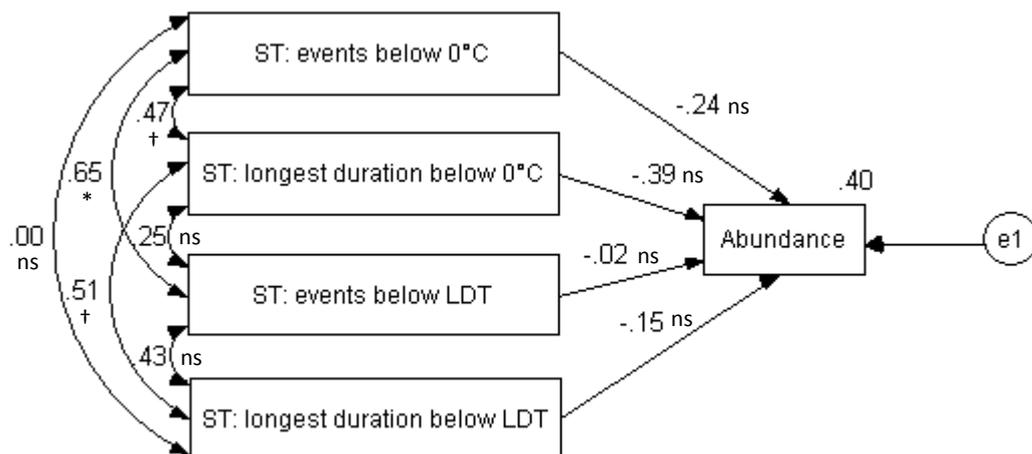
C. Combined species

D. Indigenous species
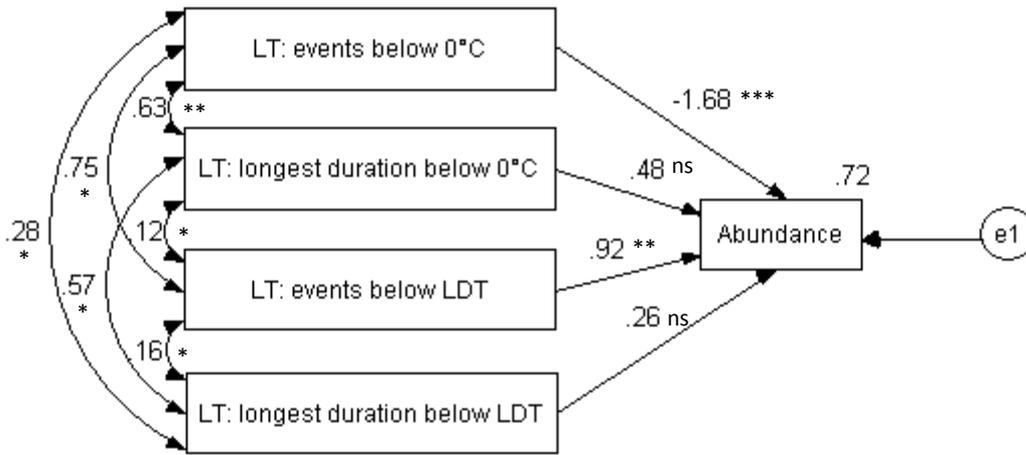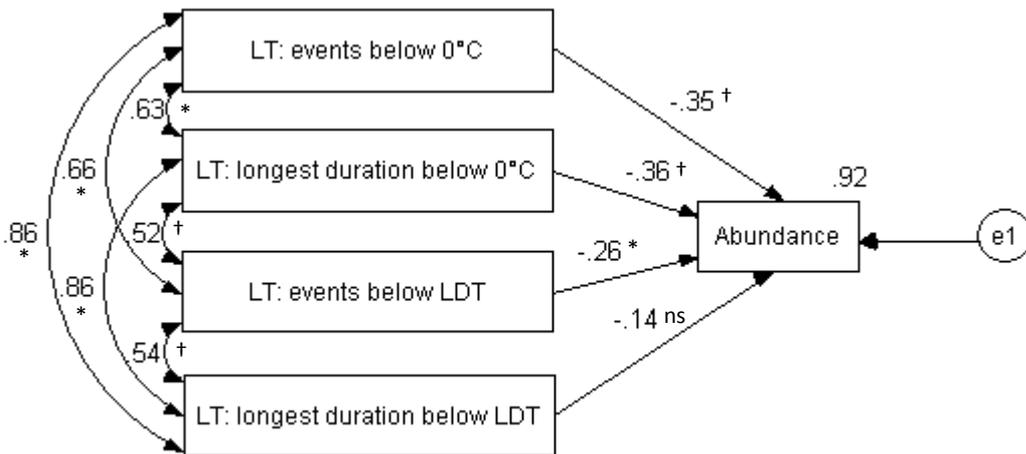
E. Invasive species
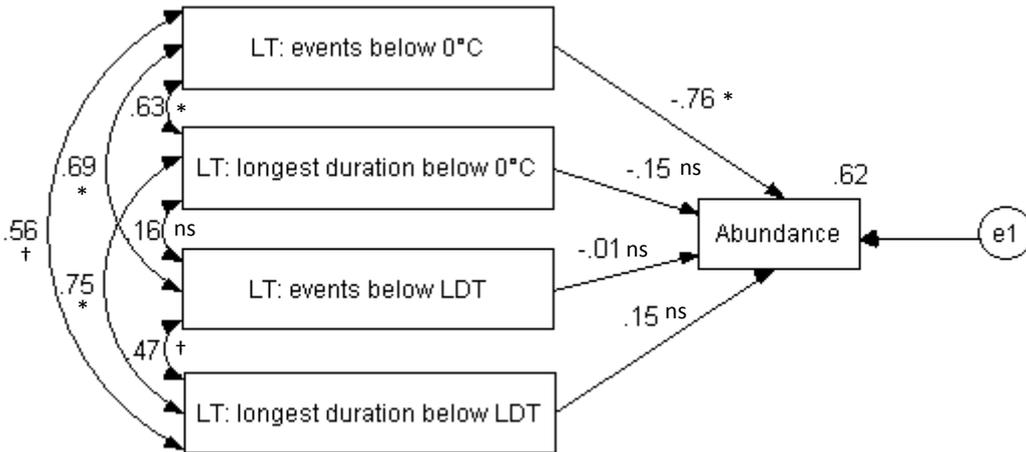
F. Combined species

Figure 4. Dynamic equilibrium mechanism: path analyses of the effect of thresholds (number of events and longest duration below 0°C and LDT) on abundance of (A)

indigenous, (B) invasive and (C) combined species calculated from the short-term temperature data set (ST), and of (D) indigenous, (E) invasive and (F) combined species calculated from the long-term data set (LT). Single headed arrows represent directional effects of exogenous indicator variables on abundance; numbers above arrows represent the standardised path estimates with significance indicated (ns = not significant; † = $p < 0.1$; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$); double headed arrows indicate covariance between variables with their correlation values and significance indicated. The e1 represents the unexplained (error) variable affecting springtail abundance. Model fit or variance explained (r^2) is indicated above the box for the endogenous abundance variable.

Range limitation mechanism

Different amounts of variation in abundance were explained over the short-term for indigenous ($r^2 = 0.31$), invasive ($r^2 = 0.83$) and combined ($r^2 = 0.50$) species, as well as over the long-term for indigenous ($r^2 = 0.50$), invasive ($r^2 = 0.88$) and combined ($r^2 = 0.65$) species (Fig. 5). Abundance of invasive species declines when minimum temperatures decline over both the short-term (0.68, $p < 0.001$) and long-term (1.15, $p < 0.05$) (Fig. 5). Over the short-term, indigenous (0.60, $p < 0.1$) and combined (0.60, $p < 0.1$) species abundance declines with a decline in minimum temperatures, although these relationships are not significant. Over the long-term, a weak effect of possible number of generations on indigenous species abundance was found (-1.61, $p < 0.1$) (Fig. 5).

A. Indigenous species
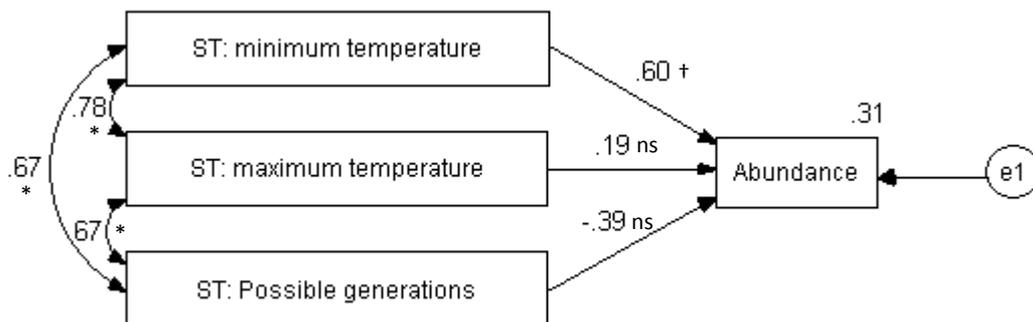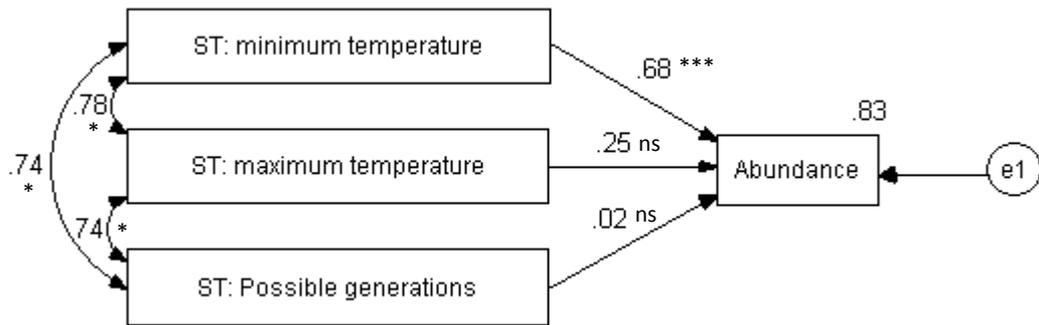
B. Invasive species
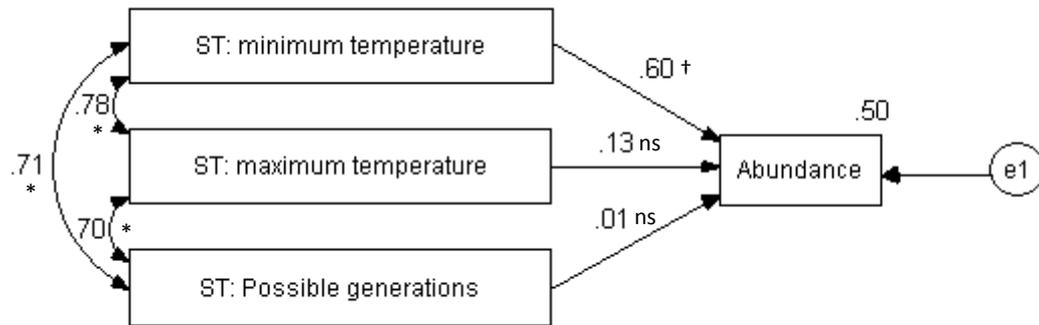
C. Combined species

D. Indigenous species
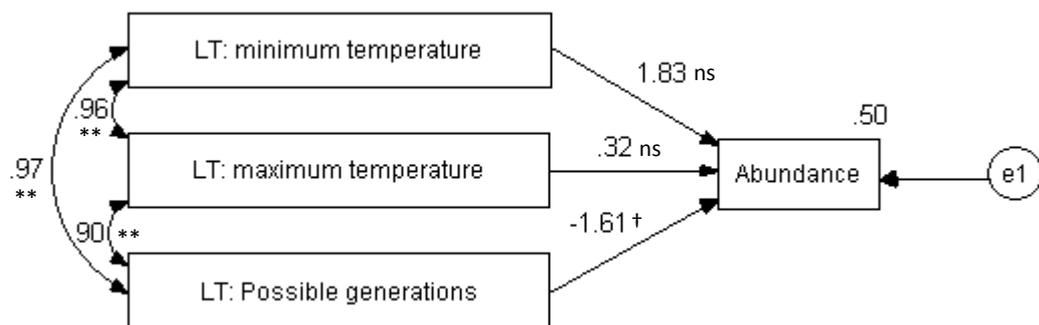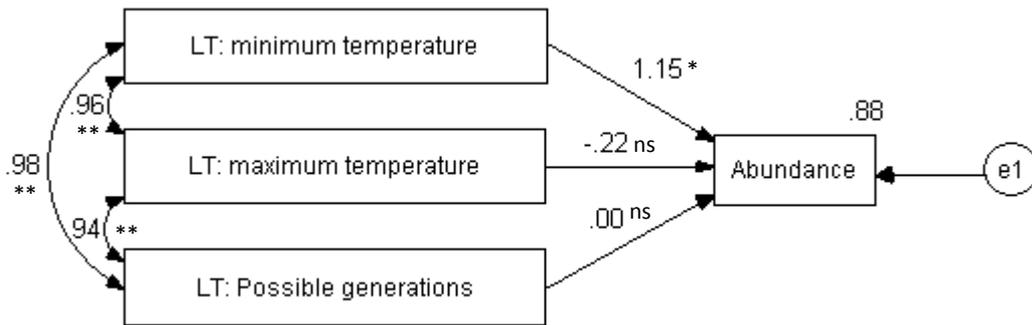
E. Invasive species
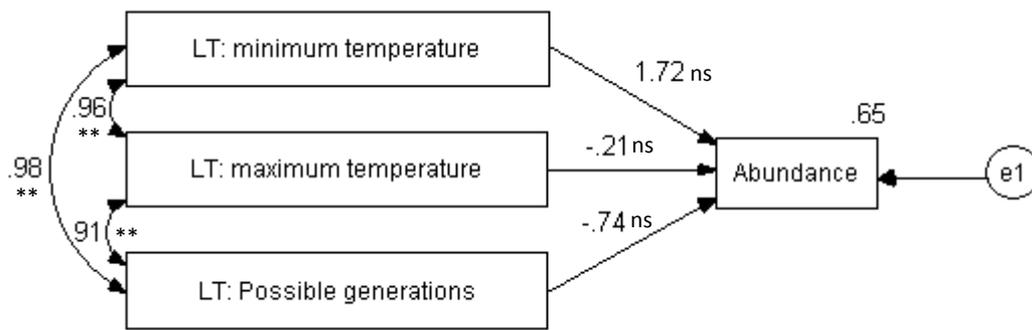
F. Combined species

Figure 5. Range limitation mechanism: path analyses of the effect of minimum and maximum temperatures, as well as generations possible, on abundance of (A) indigenous, (B) invasive and (C) combined species calculated from the short-term temperature data set (ST), and of (D) indigenous, (E) invasive and (F) combined species calculated from the long-term data set (LT). Single headed arrows represent directional effects of exogenous indicator variables on abundance; numbers above arrows represent the standardised path estimates with significance indicated (ns = not significant; † = $p < 0.1$; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$); double headed arrows indicate covariance between variables with their correlation values and significance indicated. The e1 represents the unexplained (error) variable affecting springtail abundance. Model fit or variance explained (r^2) is indicated above the box for the endogenous abundance variable.

Discussion

Positive species-energy relationships were found for both indigenous and invasive springtail species. Moreover, richness of both indigenous and invasive species declines with elevation and a mid-domain effect (MDE) of species richness does not occur. This could have been predicted, as not only is the spatial scale of this study small (MDE predictions are expected to explain species richness patterns at large spatial scales (Dunn *et al.* 2007)), but environmental conditions have been argued to be principal constraints to species distributions (Gaston 2003; Zapata *et al.* 2005). In addition, surface area is unlikely to be the main cause of species richness patterns and in any case variation in energy may determine species-area relationships (Storch *et al.* 2005; Kalmar & Currie 2007).

Of particular interest is that the same mechanisms responsible for positive species-energy relationships in indigenous species result in positive relationships in invasive species. It is therefore not surprising that the relationships among the invasive and indigenous species richness and abundance were largely positive. The most frequently cited pathway linking energy to species richness is the more individuals hypothesis (MIH) or increased population size mechanism (Gaston 2000; Evans *et al.* 2005c). However, the data in this study did not support the patterns of the MIH and the hypothesis is thus rejected. This concurs with other work that questions the MIH as a general mechanism underlying species-energy relationships (Srivastava & Lawton 1998; Currie *et al.* 2004; Hurlbert 2004; Evans *et al.* 2005c; Šímová *et al.* 2011; but see Mönkkönen *et al.* 2006). Rather, strong support was found for the dynamic equilibrium mechanism and the range limitation mechanism, indicating that the species-energy relationships for both indigenous and invasive species are determined by the physiological tolerances of the species.

Support for the dynamic equilibrium mechanism indicates that springtail species not resilient to the crossing of disturbance thresholds have lower abundances in areas where more of these disturbances occur compared to those areas with fewer disturbances. The predictions of the range limitation mechanism are also supported, i.e. in most environments, minimum temperatures are more stressful than maxima. Thus it is possible that the strong relationships found in the present study could be the general case across the whole of the sub-Antarctic, where disturbance thresholds and stressful temperatures are more important mechanisms underlying species-energy relationships than increased

population sizes with increased thermal energy availability. This certainly seems to be the case on Macquarie Island where positive relationships between diversity and environmental favourability have been found (Terauds *et al.* 2011). Moreover, as similar mechanisms underlie species-energy relationships for both indigenous and invasive species, factors such as speciation and evolutionary rates are less significant in determining species richness patterns than those where energy gives rise to higher numbers of species. Evolutionary factors do however affect species identity and may over the short-term be replaced by effects of human agency (such as preference) for alien species (Blackburn *et al.* 2009).

Nevertheless, while the mechanisms underlying species-energy relationships are the same for both groups of species, the responses of these two groups of species differ. Most notably, the range limitation mechanism in particular indicated more variance explained by species-energy relationships for invasive species than for indigenous species or the combined assemblage. The stronger response of invasive species to minimum temperature might be because they are potentially pre-adapted to warmer, high-energy environments and therefore may be more sensitive to thermal energy availability than indigenous species (Evans *et al.* 2005a). Furthermore, that range limitation is more strongly supported for invasive species than indigenous species is not surprising, given that the invasives are temperate and not sub-Antarctic species. The stronger species-energy relationship of invasive species than of indigenous species has also been documented for plants (McDougall *et al.* 2005; Marini *et al.* 2009) and birds (Evans *et al.* 2005a; Evans & Gaston 2005).

The positive relationships among invasive and indigenous species richness and abundance seem to be because both groups of species respond positively to increasing thermal energy availability. While some may argue landscape heterogeneity could be responsible for these positive relationships (Davies *et al.* 2005), the results nonetheless show that the 'rich get richer' hypothesis (Stohlgren *et al.* 2003) cannot be rejected. This concurs with findings for other taxa, including plants (Herben *et al.* 2004; Stohlgren *et al.* 2005; Marini *et al.* 2009), invertebrates (Borges *et al.* 2006; Terauds *et al.* 2011) and birds (Evans *et al.* 2005a). The likely reason behind the findings in these studies is that the same species-energy mechanisms underlie both indigenous and invasive diversity patterns.

In summary, few studies have investigated how thermal energy availability influences patterns of both indigenous and invasive species richness and what the underlying mechanisms of this might be. The present study provides one of the only tests of the nine principal mechanisms as outlined by Evans *et al.* (2005b). In particular, the results have reinforced the view that rather than merely testing a single mechanism, alternative mechanisms should be explored simultaneously. While no single mechanism currently has unequivocal support (Evans *et al.* 2005b), considering all of them can potentially provide conclusive evidence for which mechanism underlies species-energy relationships. Understanding these mechanisms is significant, particularly as thermal energy availability and thus species richness patterns are expected to alter with changing environmental conditions. Any increase in mean annual temperature will result in greater thermal energy availability (Convey 1997). Furthermore, it has been suggested that the greatest effects of climate change on biotic communities may be due to changing maximum and minimum temperatures (Stachowicz *et al.* 2002). That invasive species are more physiologically limited by disturbance thresholds and minimum temperatures than indigenous species implies that invasive species will be at an advantage under warming conditions relative to indigenous species. This supports previous such suggestions for springtails on Marion Island (Chown *et al.* 2007; Slabber *et al.* 2007; Janion *et al.* 2010), where mean annual temperature has increased substantially over the last 50 years (Smith 2002; le Roux & McGeoch 2008), as well as for other groups of organisms elsewhere (Walther *et al.* 2009). However, predicting species responses to changing conditions is likely to be problematic (Gabriel *et al.* 2001; McGeoch *et al.* 2006) especially as multiple factors will change concurrently and how these factors might change is unclear (le Roux & McGeoch 2008). Moreover, the strength of the responses of indigenous and invasive species will likely differ at different elevations (Marini *et al.* 2009). Nevertheless, changing patterns in indigenous and invasive species richness and abundance are likely to be seen, an effect that will be exacerbated by the predicted increase in the rate of introductions with climate change (Kennedy 1995; Bergstrom & Chown 1999; Frenot *et al.* 2005). This makes the case for managing the prevention of invasive species reaching new areas all the more important.

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Appendices

Appendix A
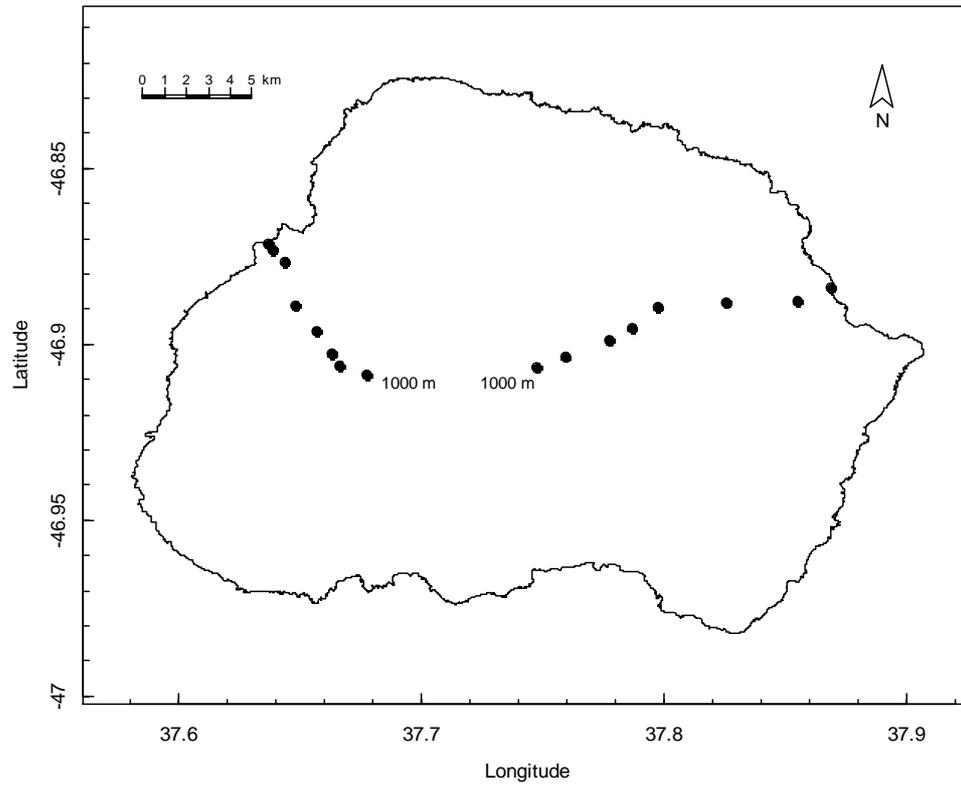
Figure S1. Position of sites sampled along two altitudinal transects on Marion Island, one on the eastern side of the island and one on the west, from the coast to 1000 m.a.s.l.

Appendix B

Figure S2. Samples drying in hanging funnels in the Mixed Pickle Cove field hut drying room.

Appendix C. Summary of soil temperatures recorded at the altitudinal sites of the present study for winter and summer months.

Site & altitude (m.a.s.l.)	Winter (mid May-08 to mid Aug-08) (°C)							Summer (mid Nov-08 to mid Feb-09) (°C)						
	n	Mean	Absolute min	Absolute max	Range	Mean daily min	Mean daily max	*n*	Mean	Absolute min	Absolute max	Range	Mean daily min	Mean daily max
East														
10	3561	4.94	1.12	9.66	8.54	4.04	5.74	2652	8.27	2.00	18.00	16.00	6.90	10.36
50	3559	4.93	2.09	9.64	7.55	4.39	5.28	2654	7.20	5.00	18.69	13.69	6.64	8.11
200	3558	4.07	1.11	8.67	7.56	3.50	4.39	4750	7.89	4.13	12.67	8.54	6.94	9.01
400	3557	3.33	-0.89	16.66	17.55	2.11	4.32	4754	6.40	1.08	16.12	15.04	4.03	9.18
600	3557	2.80	0.57	10.63	10.06	1.97	3.38	3492	5.08	0.00	21.50	21.50	3.11	8.66
750	3530	2.21	-1.97	11.16	13.13	1.15	2.94	4751	4.44	0.05	20.62	20.57	2.54	7.02
850	3526	1.83	-0.39	10.15	10.54	0.99	2.31	3494	2.69	-0.91	16.69	17.60	1.35	5.38
1000	3641	1.47	-1.39	10.16	11.55	0.78	1.92	4745	1.96	-0.97	14.22	15.19	1.09	3.04
West														
10	3656	5.42	1.60	9.16	7.56	4.68	5.81	4751	8.17	3.64	14.70	11.06	7.28	9.28
50	3674	5.11	2.12	7.64	5.52	4.61	5.31	4751	7.09	4.17	11.71	7.54	6.44	7.86
200	3655	4.66	1.09	9.14	8.05	3.95	5.16	4750	6.98	3.63	13.17	9.54	6.30	7.92
400	3651	3.23	0.10	8.59	8.49	2.36	3.64	4751	5.36	1.58	14.14	12.56	4.24	6.56
600	3634	2.90	0.07	9.61	9.54	2.03	3.43	3923	3.25	0.00	13.50	13.50	2.43	4.73
750	3632	2.28	-0.42	9.66	10.08	1.47	2.78	2374	2.27	-1.50	15.00	16.50	1.06	3.74
850	3629	1.58	-0.91	8.19	9.10	0.78	2.07	4750	2.14	0.07	10.17	10.10	1.46	2.97
1000	3625	1.01	-2.38	8.69	11.07	0.24	1.32	3924	1.18	-1.50	15.00	16.50	0.48	2.51

m.a.s.l. = metres above sea level

Appendix D
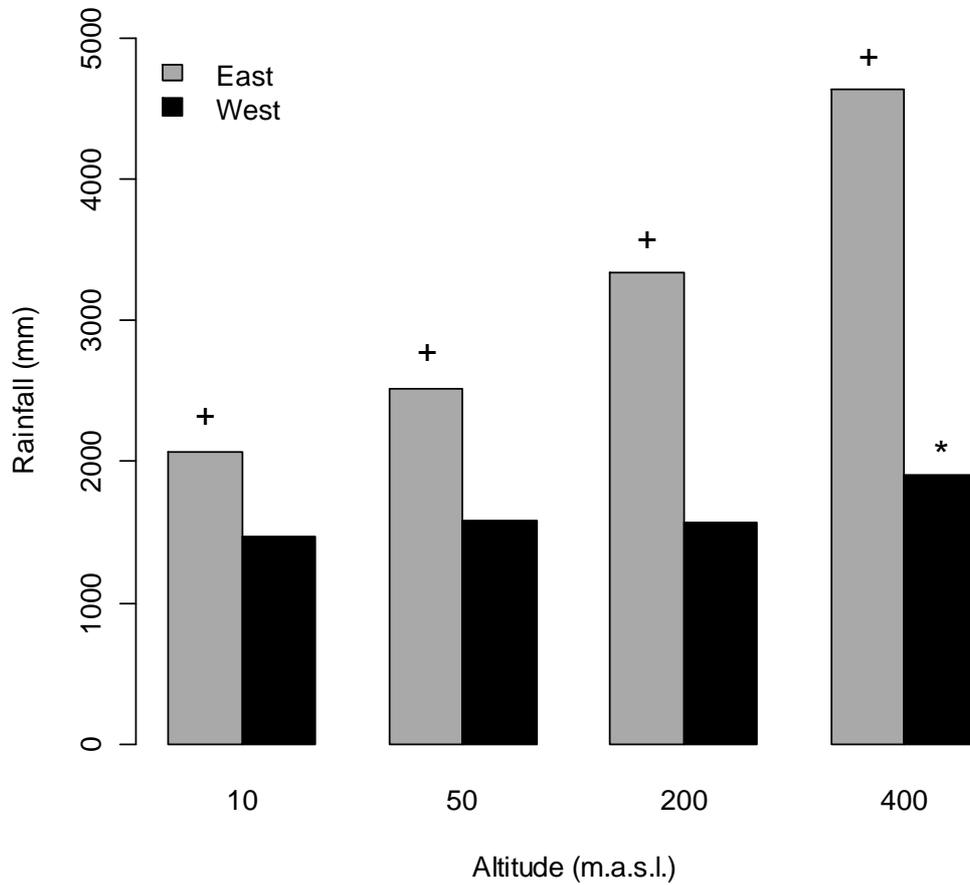
Figure S3. Annual rainfall (May 2008 to April 2009) recorded at the four lower elevations along the east and west transects on Marion Island (+ = these gauges were found to be overflowing in at least one month and rainfall estimates are therefore conservative and minimum amounts; * = estimated rainfall as the west 400 m.a.s.l. rain gauge was damaged by ice and discontinued; m.a.s.l. = metres above sea level).

Appendix E

A

B
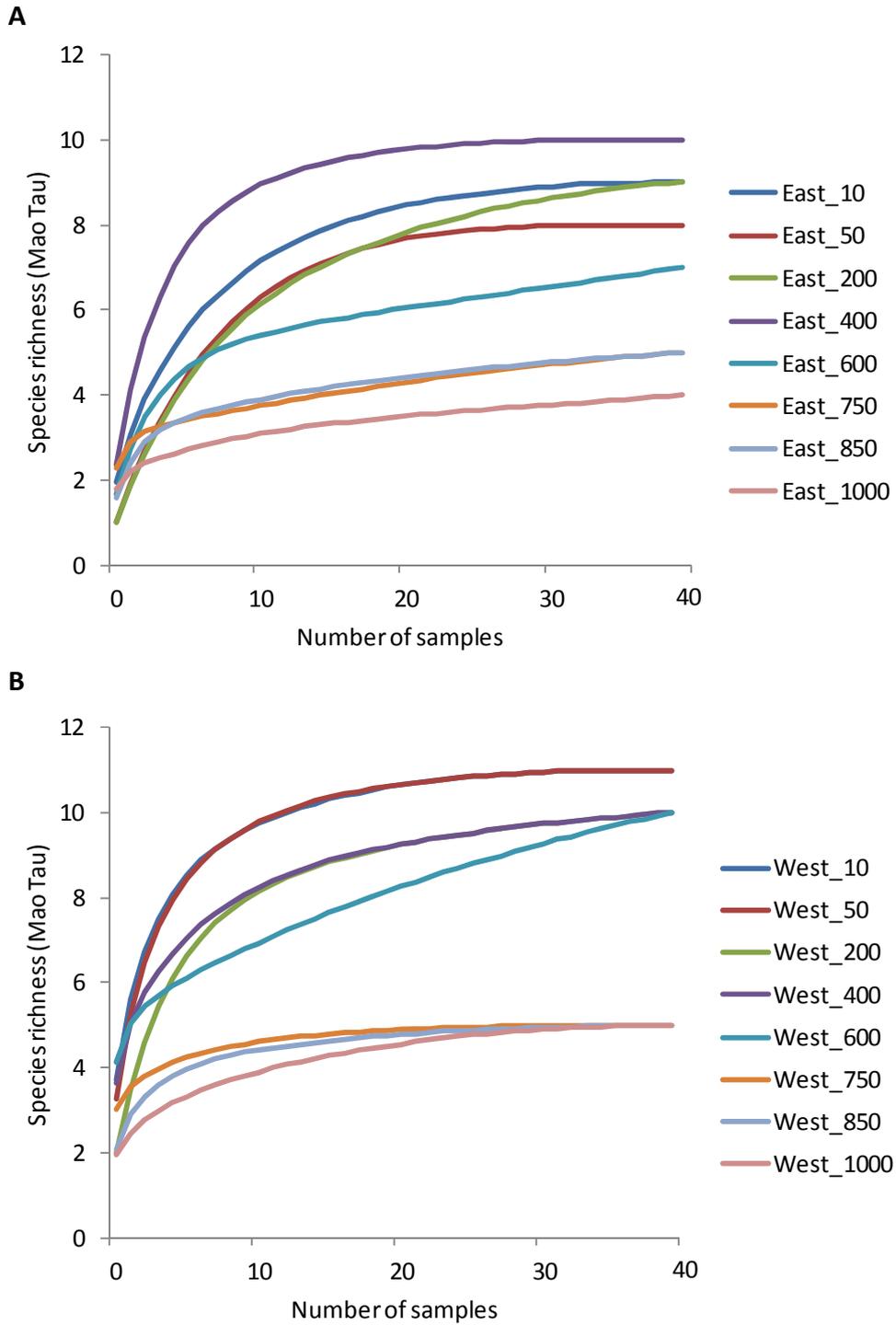
Figure S4. Rarefaction curves for eight elevations on (A) the east transect and (B) the west transect on Marion Island standardised by sample effort of springtails.

Appendix F. Abundance ($\log_{10}(x+1)$ transformed), observed species richness (S_{obs}) and estimated species richness (Jacknife2) of indigenous and invasive springtail species along two altitudinal gradients on Marion Island.

Transect	Altitude (m.a.s.l.)	Species group	Log(Abundance)	S_{obs}	Jacknife2
East	10	Indigenous	2.56	6	4.1
East	50	Indigenous	2.08	5	5.0
East	200	Indigenous	2.32	6	4.1
East	400	Indigenous	2.90	7	7.0
East	600	Indigenous	2.77	5	5.0
East	750	Indigenous	1.95	4	3.1
East	850	Indigenous	1.56	4	5.9
East	1000	Indigenous	1.84	4	5.9
East	10	Invasive	4.00	3	3.0
East	50	Invasive	2.65	3	3.0
East	200	Invasive	1.97	3	4.0
East	400	Invasive	2.75	3	3.0
East	600	Invasive	1.22	2	5.9
East	750	Invasive	0.21	1	2.9
East	850	Invasive	0.46	1	1.0
East	1000	Invasive	0.00	0	0.0
West	10	Indigenous	3.34	7	6.1
West	50	Indigenous	2.98	7	6.1
West	200	Indigenous	2.62	7	8.0
West	400	Indigenous	3.24	7	7.0
West	600	Indigenous	3.62	6	7.9
West	750	Indigenous	2.34	5	5.0
West	850	Indigenous	2.04	4	4.0
West	1000	Indigenous	1.99	4	3.1
West	10	Invasive	4.30	4	4.0
West	50	Invasive	3.04	4	4.0
West	200	Invasive	2.97	3	3.0
West	400	Invasive	2.12	3	4.0
West	600	Invasive	1.85	4	6.9
West	750	Invasive	0.00	0	0.0
West	850	Invasive	0.35	1	0.1
West	1000	Invasive	0.35	1	0.1

m.a.s.l. = metres above sea level, s.e. = standard error

Appendix G

A
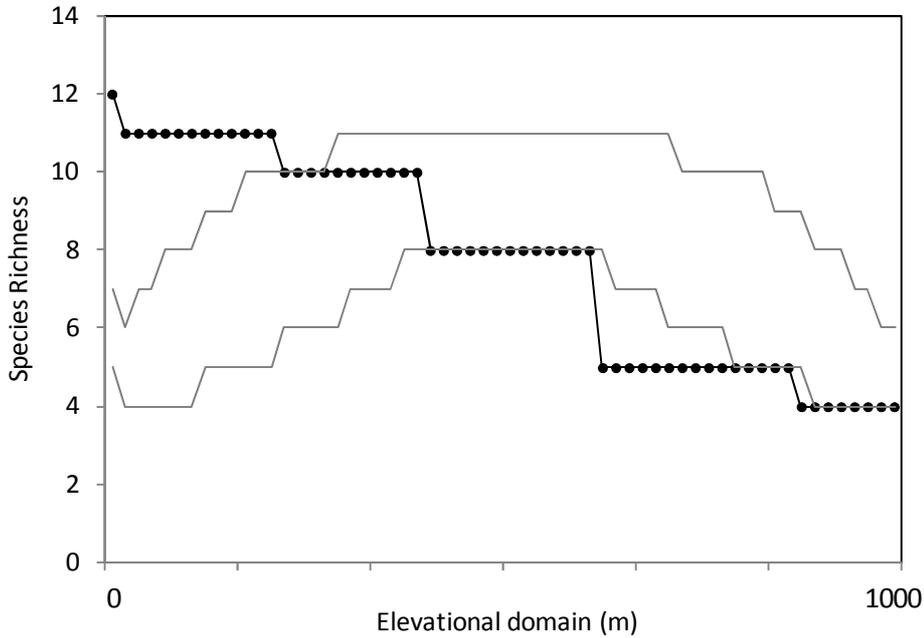
B
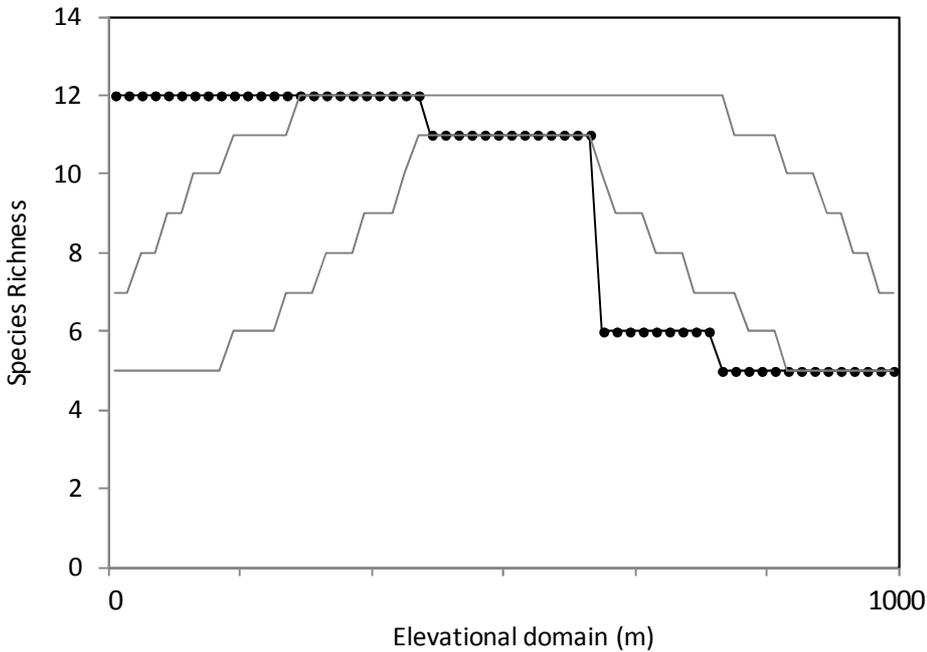
Figure S5. Springtail species richness patterns along elevational gradients (black circles and lines) for (A) the east transect and (B) the west transect on Marion Island including 95% simulation limits (lines only) of the mid-domain analysis from 50 000 range size simulations sampled without replacement using Mid-Domain Null (McCain 2004).

Chapter 3 – Contingent absences account for range limits but not the local abundance structure of an invasive springtail[1]

The invasive springtail *Pogonognathellus flavescens* (Tullberg 1871) coated
with fluorescent powder on Marion Island

Introduction

The structure, limits and dynamics of species' ranges have long formed a staple of ecology. Nonetheless, the field has gained much impetus recently owing to the realisation that various fundamental biodiversity patterns can be derived from spatial variation in abundance (Ricklefs 2008; Storch *et al.* 2008), and that understanding such variation is essential for forecasts of and conservation responses to environmental change impacts on diversity (Channel & Lomolino 2000; Gaston 2003; Thuiller 2007; Chown & Gaston 2008; Gallien *et al.* 2010; Dawson *et al.* 2011). Despite substantial progress, several major questions in the field remain, especially from an empirical perspective. Indeed, Gaston (2009), in providing a critical review of range limits, noted that '...*for no single species do we yet have a comprehensive understanding*.' Previously, Gaston (2003) had also concluded that the causal basis of abundance structure remains largely unanswered, although this challenge is now increasingly being taken up (e.g. McGeoch & Price 2004; Sagarin *et al.* 2006; Lee *et al.* 2009; Tam & Scrosati 2011). By contrast, theory concerning the processes underpinning spatial and temporal variation in abundance, occupancy, and range limits has continued to proliferate (e.g. McGeoch & Gaston 2002; He & Gaston 2003; Holt & Keitt 2005; Goldberg & Lande 2007; Berggren *et al.* 2009; Gaston 2009; Gotelli *et al.* 2009; Gilman *et al.* 2010).

Perhaps one of the most significant conceptual developments in this area has been a re-emphasis on distinguishing the effects on population dynamics of environmental conditions that are related to resource consumption and/or interactions (competition or predation), those which are not, and those of movement or dispersal. Whilst these concepts have a long and continuous history in the ecological literature (e.g. Shelford 1911; Andrewartha & Birch 1954; Park 1962; MacArthur 1972; Holt *et al.* 1997; Holt & Keitt 2000; Gaston 2003; Austin 2007), they have recently been clearly laid out by Soberón (2007, 2010). In his BAM (i.e. Biotic, Abiotic, Movement) framework, the area where a population may persist is defined as the intersection of those: where it can cope with competition and other interactions (B), where it has intrinsic abilities to survive, grow and reproduce under given environmental conditions (A), and to which individuals can readily disperse to found the population (M). Populations may also occur in other sites owing to source-sink dynamics (Gaston 2003), and may also disappear from some owing to demographic

stochasticity (Melbourne & Hastings 2008; Willi & Hoffmann 2009), giving rise to the metapopulation structure that is characteristic of many landscapes (Lennon *et al.* 1997; Oborny *et al.* 2009). The BAM framework focuses explicitly on those areas where population growth is positive and therefore where a given species will occur. However, in the context of species distribution modelling, and by definition in the consideration of abundance structure and range limits, what determines absence is similarly significant. In many ways these determinants are simply the converse of the BAM measures, with environmental absences incorporating the effects of both direct and resource constraints (in Austin's 2007 terminology), and contingent absences reflecting dispersal or historical limitation (the converse of Soberón's 2007 – M) (Lobo *et al.* 2010). However, methodological absences are different, and are a consequence of the problems associated with sampling and detecting individuals of a given species (see Garrard *et al.* 2008; Magurran & McGill 2011).

Despite the existence of this clearly useful scheme for understanding how abiotic, biotic and dispersal limitation factors might interact to give rise to non-zero abundance, few studies have sought to examine the extent to which the various components contribute to abundance structure and range limitation, reflecting a more general situation where many of the facets of the study of abundance structure and range limits are often not simultaneously examined (Gaston 2009). Such a situation is perhaps not surprising. Assessments of abundance structure require substantial sampling effort across broad spatial scales and, with the possible exception of volunteer-based schemes for counts of typically charismatic groups (Devictor *et al.* 2010), are by no means easily undertaken (Brewer & Gaston 2002). Moreover, to assess the extent to which the BAM components set range limits and influence abundance structure requires information on intrinsic physiological capabilities of the species concerned (typically considered data intensive - e.g. Guisan & Thuiller 2005; Buckley *et al.* 2010), the likely role of competition and predation (again data intensive, though some ways to alleviate the data intensity required have been suggested – Soberón 2010), and the dispersal capabilities of the organisms concerned. Nonetheless, much merit clearly exists in disentangling the BAM components, especially since it has been suggested that they are likely to operate differently in different parts of a species range and during different stages in a population's history (e.g. MacArthur 1972; Channell & Lomolino 2000; Ricklefs & Bermingham 2002; Richardson & Pyšek 2006;

Lockwood *et al.* 2009). Should generalities exist about where and when particular components of the BAM predominate, they should provide substantial insight into the extent to which predicted changes for species or higher taxa, where the full set of data are unavailable, will prove reliable or might need to be modified (see Dawson *et al.* 2011), especially since many life history and physiological variables show strong phylogenetic and spatial signals (Chown 2001; Freckleton & Jetz 2009; Cooper *et al.* 2011; Clusella-Trullas *et al.* 2011).

Here I therefore examine the extent to which the different components of the BAM model, and its converse (i.e. environmental and contingent absences) contribute to the range limits and abundance structure of an invasive springtail species in a cold temperate island setting. The rationale for selecting this system is threefold. First, much interest exists in determining the factors that are likely to set the abundance and range limits of alien species. In particular, the mechanisms underlying the shift of populations from being alien to invasive are of particular interest to invasion biologists, and reflect the more general question of how small populations grow larger and then spread through a landscape (reviewed in Gaston 2003, and see also Wilson *et al.* 2009). Second, islands provide tractable study areas and have previously been used successfully to examine local range limits and abundance structure in a variety of species (Gaston *et al.* 2006; Lee *et al.* 2009). Moreover, such local examinations are useful for understanding the more general features of abundance variation across landscapes (Brown 1984; Hengeveld 1990; Gaston 2003). Finally, although much is known about the biology and ecology of springtails (e.g. Ponge 1993; Hopkin 1997; Rusek 1998; Ferguson & Joly 2002; Ims *et al.* 2004; Salamon *et al.* 2004; Ulrich & Fiera 2009), the ways in which range limits and abundance structure of this group might be determined are not well understood (see also Hayward *et al.* 2004).

I used a three stage approach. First, I examine the physiological tolerances of this generalist species, and specifically thermal limits, desiccation resistance, and the optimal conditions for egg development (as a proxy for development overall). I then compare these characteristics with those of the local environment based on long-term soil temperature information and short-term investigations of other microclimatic variables. Based on these data I make straightforward predictions of where the species should occur on the island, given that in its indigenous range it is known as a temperate-climate, habitat generalist. Next I undertake island-wide and local abundance surveys to determine exactly where the

species does occur on the island in question and what the correlates are of abundance variation. I include in these assessments observational data on the predators and competitors encountered by the species, supplemented with information from previous investigations of community interactions on the island (Gabriel *et al.* 2001). Finally, I use both laboratory and field experiments to investigate the potential and actual dispersal distances of the species in the field. Based on these data, I show that current range limits are set by dispersal limitation (i.e. M or contingent absences are most significant), whereas abundance structure is most notably influenced by soil properties and habitat selection (A).

Materials and methods

Study area and species

Sub-Antarctic Marion Island (46°54'S, 37°45'E) is part of the Prince Edward Island group, which lies approximately 2300 km southeast of Cape Town, South Africa. The island is highly oceanic with a mean annual temperature of *c*. 6.5°C, total annual precipitation of *c*. 1900 mm, generally high humidity, and strong winds. It has two major biomes: tundra, which predominates in lowland areas, and polar desert, restricted to high elevations (*c*. > 650 m) (Smith & Mucina 2006; Gremmen & Smith 2008). Species richness of plants and invertebrates is low and no indigenous terrestrial vertebrates are found except for the Lesser Sheathbill (*Chionis minor marionensis* Reichenow). House Mice (*Mus musculus* L.) were accidentally introduced in the 1800s. A comprehensive review of the geology, biology and human history of the islands is provided by Chown & Froneman (2008).

Of the sixteen species of springtails recorded from the island, six have been introduced and are considered invasive (Deharveng 1981; Chown & Froneman 2008), including *Pogonognathellus flavescens* (Tullberg 1871), the species investigated here. A large, active, surface-living species (Peterson 1981; Ponge 2000a; Fjellberg 2007), *P. flavescens* is indigenous to and widely distributed across a variety of habitats in Europe and North America (Chagnon *et al.* 2000; Ponge 2000a; Berch *et al.* 2001; Fjellberg 2007; Soto-Adames & Taylor 2010). The exact date of its introduction to Marion Island is not clear, but may have been between 1981 and 1983 (Chown & Froneman 2008). Previous work suggested that the species is restricted on Marion Island to eutrophic *Poa cookii* Hook. f. tussock grasslands (Gabriel *et al.* 2001), which are widespread in coastal areas

adjacent to seal and seabird colonies (some of which may include > 100 000 birds), and occur in small patches up to elevations of *c*. 500 m owing to manuring by burrowing petrels (Gremmen & Smith 2008). However, Gabriel *et al.* (2001) only sampled a small number of sites on the east coast of the island.

Physiological information, microclimate variables, and expected distribution

Using the substantial information that exists on the thermal and water relations, and egg development rate-temperature relationship of the *P. flavescens* population on Marion Island (Chown *et al.* 2007; Slabber *et al.* 2007; Janion *et al.* 2009, 2010; Leinaas *et al.* 2009), I assessed the physiological capabilities of the species.

Soil temperatures have been logged on an hourly basis, at *c*. 100 m intervals from sea level to 800 m (the island is 1230 m high), across the eastern slope of Marion Island since 2002 using I-button Thermochron dataloggers (e.g. Deere *et al.* 2006). These data were used to obtain mean temperatures, absolute minima and maxima and occurrences equal to or below 0°C and -10°C on an annual basis (2002 to 2009 information). The latter were based on the freezing point of water and a temperature that represents *c*. 50% mortality for the species from freezing (Slabber *et al.* 2007; Janion *et al.* 2009). To compare potential humidity changes with elevation, Thermohygrochron I-button dataloggers (Model DS 1922H; accurate to ± 0.5°C, Dallas Semiconductors, Dallas, TX, USA) were installed on the eastern side of the island at 50 m, 400 m and 750 m for 6 months from November 2008 to April 2009. Above ground humidity was logged on an hourly basis and the data used to calculate mean daily humidity, and absolute minima and maxima for the period.

I hypothesised that habitat specificity of *P. flavescens* to tussock grasslands on Marion Island, suggested by Gabriel *et al.* (2001) might be related to the temperature and humidity conditions that characterise this habitat. Thus, the thermal and humidity buffering capacity of tussock grasslands was compared with four other habitat types by measuring temperature and humidity both within the vegetation and at 0.3 m above the vegetation (see Lee *et al.* 2009 for rationale and approach). A Vaisala HUMICAP© HM 34 temperature and humidity meter (Vaisala Inc, Woburn, MA, USA) was used to measure temperature and relative humidity at 0.3 m above ground level and, to represent the space inhabited by *P. flavescens*, underneath the vegetation canopy. Measurements were taken

in drainage line, fernbrake, tussock grasslands (including both inside and outside *P. cookii* tussocks), fellfield and mire (see Gremmen & Smith 2008 for a description of these habitat types). The buffering capacity of vegetation in the five different habitat types was estimated by calculating the differences between the temperature and humidity, respectively, at 0.3 m above ground and within the vegetation in each type. Single classification analysis of variance (ANOVA) and Tukey's Honest Significant Difference tests were used to assess the extent to which these differences vary among habitat types using R2.12.0 (R Development Core Team 2010).

Because some tussock grasslands were unoccupied by the species (see Results) I compared temperature and humidity conditions within tussocks at Trypot Beach (a sea-level site where *P. flavescens* occurs) and Mixed Pickle Cove (a sea level site where *P. flavescens* is absent). Two Thermohygrochron I-button dataloggers (Model DS 1922H; accurate to ± 0.5°C, Dallas Semiconductors, Dallas, TX, USA) were installed into three randomly chosen *P. cookii* tussocks at each location for two months from May-June 2010. The loggers were encased in PVC poles to protect them from moisture and holes were drilled into the pipes to equilibrate the humidity inside and outside the pipes. The loggers were placed amongst the dead and decaying matter in the base of each tussock as this is the area where *P. flavescens* occurs. Tussocks were all approximately 30 cm in diameter and at least 5 m apart. Aspect, altitude and GPS coordinates were recorded for all tussocks. Temperature and humidity readings were converted to time-series and detrended using the REISS package in R2.12.0. Daily summary statistics for temperature and humidity (mean, maximum, minimum) were calculated for each site and differences between the sites investigated using t-tests.

To verify the significance of these abiotic findings, I examined survival of *P. flavescens* under field conditions within a tussock grassland and on a mire site which is characterised by much less extensively developed vertical vegetation structure (*P. cookii* is not present in mires) (Gremmen & Smith 2008) and which has much smaller humidity differences between the inside and outside of the plant 'canopy' than is the case in the tussock grasslands (see below). For each habitat, five springtails, collected from the tussock grassland at Trypot Beach, were placed in each of 20 plastic vials and acclimated at 5°C for 48 hours in a climate chamber (LABCON, Johannesburg, South Africa, accurate to ± 1°C) with a photoperiod set to match the day light cycle at the time of year that the study was

conducted (12 L : 12 D). Vials were then returned to the field. In both habitats, a large wire mesh cage was placed over the vials to prevent interference by birds. I-button Thermochron dataloggers (Models DS 1921G and DS 1922H; accurate to $\pm$ 0.5°C, Dallas Semiconductors, Dallas, TX, USA) were used to monitor the temperature within *P. cookii* tussocks and on the mire surface. Vials were inspected twice a day for eight days and the number of springtails alive/dead counted. Comparisons between treatments over time were made using a generalised linear model for proportion data (correcting for overdispersion and using a logit link function) with the glm package in R2.12.0.

The information on physiological tolerances of the population at Marion Island was then compared with the environmental information to determine likely survival and development in 100 m bands across the elevational gradient. Because little effect of variable humidity (see Results) on survival in the field was found, I used the temperature data for the bands, an estimate of mean abundance where the species was present (see below), and the frequency distribution of crystallisation temperatures (the point of death for an individual) to estimate likely occurrence based on thermal limits (see Janion *et al.* 2009; Lee *et al.* 2009 for similar approaches). I did not use development rates because data from this species (Janion *et al.* 2010; C. Janion unpublished data) suggested that several generations could be completed at most low elevation sites. Whilst differences in temperature have been recorded between the eastern and western sides of the island (Nyakatya & McGeoch 2008), the differences are relatively small and long-term temperature data are not available for the remainder of the island. Although a more sophisticated biophysical modelling approach could have been adopted (e.g. Porter 1989), the small size of this species, its tendency to live within the vegetation, and the very high cloud cover on the island (le Roux & McGeoch 2008a) suggested that it would provide little additional insight, at least not for estimating likely range limits based on the available physiological information.

Island-wide and local abundance surveys

The first island-wide survey consisted of an investigation of 17 *P. cookii* tussock grasslands (at least 100 m x 100 m in extent) around the island based on Gabriel *et al.*'s (2001)
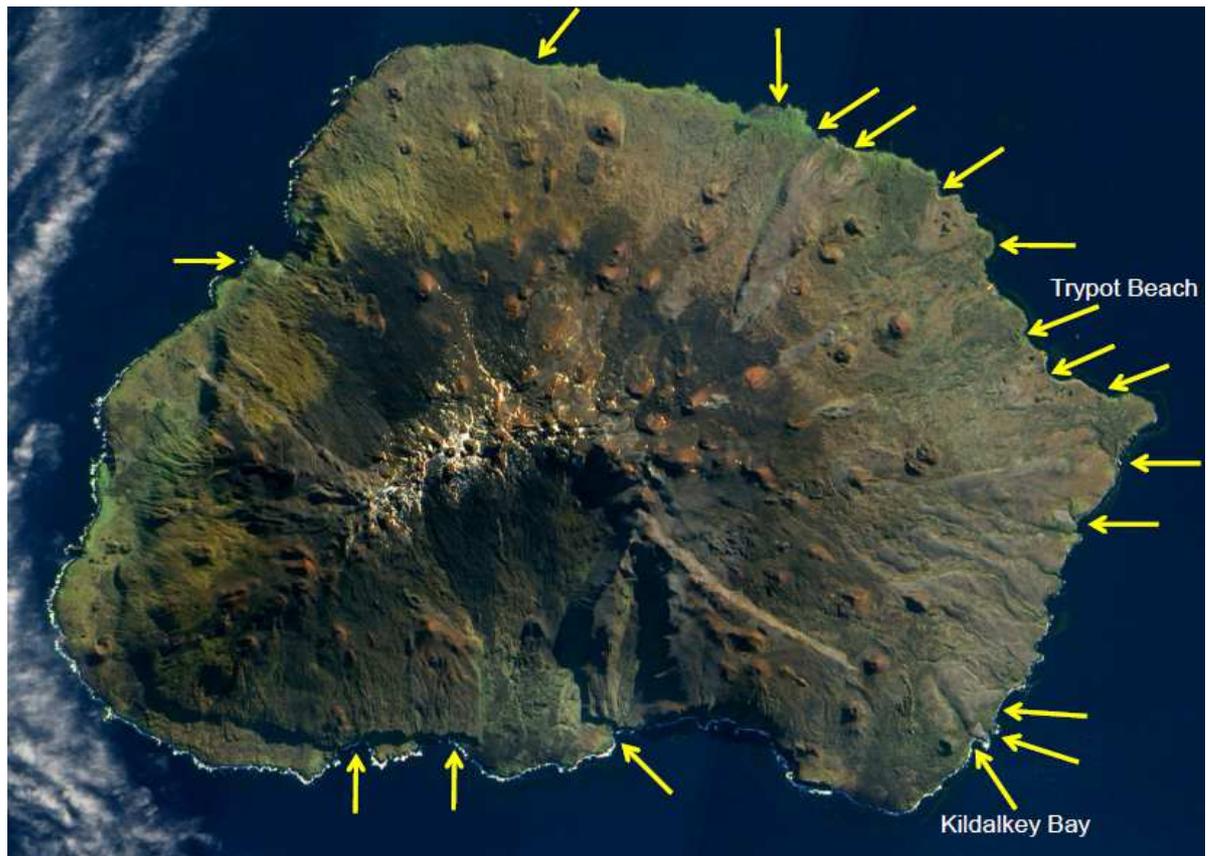
Figure 1. Areas of significant *Poa cookii* habitat on Marion Island, as indicated by the yellow arrows (Image courtesy of NASA's Earth Observatory, http://earthobservatory.nasa.gov/IOTD/view.php?id=40806).

observation that *P. flavescens* is restricted to such sites (Fig. 1). At each site, twenty, 4 m x 4 m quadrats were placed at least 15 m apart and, where possible, at least 15 m away from the edge of the *P. cookii* habitat. Five *P. cookii* tussocks were sampled in each quadrat - one at each corner and one in the centre. All tussocks were sampled by beating for one minute each and *P. flavescens* individuals collected into a sorting tray. For each tussock, *P. flavescens* individuals in the tray were collected with an aspirator, counted, and released once the quadrat sampling was completed. For each quadrat, latitude, longitude, altitude and aspect were also recorded. Additional sites consisting of one, 4 m x 4 m quadrat each were randomly surveyed around the island in a mixture of habitats and at various altitudes. Quadrats in *P. cookii* tussock grassland were sampled as above. For other vegetation types as well as habitats consisting of soil and stones, five areas of

25 cm x 25 cm each were inspected by hand for one minute in each quadrat - one in the centre and one at each quadrat corner. For each quadrat, latitude, longitude, altitude, aspect, vegetation or substrate type sampled, and dominant plant species present were also recorded.

Abundance data (as springtails per quadrat) were mapped spatially using R2.12.0 and then interpolated using a natural neighbour algorithm in ArcMap (v. 9.3.1 ESRI, CA, USA) to represent visually the abundance structure of this species. To investigate habitat specificity, the abundance data were transformed to presence/absence data, and a Fisher's Exact test (fisher.test, R2.12.0) was used to determine the likelihood of presence in *P. cookii* relative to other habitat types based on previous information suggesting such specificity (Gabriel *et al.* 2001).

Strong habitat specificity to *P. cookii* and a high abundance particularly around biotically influenced sites around bird and seal colonies was detected. Therefore, two additional surveys were carried out to further investigate the high abundances of *P. flavescens* around biotically influenced areas. In the first, one, 4 m x 4 m quadrat was sampled in *P. cookii* every 100 m where possible along 6.5 km of the coastline incorporating most of the distribution of *P. flavescens* identified following preliminary analyses of data from the island-wide survey. Quadrats were sampled as described above. For each quadrat, latitude, longitude, distance from the edge of the *P. cookii* habitat, aspect and presence of vertebrate species in the vicinity were recorded. To investigate correlates of abundance variation, a generalised linear model assuming a Poisson distribution with a log-link function and corrected for overdispersion was implemented using the glm function in R2.12.0. The model included aspect, distance from habitat boundary and presence of vertebrate species in the vicinity as categorical predictors and abundance of *P. flavescens* as the response variable. Interaction effects between the categorical predictors were also investigated.

The second additional survey examined abundance structure at a finer scale in *P. cookii* at a biotically influenced site. Four transects were sampled perpendicularly from the island's largest penguin colony at Kildalkey Bay (Fig. 1). Along each transect, 4 m x 4 m quadrats were sampled from the colony edge outwards at a minimum of 3 m - 5 m intervals in various vegetation complexes until zero abundance of *P. flavescens* was reached (Appendix A). Habitat specificity of *P. flavescens* to *P. cookii* as well as the high

abundance of *P. flavescens* around biotically influenced sites suggested that a change in substrate quality might be responsible for the range edges found away from these sites. I hypothesised that the most likely driver of these range edges would be soil nutrients. To investigate the effect of soil parameters on the fine scale abundance structure of *P. flavescens*, a soil sample was collected at each quadrat. The soil samples were returned to the laboratory where they were oven dried at 60°C for 36 hrs. They were transported back to South Africa and analysed for N (%), C (%), P (%), K (%), Ca (%), Mg (%) and Na (mg.kg^{-1}) (BemLab, Somerset West, South Africa). The soil pH and conductivity of each site was also measured in the laboratory at the island (Appendix B). Relationships between abundance of *P. flavescens* and environmental variables were explored with scatterplots drawn in R2.12.0. A generalised additive model (GAM) was fitted with a Poisson distribution and logit link function using the mgcv package in R2.12.0. The model was corrected for overdispersion and included cubic spline smoothers. Highly collinear variables were excluded by investigating the Spearman rank correlations and Variance Inflation Factors (adopting a VIF threshold of 5, see Zuur *et al.* 2010). The best fit model was sought by running models with different combinations of variables as well as by using a forward selection process by comparing model Generalised Cross-Validation (GCV) scores and percentage deviances explained.

Locomotion and dispersal capabilities

Physiological information was used in conjunction with data obtained from two experiments to assess dispersal in the species. For these studies, all animals were collected from the population found at Trypot Beach (Fig. 1), about 1 km southeast of the scientific station.

The first experiment assessed innate locomotion speed and consisted of an investigation of temperature effects on locomotion in the laboratory. The protocol adopted by Deere & Chown (2006) was modified for use with this species, which runs rapidly in most situations. Individuals were acclimated for one week at 7°C and the fastest speed from three repetitions on a temperature-controlled stage was used for each of ten different individuals at eight temperatures (0°C, 7°C, 14°C, 21°C, 24°C, 28°C, 32°C and 35°C) (Appendix C). To estimate the maximum locomotion speed (U_{max}) and the temperature at

which this occurs (T_{opt}) the best fit curve was estimated using TableCurve 2D (SYSTAT Inc, 2002, San Jose, California, USA) (Janion *et al.* 2010). Performance speeds at lower temperatures (those typical of long-term climate information) were also obtained from the best fit curve above. These various values were converted to distance per year (i.e. m.yr^{-1}) assuming non-stop linear travel, and used to calculate a series of maximum potential rates of spread.

The second experiment, to provide a conservative rate of spread, investigated field dispersal ability using animals marked with orange fluorescent powder (Day-Glo® powder, Day Glo Corp, Cleveland, OH) (see Kristensen *et al.* 2008 for rationale). Initial laboratory trials indicated that the fluorescent powder does not affect the survival of *P. flavescens* relative to a control group (Survival Analysis: x^2 = 0.3, *p* = 0.564, (Appendix D)), but that after 72 hours all of the fluorescent powder had rubbed off from most springtails. Therefore field trials were not run for longer than 60 hours. For these trials, 150 springtails were marked by lightly shaking them with 1.5 (± 0.5) mg powder in a plastic vial (see Kristensen *et al.* 2008), and then releasing 50 of them into each of three *P. cookii* tussocks at Trypot Beach. Each tussock was in the centre of a separate marked out grid in a tussock grassland. Movements were tracked 12 and 59 hours later at night using a Futronic rechargeable stand-by light (KN-886) with one 8W 12 inch black-light-blue bulb and one fluorescent bulb (Appendix D). Re-sight rate was calculated as the percent of the total number of powdered springtails released that were re-sighted for each night. The distances travelled per unit time (i.e. m.hr^{-1}) of the re-sighted springtails were converted to a rate per year (m.yr^{-1}) and used to estimate a rate of spread, i.e. the amount of time needed to populate the entire island, assuming a homogenous environment, no jump dispersal, and linear travel. Diffusion rate (*D*) was calculated (Turchin 1998) as:

$$D = \frac{\sum_{i=1}^{n} \iota_i^2}{4 \sum_{i=1}^{n} t_i} \tag{1}$$

where ι_i is the distance of the *i*-th move and t_i is its duration.

This diffusion rate was then used to calculate the rate of spread (v) (Turchin 1998; Hui *et al.* 2011):

$$v = 2\sqrt{rD} \tag{2}$$

where r is the intrinsic population growth rate. No literature data are available for *P. flavescens* population growth rate and a value for a similar large, surface-living species, *Orchesella cincta* (L.), was therefore used, i.e. $r = 0.6$ per year (van Straalen *et al.* 1989). The time needed to populate the entire island (T) was then estimated as:

$$T = \frac{R}{v} \tag{3}$$

where R is the maximum distance across the island from the estimated point of introduction of the springtail.

Results

Pogonognathellus flavescens is a relatively desiccation tolerant springtail species (surviving for 17-30 hours at 70% relative humidity and at 15 or 5°C, respectively), with a lower lethal temperature (= crystallisation temperature) of *c*. -12.0°C, and capable of developing in the egg stage down to at least 5°C (Table 1).

Along the altitudinal transect, mean annual soil temperature declines with altitude with the absolute minima dropping below -11°C from 700 m upwards (Table 2). Importantly, a band of low minimum temperature is also found at 200 m, likely reflecting an area not subject to continuous snow cover, while a band of much higher minimum temperature is found at 600 m, indicating winter snow cover. Mean humidity increases slightly with elevation, while the minimum humidity declines and the maximum humidity remains unchanged (Table 2). Temperature differences between sites below the vegetation and above it did not differ among habitat types (Table 3). By contrast, *P. cookii* and drainage line habitats had a significantly higher humidity within the vegetation than outside it, compared to the other habitat types (Table 3). Although these results suggest

that the *P. cookii* tussock grassland habitat might offer protection against desiccation owing to its high humidity buffering capacity, no significant effect of habitat (F = 1.23, p = 0.27) or time (F = 1.38, p = 0.24) was found between springtail survival in tussock grassland and on a mire. In addition, no significant differences in either temperature (t = 0, p = 1) or humidity (t = 0, p = 1) were found between *P. cookii* tussocks at Trypot Beach, one of the sites where *P. flavescens* occurs, and Mixed Pickle Cove, a site where the species has not been found.

Table 1. A summary of the physiological data available for the thermal and water relations, and egg development rate-temperature relationship of the *P. flavescens* population on Marion Island.

Trait	Main findings	Reference
Crystallisation temperature (= supercooling point (SCP))	-12.0 ± 1.00 (°C mean ± s.e.)	Slabber *et al.* (2007)
	Range: -21.5°C to -3.2°C	
	Acclimation to 20°C results in a pronounced decline of SCP	
T_{opt} for egg development	24.6°C	Janion *et al.* (2010)
	Higher egg hatching success at 25°C than at 15°C, but hatching continues to 5°C	
Desiccation tolerance	Survival times of 600 min (5°C) to 1800 min (15°C) at 70% relative humidity.	Chown *et al.* (2007)
Effect of thermal acclimation on water loss rate	Water loss in animals acclimated to 15°C is greatly reduced by acclimation to 5°C	Leinaas *et al.* (2009)
	Acclimation to 15°C protects sensitive smaller animals against rapid water loss (relative amount of water content in fully hydrated animals decreases with increasing body size)	
ULT50	33.3 ± 0.5 (°C mean ± s.e.)	Slabber *et al.* (2007)
	Increases with acclimation temperature	

s.e. = standard error

Table 2. (A) Descriptive statistics of soil temperatures (°C) across an altitudinal transect on the eastern side of Marion Island from 2002 to 2009, and the number of occurrences and number of hours that temperatures were less than or equal to 0°C, and less than or equal to -10°C during that period (P. le Roux, unpublished data). (B) Descriptive statistics of above ground humidity in air at three elevations on the eastern side of Marion Island for six months from November 2008 to April 2009.

A. Temperature

Altitude	n	Mean	s.d.	Minimum	Maximum	Range	Occurrences (hours) ≤ 0°C	Occurrences (hours) ≤ -10°C
0	54899	6.16	2.7	-1.0	22.5	23.5	3 (42)	0 (0)
100	54684	5.56	3.0	-2.2	20.0	22.2	32 (380)	0 (0)
200	56872	5.02	3.2	-10.0	18.5	28.5	55 (646)	0 (0)
300	56393	4.46	3.3	-4.0	18.8	22.8	143 (2245)	0 (0)
400	53471	3.82	3.3	-8.0	20.0	28.0	123 (1412)	0 (0)
500	58201	3.74	3.5	-6.5	36.0	42.5	83 (756)	0 (0)
600	55878	3.30	3.1	-1.0	25.8	26.8	86 (3011)	0 (0)
700	53992	2.25	3.8	-11.0	24.7	35.7	349 (8020)	19 (43)
800	56998	1.68	3.3	-13.5	23.0	36.5	690 (11301)	44 (467)

B. Humidity

Altitude	n	Mean	s.d.	Minimum	Maximum	Range
50	7174	88.31	15.10	29.84	110.33	80.49
400	8621	93.87	15.30	22.11	112.18	90.07
750	7215	96.33	18.59	8.32	111.13	102.81

s.d. = standard deviation

Table 3. The thermal and humidity buffering capacity of five habitat types as determined by measuring temperature and humidity both within the vegetation and at 0.3 m above the vegetation. Differences between habitats determined using ANOVAs are also indicated. Different letters denote significant differences among habitats based on Tukey's Honest Significant Difference.

Habitat	Temperature (°C ± s.e.)			Humidity (± s.e.)		
	Under canopy	Above canopy	Difference	Under canopy	Above canopy	Difference
Drainage line	6.80 ± 0.45	6.35 ± 0.51	-0.45 ± 0.16	86.13 ± 2.04	69.45 ± 3.82	-16.68 ± 4.13 [a]
Fellfield	5.03 ± 0.24	4.55 ± 0.19	-0.48 ± 0.06	74.25 ± 2.31	75.78 ± 2.97	1.53 ± 1.34 [c]
Fernbrake	7.11 ± 0.59	6.34 ± 0.53	-0.77 ± 0.31	79.70 ± 2.02	72.85 ± 2.56	-6.85 ± 3.07 [b]
Mire	7.53 ± 0.66	6.66 ± 0.45	-0.87 ± 0.27	82.04 ± 1.80	77.64 ± 3.74	-4.40 ± 4.58 [b]
Poa cookii	7.24 ± 0.29	6.79 ± 0.18	-0.45 ± 0.15	81.66 ± 0.91	65.23 ± 1.41	-16.43 ± 1.56 [a]
Habitat differences:	$F_{4,38} = 0.36$, $p = 0.84$			$F_{4,38} = 3.30$, $p = 0.02$		

s.e. = standard error

The above data suggest that *P. flavescens* should be able to survive at least to an altitude of 200 m, and perhaps, if able to traverse this low temperature area, also from 300 m to 700 m (Fig. 2a). However, if *P. cookii* is a habitat requirement for this springtail (as suggested by Gabriel *et al.* 2001), sites at higher elevations would be unavailable because tussock grasslands in small patches have an upper elevational limit of 500 m (Gremmen & Smith 2008) (Appendix E). The Fisher's Exact test, based on the island-wide survey and habitat data, indicated a highly significant habitat specificity of *P. flavescens* to *P. cookii* ($p < 0.0001$). Thus, because *P. flavescens* is clearly restricted to *P. cookii*, it would be expected to show a patchy distribution up to at least 200 m, and perhaps above that.

Contrary to these predictions, the island-wide survey revealed a restriction of the species to the southeast side of the island (Fig. 2b). Small populations also occur at Trypot Beach and Watertunnel Stream (Fig. 2b). Surveys of this southeast coastal distribution showed that the presence of vertebrate species in the vicinity of tussock grasslands is also a strong predictor of *P. flavescens* abundance ($X^2 = 30.72$, $p < 0.001$) with the highest abundances of the springtail found around biotically influenced areas near nesting birds and vertebrate colonies (Appendix F). Neither distance to the edge of *P. cookii* habitat ($X^2 = 2.44$, $p = 0.295$) nor aspect ($X^2 = 10.82$, $p = 0.094$) were significant explanatory variables and there were also no significant interactions between these variables. Zero abundance of *P. flavescens* was also found in *P. cookii* habitat to the north of a 720 m stretch of continuous non-*P. cookii* habitat at the north-easterly end of the coastal survey.
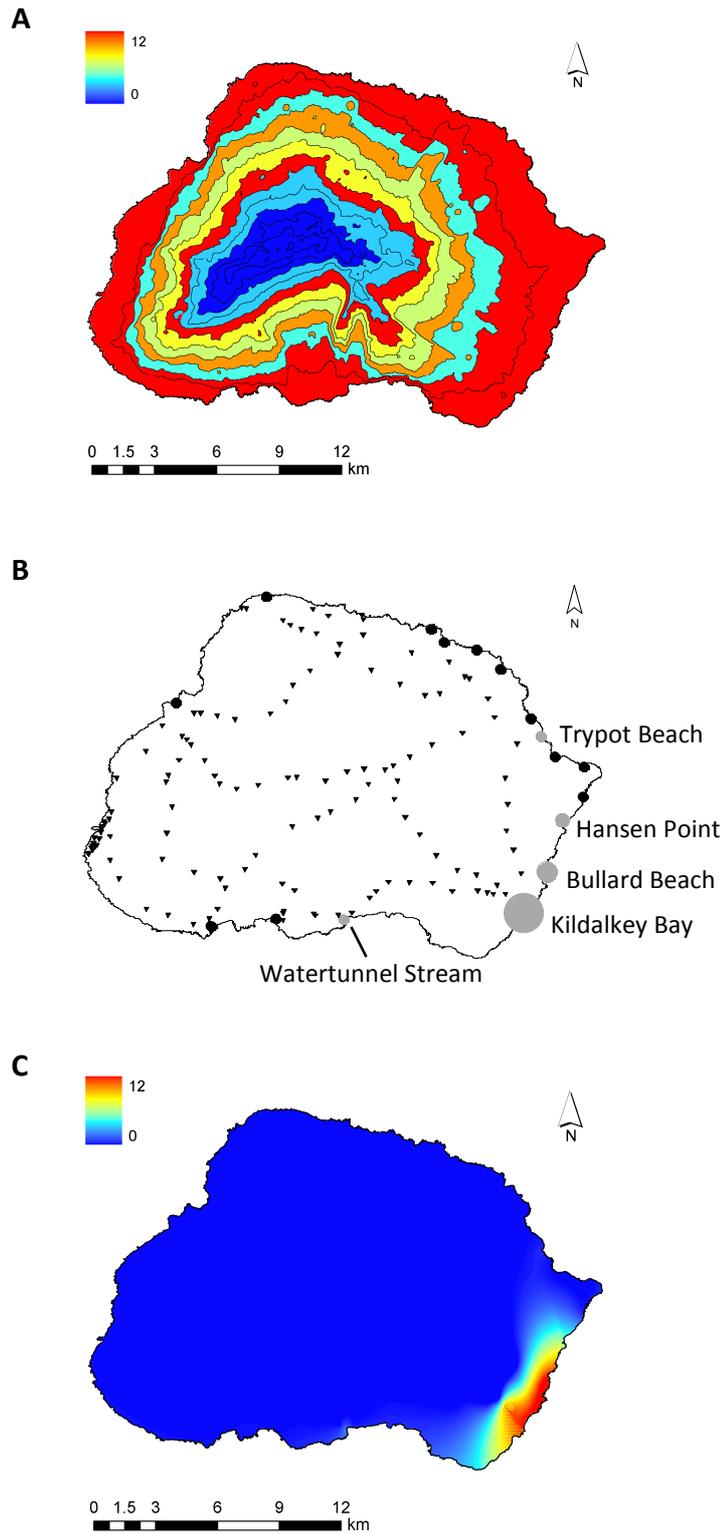
Figure 2. (A) The predicted likely occurrence of *P. flavescens* (as mean springtails per quadrat) in 100 m bands on Marion Island based on thermal limits, using temperature data for the bands, an estimate of mean abundance where the species was found and the

frequency distribution of crystallisation temperatures (the point of death for an individual). (B) Abundance data (as mean springtails per quadrat) as determined in the island-wide surveys. Circles indicate the 17 *P. cookii* sites investigated for *P. flavescens* abundance. Black circles indicate zero abundance, while grey circles indicate presence. The size of the grey circles correlates to abundance at those sites. Triangles indicate the locations of the additional quadrats sampled haphazardly around the island, all of which had zero abundance. (C) Natural neighbour interpolation of *P. flavescens* island wide abundance (numbers.m^{-2}) across the island based on the data shown in (B).

The small scale survey in an area of high *P. flavescens* abundance revealed high abundances of the springtail in *P. cookii*, with the numbers decreasing as the vegetation type changed from *P. cookii* to other vegetation types and with distance from the penguin colony (Fig. 3). Large numbers of zero counts and quadrats with very few numbers of *P. flavescens* were also found. Of the vegetation types sampled, the soils of coastal *P. cookii* tussock grassland were characterised by high N, high C, low pH, low Ca and low Mg. Relationships between the abundance of *P. flavescens* and environmental variables were evident, particularly with N, Ca, Mg, soil pH and soil conductivity (Appendix G). However, collinearity between explanatory variables was detected, with the strongest correlations occurring between vegetation habitat type, N, Mg, soil pH and soil conductivity. These variables also had the highest Variance Inflation Factors (VIF), particularly soil pH and soil conductivity. Soil conductivity, C, vegetation type and N were removed, while soil pH and Mg were kept as these variables have been found to be important determinants in springtail and *P. flavescens* distribution (van Straalen & Verhoef 1997; Chagnon *et al.* 2000; Ponge 2000b; Chagnon *et al.* 2001; Loranger *et al.* 2001; Auclerc *et al.* 2009). After the sequential removal of these variables, the VIF values of the remaining variables (P, K, Ca, Mg, Na, pH) were all less than 5. The optimal GAM model explaining the most variance (82.8%) included the terms K, Ca, Na and soil pH (Appendix H). Only Ca ($F = 4.9$, $p < 0.01$) and soil pH ($F = 3.7$, $p < 0.01$) were significant at the 0.05 level. The model was rerun excluding K and Na, but the explained variance declined (67.8%). The results indicate that Ca and soil pH are the strongest variables explaining the small scale abundance of *P.*

flavescens and the relationship between these variables and the abundance of *P. flavescens* showed a clear threshold effect (Fig. 4).
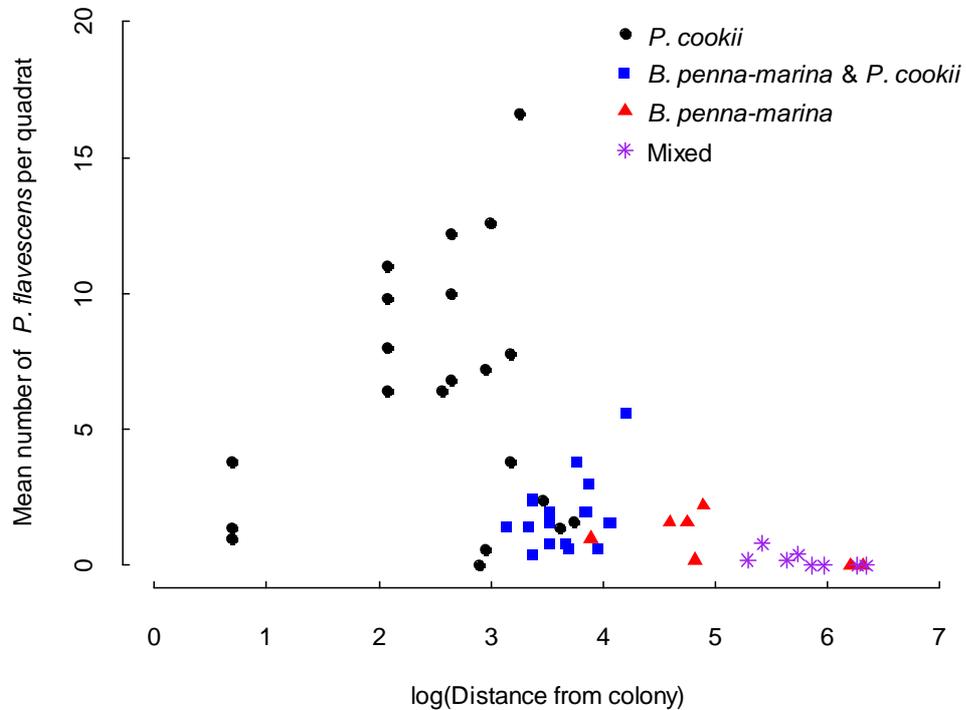
Figure 3. Change in abundance of *P. flavescens* at a small scale with distance from a penguin colony by vegetation type. The change in vegetation habitats from the penguin colony outwards were classified into the following complexes: habitat one was dominated by *P. cookii*; habitat two was dominated by *Blechnum penna-marina* (Poir.) Kuhn, but *P. cookii* was still present; habitat three was dominated by *B. penna-marina*; and, habitat four consisted of mixed vegetation with two or more of soil, stone, rock, *Azorella selago* Hook. f., *Agrostis magellanica* Lam., *Sagina procumbens* L., *B. penna-marina*, *Acaena magellanica* (Lam.) Vahl. and bryophytes.

The laboratory performance data indicated a maximum locomotion speed (U_{max}) of 0.97 cm.sec^{-1} at a T_{opt} (the optimum temperature of performance) of 26.1°C (Appendix I). The performance breadth (the index of the breadth of the curve, T_{br}) (Huey & Stevenson 1979; Gilchrist 1996) was 19.25. This optimum performance temperature, T_{opt}, and speed,

U_{max}, translates to a rate of movement of 306 089 m.yr^{-1} if a springtail moved in a non-stop linear fashion. At a lower temperature of 6.5°C which is approximately the mean annual temperature at the coast on Marion, a corresponding locomotion speed of 0.42 cm.sec^{-1} gives a rate of movement of 131 375 m.yr^{-1}. These liberal (maximum) estimates of movement suggest that it would take the species two to four years to populate the entire island, presuming non-stop linear travel on a homogenous surface (Table 4). By contrast, field dispersal trials indicated a slower rate of dispersal. The fluorescent powder was easily detected in the field dispersal experiments and both powdered *P. flavescens* individuals and powder residue indicating the presence of springtails were recorded on both *P. cookii* tussocks and on the ground during both nights. The re-sight rate of powdered springtails was 26.7% for the first night and 14.0% for the second. Using the distances travelled of the springtails re-sighted on the first night, a diffusion rate of 0.001 m.hr^{-1} or 10.942 m.yr^{-1} was calculated. When used in conjunction with an estimated population increase of 0.6 per year, the diffusion model indicated that 4 305 years would be required for *P. flavescens* to populate the entire island, assuming a homogenous environment, linear travel and no jump dispersal.
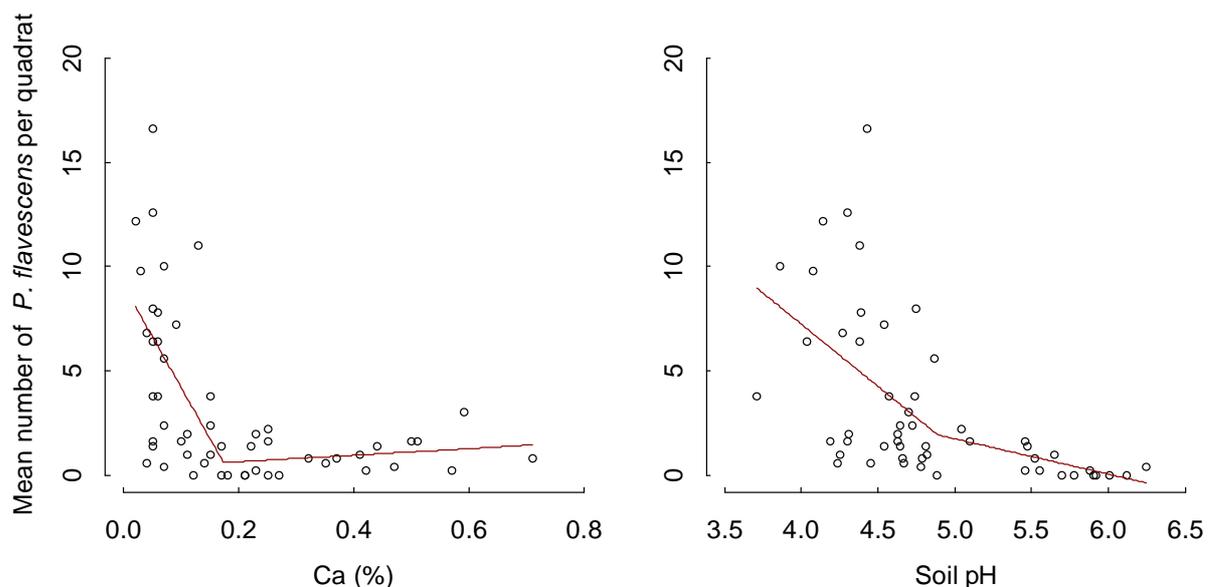
Figure 4. Small scale abundance of *P. flavescens* plotted against Ca (%) and soil pH on Marion Island.

Table 4. The estimated number of years it would take for *P. flavescens* to populate Marion Island by natural diffusion at a range of temperatures based on laboratory locomotion performance experiments and using a diffusion model (Turchin 1998; Hui *et al.* 2011) and an intrinsic rate of population increase of 0.6 per year (van Straalen *et al.* 1989). These are experimental values based on an individual running on a straight, flat, homogenous surface under laboratory conditions.

Temperature (°C)	Locomotion speed (m.yr^{-1})	Time to populate island (years)
26.1 (T_{opt})	306089.2 (U_{max})	1.8
6.5	131375.2	4.1
10	158921.7	3.4
15	208727.8	2.6
Modelled time based on field data	10.942	4 305

Discussion

Within Soberón's (2007, 2010) BAM framework, a population will persist where abiotic conditions allow survival and reproduction, where it's density is not reduced by predation or competition to such low values that extinction is inevitable, and in an area that it has been able to reach initially by dispersal (or one where dispersal maintains the population via the rescue effect (Brown & Kodric-Brown 1977). In the case of *P. flavescens* on Marion Island, temperature and humidity conditions across a range of low elevation habitats should ensure survival given what is known of the tolerances of this species (Table 1) and what was found in the survival trials in two very different habitats (mire and tussock grassland). Likewise, development data for the eggs, a significant stage in the springtail lifecycle (van Straalen 1994), suggest that lowland conditions across the island should also be conducive to reproduction. By contrast, between 200 m.a.s.l. and 300 m.a.s.l. conditions are likely to result in substantial mortality at least on an irregular basis (646 hours below -10°C across a seven-year period) and slow development given the sharp decline in

development rate with temperature. Above the 200 m band, low-temperature associated mortality increases, except at the 600 m level. At 600 m, the topography, at least on the east coast, is conducive to high snow cover, which results in ameliorated ambient minimum temperatures (Leather *et al.* 1993). However, at this elevation, vascular plants, including *P. cookii*, are largely absent (Gremmen & Smith 2008; Meiklejohn & Smith 2008). On this basis it might be expected that *P. flavescens* should occur in most of the lowland areas below 200 m on Marion Island.

Clearly, this is not the case. Despite *P. flavescens* having generalist habitat requirements elsewhere, including occurrence in moss and forest litter under both hardwood and conifers (Chagnon *et al.* 2000; Ponge 2000a; Berch *et al.* 2001; Fjellberg 2007) and has also been a commonly collected springtail in caves in the Holarctic (Soto-Adames & Taylor 2010), on Marion Island it is restricted to *P. cookii* tussock grassland habitats. Preference for this habitat seems to be associated with the presence of vertebrates and low Ca and pH. Although preferences for low Ca and pH have been found elsewhere for the species (Chagnon *et al.* 2001; Loranger *et al.* 2001) and more generally for the Tomocerid family of springtails (Fisk *et al.* 2006), the mechanistic basis thereof has not been elucidated. Why such extreme preferences for *P. cookii* habitats, biotic manuring, and low pH and Ca should be found on the island is not clear, but may have to do with a founder effect. The species is characterised by only a single MtCOI haplotype on the island, suggesting that no more than a few individuals were introduced (Myburgh *et al.* 2007). Such single introductions associated with low genetic diversity are less common for plants than for invertebrates (Wilson *et al.* 2009; but see also Dlugosh & Parker 2008). Irrespective, it is also clear that *P. flavescens* is absent from many areas of lowland *P. cookii* tussock grassland (Figs. 1 & 2), and many inland tussock grasslands also remain unoccupied, although their soil characteristics and other abiotic conditions appear suitable (Table 2; see also Smith *et al.* 2001; Gremmen & Smith 2008).

One explanation for the restricted geographic distribution could be differential predation or competition (the B in Soberón's (2007, 2010) framework). Despite an investigation of abundance co-variation and species associations (albeit over a limited set of sites) Gabriel *et al.* (2001) found no evidence of competition among the springtail species on the island. Rather, of the 13 habitats investigated, *P. cookii* tussock grasslands housed the highest species richness and highest total density of springtails. Moreover, the

other springtail species are not restricted to particular geographic quadrants of the island (as this species is to the southeast) (Hugo *et al.* 2004; see Chapter 2 of this thesis). More generally in the region, springtails appear to respond strongly to habitat quality, and if interspecific interactions are involved it appears that at fine scales certain alien species displace the indigenous fauna, rather than being excluded by them (Convey *et al.* 1999; Gabriel *et al.* 2001; Terauds *et al.* 2011). Thus, competition seems unlikely an explanation for the abundance variation in this species. Similarly, although predation has not been examined in detail, field observations suggest that spiders are the most significant predators (Appendix J). However, spiders are widely distributed across the island, and show their highest abundances in *P. cookii* tussock grassland, likely in response to high prey densities (Burger 1978; Lee *et al.* 2011). Thus, differential predation by spiders is unlikely to account for the absence of the species across most of the lowland tussock grassland areas. Other predators might include some predatory mites (Marshall *et al.* 1999), the introduced House Mouse, and the indigenous Lesser Sheathbill. No reports exist of springtails from stomach samples of mice or sheathbills (even though aphids and mites have been detected in gut samples of mice, and the sheathbills do feed on invertebrates) (Burger 1978; Smith *et al.* 2002). Likewise, whilst predatory mites may prey on the species and its eggs, most of these species are widespread around the island, although sometimes restricted to major biomes (such as the intertidal vs. vegetated areas - Barendse *et al.* 2002). Overall, therefore, interspecific interactions seem an unlikely explanation for the restricted distribution of *P. flavescens*.

By contrast, limited dispersal tendency and patchy habitats seem more likely to account for the absence of *P. flavescens* from *P. cookii* tussock grassland patches around the island. Although the locomotion speed of individuals suggests that they should easily have been able to colonise the island since the 1970s or 1980s, when they are presumed to have been introduced (Deharveng 1981 does not list them despite cataloguing virtually all other species known from the island), even within tussock grasslands individuals tend to have low dispersal rates, accounting for the long diffusion time estimated for full colonisation of the island, assuming homogeneous habitats (*c*. 4 000 years). Moreover, *P. cookii* tussock grasslands tend not to be continuous, except for some areas along the southeast coast, and even a break of 720 m, between the northernmost occupied *P. cookii* site just north of Bullard Beach and the next, unoccupied tussock grassland at Hansen Point

(Fig. 2b) is sufficient to have precluded dispersal thus far. The inability of individuals to traverse other habitat types between tussock grassland patches seems likely to be associated with a strong preference for tussock grasslands and their soil characteristics, rather than mortality in other habitat types. Even on the surface of mire vegetation, which differs in many ways from the grasslands (including pH, soil nutrients, soil moisture; Smith *et al.* 2001; Gremmen & Smith 2008), adult survival was as high as in the latter. This outcome is in keeping with the view that mortality is often overemphasised as a determinant of range limits (Gaston 2009).

Thus, in the case of *P. flavescens*, it is clear that long-distance dispersal by humans originally brought the species to the island (the entire family Tomoceridae is alien to the Southern Ocean Islands (Hopkin 1997)), and that it has subsequently invaded contiguous tussock grasslands from what must have been the point of introduction (likely Kildalkey Bay based on high densities, see Hengeveld 1989; Gaston 2003 for rationale). The isolated population at Trypot Beach further supports the idea that only jump dispersal (*sensu* Wilson *et al.* 2009) mediated by human or perhaps by bird vectors is likely to enable the species to spread further around the island. Therefore, despite a suitable 'scenopoetic' environment (Soberón 2010) in most lowland, and presumably some higher elevation, tussock grasslands, the species' habitat preferences and dispersal ability have precluded it from reaching these areas. In consequence, the most significant factors currently determining the range limits and abundance structure of the species are, respectively, contingent and environmental absences (Lobo *et al.* 2010). Range expansion seems precluded by the absence of suitable vectors for jump dispersal, whilst a decline in substrate suitability seems to account for the declines in abundance at the edge of currently occupied patches. Indeed, these sharp declines take the threshold form that Caughley *et al.* (1988) predicted would be associated with changes in soil substrate suitability. They also show that the species abundance structure at fine scales is characterised by patches of high and low density associated with *P. cookii* tussock grasslands, thus providing little support for an abundant centre distribution (Brown 1984; Sagarin *et al.* 2006). However, at a coarser spatial scale such a distribution is a plausible generalisation for the species (Fig. 2c).

More generally, the current findings and those from studies of other soil invertebrates (e.g. van Straalen & Verhoef 1997; Chagnon *et al.* 2001; Loranger *et al.* 2001;

Adams *et al.* 2006; Raschmanová *et al.* 2008; Lee *et al.* 2009; Roura-Pascual *et al.* 2011), suggest that edaphic factors are perhaps more important a determinant of abundance structure than are climatic factors. Thus, for species distribution modelling of these taxa (whether using mechanistic or correlative approaches, see Elith & Leathwick 2009; Kearney & Porter 2009; Buckley *et al.* 2010 for review), soil substrate variables or some proxy for them, such as vegetation or soil type, are likely to improve models of current distributions, and forecasts of distributions either in new areas (if the species become alien, see Blackburn *et al.* 2011) or in response to changing climates. By contrast, because of considerable habitat specificity and restricted dispersal ability, residence time may not be as important in determining the geographic range and impact of this alien species, and other habitat-specific alien soil invertebrates, as it is for other introduced species (reviewed in Wilson *et al.* 2007), in the absence of intentional or accidental movement by humans. Indeed, in the context of the limited haplotype diversity in *P. flavescens* (Myburgh *et al.* 2007), the findings of habitat specificity are unusual given that low genetic diversity appears not to affect the likelihood of invasion (Dlugosch & Parker 2008). However, these authors noted that more data concerning these relationships are required. Moreover, they also argued that introduction of additional individuals into a population should be avoided because of evidence that in some cases this can lead to increased genetic variation and invasion of previously unoccupied sites. In this particular case, given what is known of habitat preferences of *P. flavescens* elsewhere, such a recommendation seems warranted. However, over the longer term at Marion Island spread may well be driven by a widening distribution of *P. cookii*. Owing to the presence of feral cats, introduced to the island in 1947 and which by the 1970s had substantially reduced burrowing petrel populations (Ryan & Bester 2008), tussock grasslands have been much reduced in their extent away from penguin and seal colonies (Gremmen & Smith 2008). The eradication of cats in the early 1990s and a slow rise in burrowing petrel numbers (Ryan & Bester 2008) may well see *P. cookii* return to many areas (le Roux & McGeoch 2008b), which might then provide the conditions required for *P. flavescens* to spread. Such interactions among alien species, mediated in some cases by management interventions (see also Bergstrom *et al.* 2009), make the case for prevention of introductions all the more compelling.

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Appendices

Appendix A

Fine scale abundance structure

Four transects were sampled perpendicularly from the island's largest penguin colony at Kildalkey Bay. Along each transect, 4 m x 4 m quadrats were sampled from the colony edge outwards at a minimum of 3 m - 5 m intervals in various vegetation complexes until zero abundance of *Pogonognathellus flavescens* was reached. The first quadrat was placed at the colony edge and subsequent quadrats followed vegetation complex boundaries. Four vegetation complexes were identified from the edge of the penguin colony outwards: habitat one was dominated by *Poa cookii* Hook. f.; habitat two was dominated by *Blechnum penna-marina* (Poir.) Kuhn but *P. cookii* was still present; habitat three was dominated by *B. penna-marina*; and, habitat four consisted of mixed vegetation with two or more of soil, stone, rock, *Azorella selago* Hook. f., *Agrostis magellanica* Lam., *Sagina procumbens* L., *B. penna-marina*, *Acaena magellanica* (Lam.) Vahl. and bryophytes.

At least two quadrats were sampled in habitat one. Five *P. cookii* tussocks were sampled in each quadrat - one at each corner and one in the centre. All tussocks were sampled by beating for one minute each and *P. flavescens* individuals collected into a sorting tray. For each tussock, *P. flavescens* individuals in the tray were collected with an aspirator, counted, and released once the quadrat sampling was completed. For habitats two, three and four, quadrats were placed on the vegetation and five 25 cm x 25 cm vegetation samples were dug out per quadrat (one at each corner and one in the middle). These samples were placed in individually labelled ziplock bags and returned to the field hut where they were hand sorted and *P. flavescens* individuals counted. Four quadrats were sampled in habitat two, one quadrat was sampled in habitat three (only one was possible due to the small size of this habitat), and at least two quadrats were sampled in habitat four. Quadrats were sampled in habitat four until zero presence of *P. flavescens* was found for two consecutive quadrats.

Appendix B

Soil pH and conductivity measurements

A soil sample was collected from each of the Kildalkey fine scale survey sites using a 34 mm stainless steel soil corer. Each core was placed in an individually labelled ziplock plastic bag and returned to the laboratory. The litter and vegetation was removed from the surface of each core and the top 5 cm of the cores used for analysis. For each sample, 10 g of fresh soil was placed in a 100 ml glass beaker to which enough distilled water was added to cover the soil. A glass rod was used to thoroughly stir the sample after which the sample was left to settle for 30 minutes. The bulb of a series 50 50 fixed-wire cable pH glass electrode (Crison Instruments, SA) was then placed into the soil at the bottom of the liquid (measurement in a soil slurry) and the pH, conductivity and temperature were read using a Portable PH 25 pH-meter (Crison Instruments, SA). Date and time of measurement were also recorded. The pH meter was calibrated before measurement and after every ten samples using three buffer solutions: pH 4.01, pH 7.00 and pH 9.21. Care was taken to avoid cross contamination between samples. Beakers and the glass rod were washed with distilled water in-between samples and the electrode was rinsed with distilled water in-between each sample and after each calibration.

Appendix C

Locomotion performance

P. flavescens individuals were collected from the field by beating coastal *P. cookii* tussocks into a sorting tray. An aspirator was used to transfer the springtails to 100 ml plastic jars with moist plaster of Paris substrates and small pieces of *P. cookii* and other decaying plant material from the base of the *P. cookii* tussocks as a food source and for shelter. Animals were returned to the laboratory within one hour and transferred to individual plastic vials with moist plaster of Paris and pieces of vegetation as above. The springtails were placed in a climate chamber (LABCON, Johannesburg, SA, accurate to ± 1°C) at 7°C with a photoperiod set to match the day light cycle at the time of year that the study was conducted (12 L : 12 D). This acclimation temperature was chosen to represent the mean temperature as recorded inside the base of *P. cookii* tussocks on the day of collection by using a Vaisala HUMICAP© HM 34 temperature and humidity meter (Vaisala Inc, Woburn, MA, USA). A period of seven days was selected for acclimation following Hoffmann & Watson (1993) and previous studies on arthropods on both Marion (e.g. Klok & Chown 2003; Terblanche *et al.* 2007; Marais & Chown 2008) and elsewhere (e.g. Terblanche *et al.* 2006) as sufficient for phenotypic responses to temperature that could alter performance (Angilletta 2009) to equilibrate (Terblanche *et al.* 2007). Shelf effects in the climate chamber were unlikely to have had any influence on the temperature as the containers took up very little space on a single shelf. A temperature controlled walking stage was attached to a Grant LTC 12 water bath which was set to regulate stage temperature. Stage temperature was monitored at each end using Type T 20-gauge thermocouples connected to CHY 507 Digital Thermometers (CHY Firemate Co, Taiwan). A single springtail was introduced to the stage at a time using an aspirator. Springtails were given two minutes to equilibrate to the set temperature by placing a small round metal lid (radius 2 cm x height 0.5 cm) over them. The path that the individuals then ran in 10 seconds was traced using a non-permanent marker and this line was measured using string. The fastest speed from three repetitions was used for each individual (for rationale, see Garland & Losos 1994; Angilletta *et al.* 2002) and ten individuals were examined at eight temperatures (in this order: 0°C, 7°C, 14°C, 21°C, 24°C, 28°C, 32°C and 35°C). Individuals were returned to their

acclimation treatment in the climate chambers in-between temperature runs for at least 1-2 hours.

Appendix D

Laboratory trials with fluorescent powder

Laboratory experiments were conducted to determine whether fluorescent powder (Day-Glo® powder (Day Glo Corp, Cleveland, OH)) affects the survival of *P. flavescens* and also how long the powder adheres to the springtails before rubbing off onto the surrounding vegetation. Fifty *P. flavescens* individuals were collected from the field at Trypot Beach by beating coastal *P. cookii* tussocks into a sorting tray. An aspirator was used to transfer the springtails to 100 ml plastic jars with moist plaster of Paris substrates and small pieces of *P. cookii* and other decaying plant material from the base of the *P. cookii* tussocks as a food source and for shelter. Animals were returned to the laboratory within one hour. Twenty five springtails were transferred into a 35 ml plastic vial with 0.0015 (± 0.0005) g of fluorescent powder (Kristensen *et al.* 2008). The container was shaken gently to ensure that the springtails were coated in the powder. Five powdered individuals were then placed in each of five 30 ml plastic pill vials with moist plaster of Paris and small pieces of *P. cookii* and decaying plant matter. As a control, the remaining 25 un-powdered springtails were divided into five further pill vials with moist plaster of Paris and pieces of vegetation. The vials were placed in a climate chamber (LABCON, Johannesburg, SA, accurate to ± 1°C) set to a fluctuating temperature cycle of 5°C - 15°C (12 D : 12 L photoperiod). Springtail survival and powder adhesion were monitored twice daily for seven days. A survival analysis run using the survival package in R2.12.0 (R Development Core Team 2010) indicated no significant differences in survival of *P. flavescens* over time between the two treatments (X^2 = 0.3, p = 0.564) (Fig. S1).
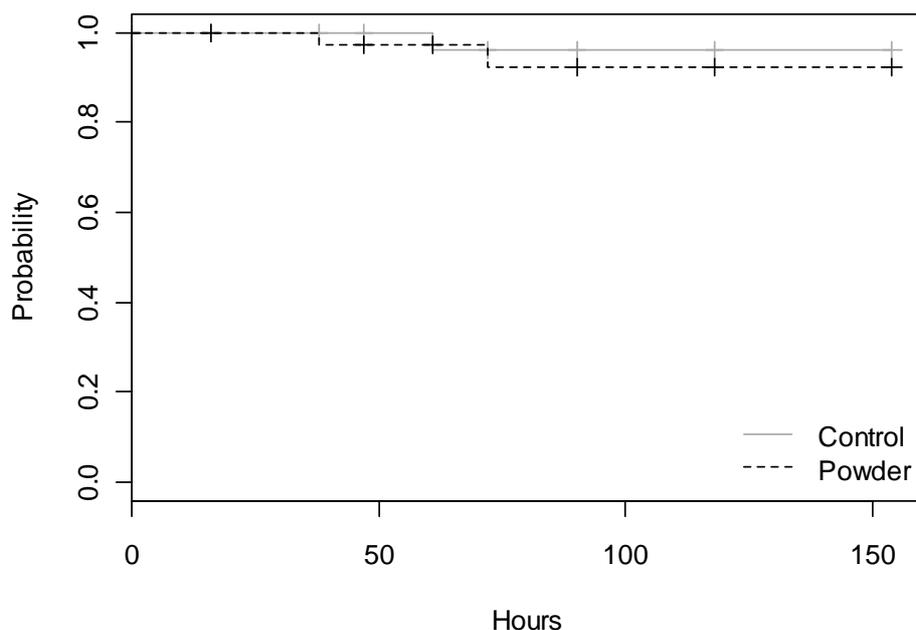
Figure S1. Survival function estimates calculated from a survival analysis of powdered versus non-powdered (Control) *P. flavescens* individuals.

Field dispersal experiments

Field dispersal experiments were conducted during April 2010. For these experiments, 150 springtails were marked by lightly shaking them with 0.0015 (± 0.0005) g fluorescent powder in a plastic vial (Kristensen *et al.* 2008), and then releasing 50 of them into each of three randomly selected *P. cookii* tussocks at Trypot Beach. Tussocks were at least 20 m apart and each was in the centre of a separate marked out grid in a tussock grassland. Care was taken to not spill any excess powder onto the tussocks or ground. Movements were tracked 12 and 59 hours later at night using a Futronic rechargeable stand-by light (KN-886) with one 8W 12 inch black-light-blue bulb and one fluorescent bulb. Powdered springtails or powder residue were easily identified. Tussocks and the ground were carefully inspected so as to cause minimal disturbance and the number of powdered *P. flavescens* and occurrences of powder residue (equivalent to the presence of a springtail) in tussocks or on the ground noted. Re-sight rate was calculated as the percent of the total number of powdered springtails released that were re-sighted for each night. Distances travelled per unit time were used to estimate a rate of spread, i.e. the amount of time needed to populate the entire island.

Appendix E

Figure S2. *P. cookii* habitat recorded at 500 m on Long Ridge South, Marion Island.

Appendix F. Outcome of a generalised linear model (assuming a Poisson distribution, using a log link function and corrected for overdispersion) investigating the effects of distance to the edge of the *P. cookii* habitat (Distance), presence of vertebrate species in the vicinity (Vertebrate) and aspect on the abundance of *P. flavescens* along a 6.5 km stretch of coastline incorporating most of the range of the springtail.

Variable (Response: *P. flavescens* abundance)	df	X^2	p
Distance	2	2.44	0.295
Vertebrate	4	30.72	< 0.001
Aspect	6	10.82	0.094
Distance * Vertebrate	1	0.41	0.522
Distance * Aspect	2	1.85	0.396
Vertebrate * Aspect	5	9.68	0.085
Distance * Vertebrate * Aspect	0		

Appendix G
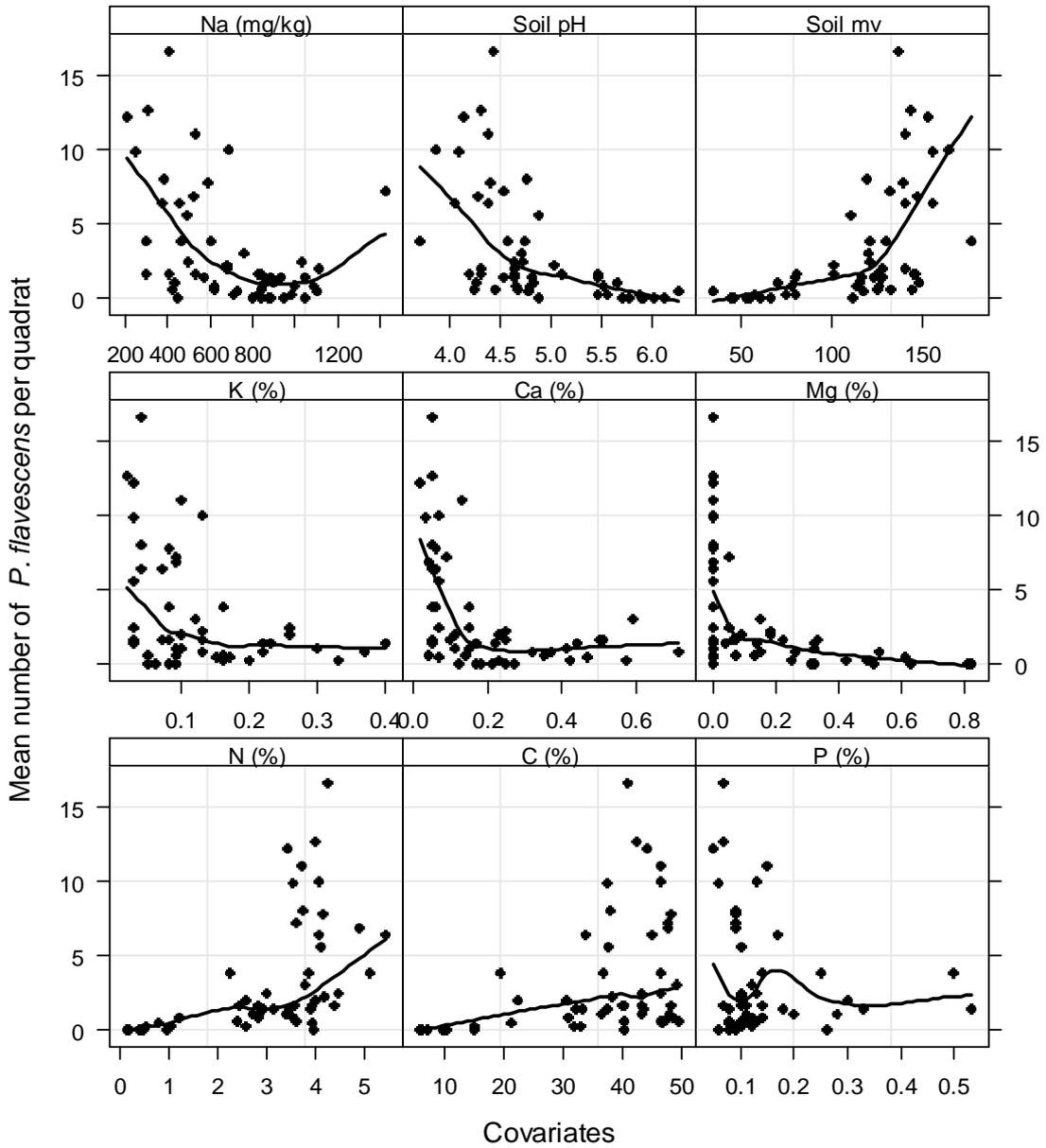
Figure S3. Multi-panel scatterplots between the mean number of *P. flavescens* per quadrat and soil nutrients, pH and conductivity (mv). LOESS smoothers were added to aid visual interpretation.

Appendix H. Outcome of a generalised additive model (with a Poisson distribution, using a logit link function, corrected for overdispersion and including cubic spline smoothers) investigating the effects of soil parameters on the small scale abundance of *P. flavescens*.

R-sq. (adj)   =   0.751

GCV score   =   1.978   Deviance explained   =   82.8 %   *n* = 52

Variable (Response: *P. flavescens* abundance)	df	Ref. df	*F*	*p*
K	7.868	8.523	1.959	0.083
Ca	2.198	2.785	4.944	0.007
Na	0.956	1.296	1.605	0.218
Soil pH	8.868	8.982	3.689	0.003

Appendix I

Performance curve fitting

For the performance curve fitting procedure, a curve was fitted to the performance data using TableCurve 2D (SYSTAT Inc, 2002, San Jose, California, USA). The curve with the largest coefficient of determination was selected, irrespective of the number of terms. The best fit was provided by an intercept form Beta curve, which has the following equation:

$$y = \frac{a\left[\dfrac{x-b+\dfrac{c(d-1)}{d+e-2}}{c}\right]^{d-1}\left[1-\dfrac{x-b+\dfrac{c(d-1)}{d+e-2}}{c}\right]^{e-1}}{\left[\dfrac{d-1}{d+e-2}\right]^{d-1}\left[\dfrac{e-1}{d+e-2}\right]^{e-1}} \qquad (1)$$

A fitted curve typically took the form indicated in Fig. S4 below.
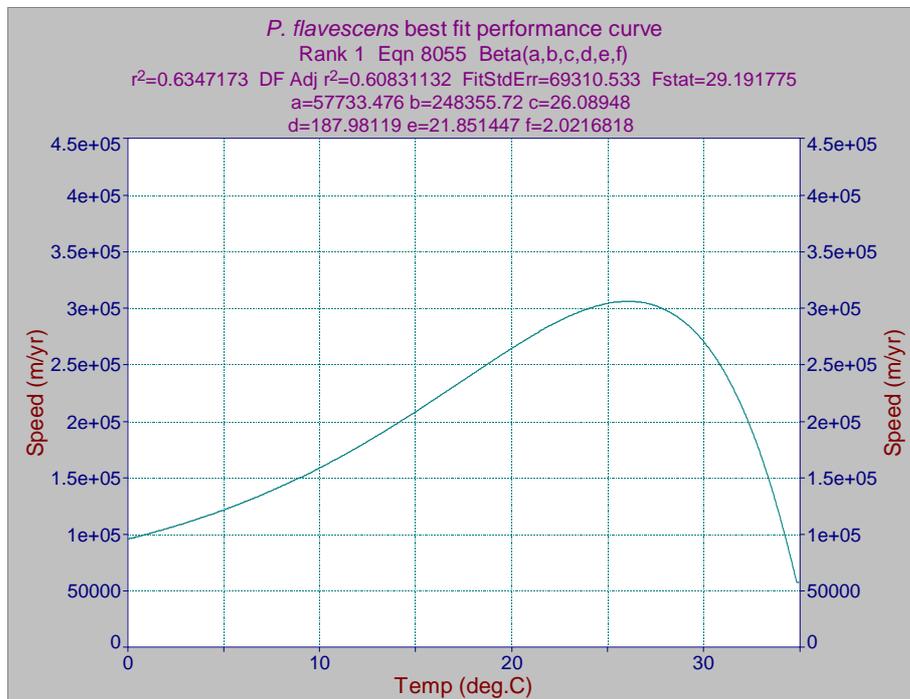
Figure S4. A beta curve fitted to the performance data for *P. flavescens.*

Appendix J

Figure S5. Spiders are thought to be the main predators of *P. flavescens* on Marion Island. No other predator on the island has been seen to eat this invasive springtail species.

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Chapter 4 – Synergistic effects of climate change and biological

invasion on ectotherm body size[1]

Bothrometopus randi (Jeannel 1953), indigenous to the Prince Edward
Island group

Introduction

Body size is one of the most significant and obvious features of animals and is of considerable ecological and physiological importance (Peters 1983; Brown *et al.* 2004). How body size varies through time and space, and what mechanisms underlie this variation have therefore long occupied biologists (for recent reviews see Angilletta 2009; Chown & Gaston 2010; Huston & Wolverton 2011). Optimal body size depends on interactions between mortality rates and production rates, which are influenced by predation and parasitism, and competition, respectively (Kozłowski & Gawelczyk 2002; Kozłowski *et al.* 2004). These rates are strongly size-dependent, and so influence the fitness of a given size, eventually leading to the intraspecific body size distribution (Kozłowski *et al.* 2004).

In ectotherms, production and mortality rates are also strongly influenced by temperature (Atkinson 1994; Angilletta 2009), and seasonal constraints in terms of the availability both of nutritional and thermal resources (Roff 1980; Clarke & Gaston 2006). In consequence, size variation is strongly influenced by temperature, both for intrinsic physiological reasons (Davidowitz & Nijhout 2004; Davidowitz *et al.* 2004, 2005) and owing to interactions among optimisation and growing season length (Chown & Gaston 1999, 2010). These interactions likely lie at the heart of both increasing size with increasing latitude and altitude (known, though often controversially, as Bergmann's Rule or Bergmann size clines – see Angiletta 2009 for a review), and declining size with decreasing latitude/altitude, sometimes also called converse Bergmann clines (Blanckenhorn & Demont 2004; Chown & Gaston 2010), though the mechanistic basis of this spatial variation in size remains much discussed (Cassell-Lundhagen *et al.* 2010; Watt *et al.* 2010; Forster *et al.* 2011; Huston & Wolverton 2011; Olalla-Tárraga 2011). Nevertheless, climate change is predicted to have significant effects on body size. Indeed, reduced body size has been described as one of the most universal ecological responses to warming (Daufresne *et al.* 2009). Surpisingly, while declines in body size in response to warming have been recorded for some taxa, including birds, mammals and insects (e.g. Smith *et al.* 1995; Smith & Betancourt 2006; Gienapp *et al.* 2008; Gardner *et al.* 2009), studies specifically addressing this are few, particularly for ectotherms (Daufresne *et al.* 2009).

At the same time, the body size of an indigenous species can also be affected by invasive species. The introduction of invasive species is one of the most significant threats

facing biodiversity today. These species have profoundly altered biotic and physical environments, and have had pronounced ecological impacts on native species (Strauss 1991; Gremmen *et al.* 1998; Mack *et al.* 2000; Frenot *et al.* 2005; Blackburn 2008). Invasive species can affect indigenous species' body sizes through predation by influencing both their mortality and production rates (e.g. Brooks & Dodson 1965; Galbraith 1967; Reznick *et al.* 1997; Cousyn *et al.* 2001; Phillips & Shine 2004; Fisk *et al.* 2007), and through more subtle interactions (Chown & Block 1997). In addition, invasive species can also change vegetation structure, which can have impacts on assemblage body size distributions (Coetzee *et al.* 2007; Chown & Gaston 2010). Typically, large individuals are lost from an assemblage, the consequences of which are similar to those of overexploitation where the loss of larger individuals of a species causes the 'erosion of large areas of phenotypic space' (Chown & Gaston 2008, pp. 1473).

While much evidence exists for the impacts of both climate change and biological invasions on species and their life history characteristics, including body size (see reviews in Lockwood *et al.* 2007; Gienapp *et al.* 2008; Gardner *et al.* 2011), the form of the interactions among these impacts are poorly understood. Several suggestions have been made that climate change and biological invasions will act synergistically to increase the impacts of invasive species (e.g. Dukes & Mooney 1999; Theoharides & Dukes 2007; Brook 2008; Brook *et al.* 2008). At least from the perspective of population dynamics, some evidence is starting to accumulate that climate change may indeed benefit invasive over indigenous species (e.g. Stachowicz *et al.* 2002; Chown *et al.* 2007). By contrast, meta-analysis of the experimental work to date has not found widespread support for synergies among environmental change drivers of mortality (Darling & Cote 2008). One reason for the latter may be the small number of studies undertaken to date. Indeed, several calls have been made that a wider range and extent of work is required to determine the extent to which synergies among environmental change drivers might be realised and might already be taking place (Brook *et al.* 2008).

The sub-Antarctic Prince Edward Island group provides an ideal opportunity to investigate the extent to which climate change and biological invasions may be synergistic. The group comprises two islands, Marion (MI) and Prince Edward (PEI), lying 19 km apart. They are experiencing rapid warming, yet have different invasive assemblages owing to very different human histories (Chown & Froneman 2008). Six indigenous weevil

(Curculionidae) species occur on the islands. They are of considerable significance in terrestrial food webs (Chown & Scholtz 1989; Smith & Steenkamp 1992, 1993), and form the main prey of invasive mice (*Mus musculus* L.) on MI, which are not present on PEI (Smith *et al.* 2002; Chown & Froneman 2008). In consequence, and given the fact that these weevils show Bergmann-type size clines with altitude, ascribed to declining temperatures, but little variation in growing season length (Chown & Klok 2003), the weevils should show size variation in step with changing climates at the islands, with the additional effects of size-selective predation (Chown & Smith 1993) on MI, but not on PEI. Here, I use a 24-year time series of body size data from the six weevil species found on the two islands to test these predictions.

Materials and methods

Study area and species

The sub-Antarctic Prince Edward Island group, comprising the larger MI (46°54'S, 37°45'E) and smaller PEI (46°37'S, 37°55'E), is located approximately 2300 km southeast of Cape Town, South Africa. The islands are volcanic in origin and the oldest recorded date for lavas on MI is 450 000 years (McDougall *et al.* 2001). The islands have an oceanic climate with a mean annual coastal temperature recorded on MI of *c*. 6.5°C, total annual precipitation of *c*. 1900 mm (as measured at the meteorological station on the north eastern coast of MI), generally high humidity, and strong winds. The climate has shown substantial change over the last 50 years, including an increase in mean annual temperature of more than 1°C and a decline in precipitation of more than 500 mm per annum (Smith 2002; le Roux & McGeoch 2008). The islands have two major biomes: polar desert at higher elevations (*c*. > 650 m), and tundra which predominates in lowland areas (Smith & Mucina 2006; Gremmen & Smith 2008). Six habitat complexes have been identified on the islands, namely mire, slope, fellfield, polar desert, saltspray and biotic (Gremmen & Smith 2008). Species richness of plants and invertebrates is low and no indigenous terrestrial vertebrates are found except for the Lesser Sheathbill (*Chionis minor marionensis* Reichenow) (see Chown & Froneman 2008 for an overview). The islands share most of the indigenous species that have been found in the archipelago, but PEI has far fewer invasive species than MI (Crafford *et al.* 1986; Crafford & Scholtz 1987; Gremmen & Smith 1999; Chown *et al.* 2002). This is due to

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the long history of human habitation on MI and the strict controls in place for human visitation to PEI. Most notably, the MI seabird fauna has suffered extensive impacts due to the now exterminated Domestic Cat (*Felis catus* L.) (cats have never occurred on PEI) (van Aarde 1980; Bester *et al.* 2000). House Mice (*Mus musculus* L.) became established on MI in the early 1800's (Watkins & Cooper 1986), but PEI has remained mouse-free. In consequence, substantial differences in invertebrate assemblages and community composition between the two islands have been recorded (Crafford & Scholtz 1987; Smith & Steenkamp 1990).

Six indigenous weevil species (Coleoptera: Curculionidae) of the *Ectemnorhinus* group of genera occur on the islands, of which two are restricted to the coast with the remainder occupying most of the altitudinal range of the islands (Table 1) (see Chown 1989 for detailed information on diet and habitat distribution). *Bothrometopus parvulus (sensu lato)* includes the species *Bothrometopus parvulus* and *Bothrometopus huntleyi* (see below). The *Ectemnorhinus* group is a monophyletic unit of weevils that is restricted to South Indian Ocean Province Islands in the Southern Ocean, on which they are crucial to nutrient cycling and thus constitute an important part of the terrestrial arthropod fauna and food webs (Burger 1985; Smith & Steenkamp 1992; Chown 1993a; Kuschel & Chown 1995; Barendse & Chown 2000; Grobler *et al.* 2011). Weevils are a preferred food item of mice on MI (Smith *et al.* 2002), with some species as well as larger individuals being favoured over others (Chown & Smith 1993). Evidence for consumption of the different species shows that the vegetation-dwelling and rock face-dwelling species *E. marioni* and *B. randi* are regularly consumed. *Bothrometopus parvulus* (*sensu lato*) is also taken by mice, and circumstantial evidence from higher elevations indicates that *B. elongatus* is also consumed. Although this species is small, mice have also been found with high numbers of aphids and mites in their gut contents (Smith *et al.* 2002). By contrast, no evidence has been found that mice consume *P. eatoni*, mostly because they occur in the supralittoral and intertidal zones on vertical rock faces, which are habitats not used by mice (Chown 1989; Chown & Smith 1993).

Table 1. The indigenous weevil species and their altitudinal ranges on Marion Island (MI) and Prince Edward Island (PEI) as sampled in the present study.

Species	Altitudinal range MI (m)	Altitudinal range PEI (m)
Palirhoeus eatoni	3 - 10	3 - 9
Bothrometopus elongatus	54 - 1261	284 - 662
Bothrometopus parvulus (sensu lato)[1]	3 - 1005	3 - 681
Bothrometopus randi	3 - 36	3 - 20
Ectemnorhinus marioni	3 - 1026	3 - 681

[1] Includes the species *Bothrometopus huntleyi* and *Bothrometopus parvulus* - see Methods.

Collection and measurement

A 24-year time series, incorporating four well-spaced sampling events on both islands, was used to determine the relative effects of climate change and mouse predation on adult weevil body size distributions. Adult weevils of all species were hand-collected from various vegetation types on MI and PEI in the following year groups: 1986-1988, 1992, 1999-2003 and 2008-2010. Location (latitude, longitude and altitude) was also recorded where possible. All weevils were sexed to investigate sexual size dimorphism and measured from the anterior margin of the eyes to the posterior edge of the elytra using a light microscope fitted with a calibrated eye-piece micrometer (Fig. 1).

Data analysis

Strong sexual size dimorphism was found for all species (see Results) and sex was therefore always included in statistical models. *Bothrometopus parvulus (sensu lato)* (Table 1) includes the cryptic species *Bothrometopus parvulus* and *Bothrometopus huntleyi*. *B. huntleyi* was recently split from *B. parvulus* largely on molecular and cryptic morphological grounds (Grobler *et al.* 2011). Due to missing historical specimens, it was not possible to re-identify all individuals for this study. Therefore these two related species were analysed as a single group, which was not considered problematic, as morphologically the two species are exceptionally similar (Grobler *et al.* 2011).
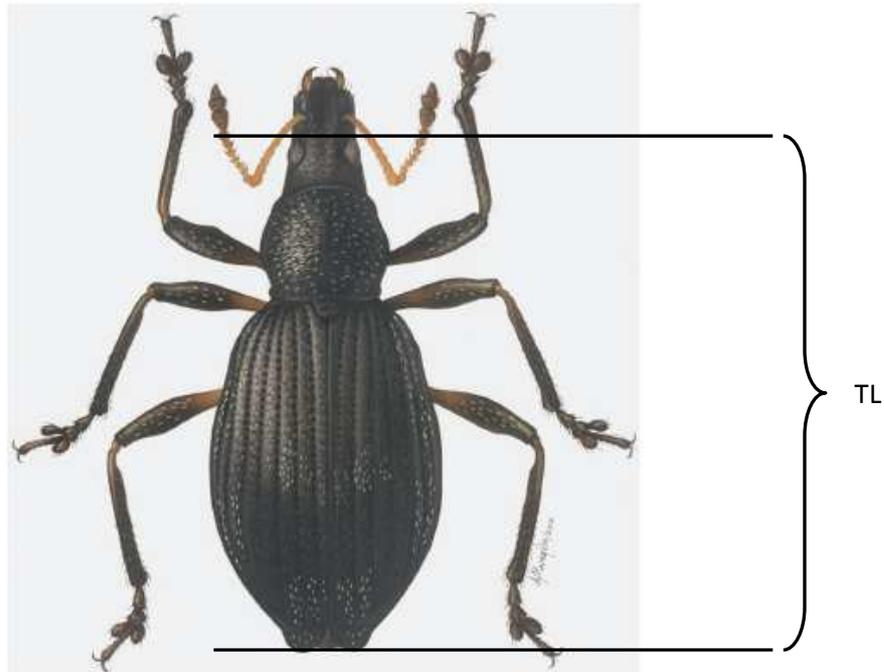
Figure 1. The individual total body length (TL) measured in adult weevils from the anterior margin of the eyes to the posterior edge of the elytra (drawing from Grobler *et al.* 2011).

Generalised linear models (GLZs, each assuming a Gaussian distribution with an identity link function) were used to determine the relative effects of sex, island (including an interaction between sex and island), altitude, and mean annual temperature on weevil body size, with the glm function in R2.12.0 (R Development Core Team 2010). Altitude was included as a variable because body size increases with altitude have been observed for the weevils on MI (Chown & Klok 2003). However, altitude was excluded from models for the coastal species *P. eatoni* and *B. randi*. As it is the temperature at the development stage that determines final (adult) body size (Atkinson 1994; Kingsolver 2009) and the life cycles of the weevils span at least one year (Chown & Scholtz 1989; Chown 1990), the mean annual temperature (as recorded at the meteorological station on the north eastern coast of MI) of each of the years preceding the years of collection were used in the statistical models. These were averaged for the four time periods to give a mean annual temperature for each time period for use in the models. Optimal models were sought by sequentially

removing non-significant terms until only significant terms remained. Full and optimal models were then ranked in accordance with Akaike's Information Criterion values (AIC$_c$) and weights (AIC$_w$). Because MI rises to 1230 m.a.s.l. and PEI only to 681 m.a.s.l., models for the species occurring across the elevational range were restricted to individuals collected below 681 m on MI to ensure compatability across the islands.

Because the island term was significant in most of the models, generalised linear models were then used to determine the relative effects of sex, altitude and mean annual temperature on weevil body size on MI and PEI separately (each model assuming a Gaussian distribution with an identity link function).

Further to explore variation in sizes among years and islands, body size frequency distributions of the preferred prey species, *E. marioni*, and the species least likely to be consumed, *P. eatoni*, were constructed for the year groups 1986-1988 and 2008-2010. For this analysis, only *E. marioni* individuals collected from *Azorella selago* Hook. f. were used to compare the current findings with those of Chown & Smith (1993). Bin size is known to influence body size frequency distributions (Loder *et al.* 1997). In this study equal sized bins were selected manually for both species to facilitate visual comparison, which was considered appropriate as the size frequencies were only used to qualitatively view and illustrate changes over time.

Results

Substantial interannual variation was found for both mean annual temperature (6.17°C, 6.09°C, 6.41°C and 5.62°C for 1986-1988, 1992, 1999-2003 and 2008-2010, respectively) and body size of all weevil species (Fig. 2). In addition, all species displayed strong sexual size dimorphism with females significantly larger than males (Appendix A; Fig. 2; Table 2). On PEI, the body size of all weevil species showed a significant decline with increasing mean annual temperature (Table 3). On MI however, positive relationships were found between mean annual temperature and body size for all weevil species except *P. eatoni* (Table 4). For this species, *P. eatoni* (the non-preferred prey species), a striking similarity in the direction and strength of the relationships was found across the populations inhabiting the two islands (Tables 3 & 4). Indeed, the estimates are statistically indistinguishable (post-hoc test, T'-method of minimum significant differences at α = 0.05 (Sokal & Rohlf

1995), indicated overlap of confidence intervals: MI upper = -0.084, lower = -0.206; PEI upper = -0.097, lower = -0.285). Altitude has a significant effect on *B. elongatus* and *B. parvulus (sensu lato)*, on both MI and PEI (Tables 3 & 4).

The body size frequency distribution comparison between the preferred prey species, *E. marioni*, and the non-preferred species, *P. eatoni*, showed substantial differences between the two (Fig. 3). The frequency of the larger body sizes of *E. marioni* males and females on MI decreased significantly from 1986-1988 to 2008-2010, while on PEI there was no obvious effect (Fig. 3a). Conversely, no obvious changes in body size frequencies were observed for *P. eatoni* on either MI or PEI (Fig. 3b). *E. marioni* collected from *A. selago* displayed bimodality of body size, in line with previous findings for this species (Chown 1990; Chown & Smith 1993).

Males   **Females**

A. *P. eatoni*

B. *B. elongatus*

C. *B. parvulus (sensu lato)*

D. *B. randi*
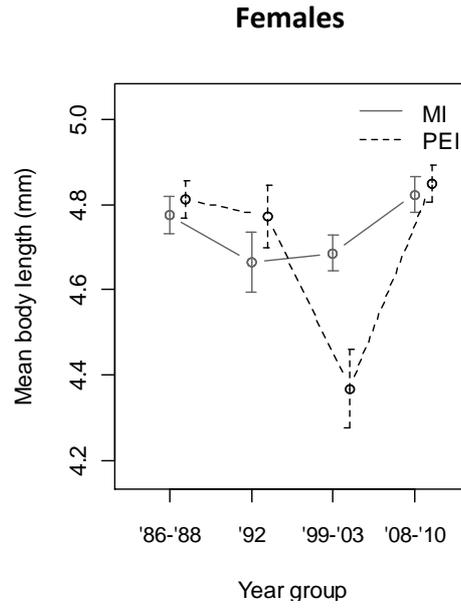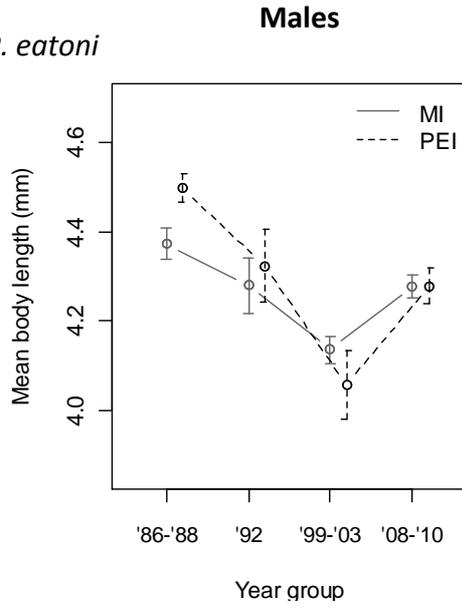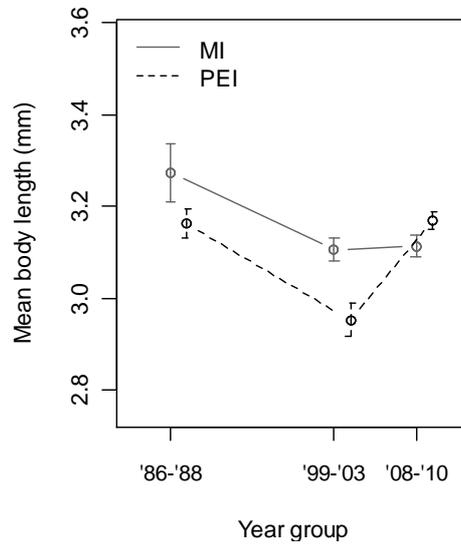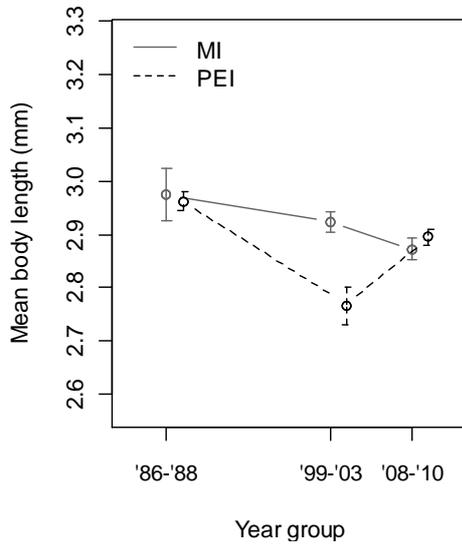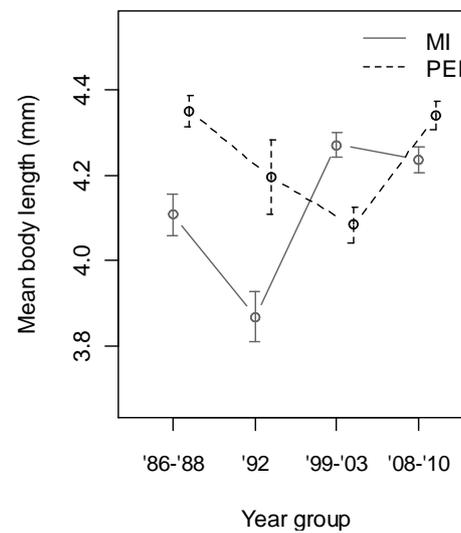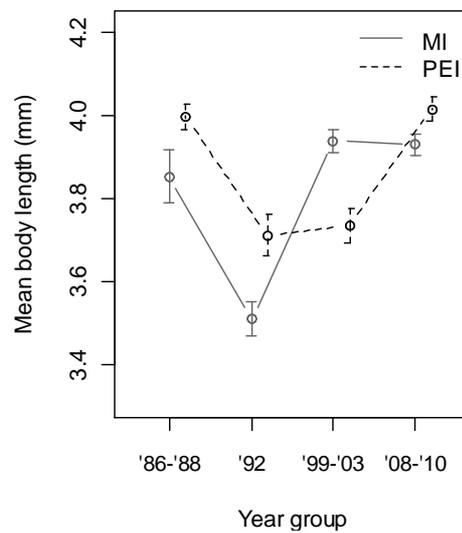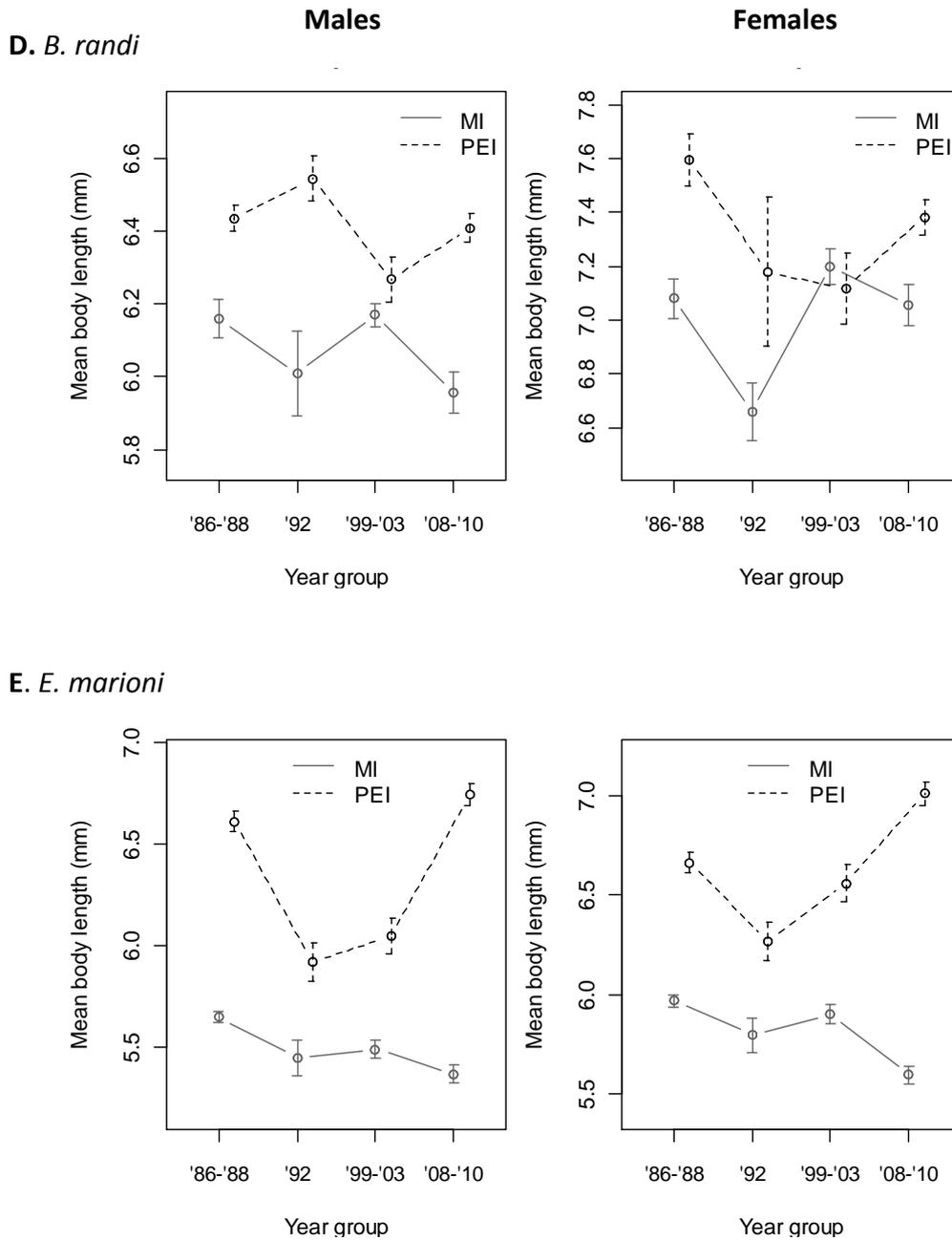
E. *E. marioni*

Figure 2. Mean body length against year groups for weevil species on Marion Island (MI) and Prince Edward Island (PEI) for males (left) and females (right). Vertical bars indicate standard errors and means are actual means. Note that females are larger than males for all species and that Y axes for the sexes are not equal. A = *P. eatoni*, B = *B. elongatus*, C = *B. parvulus (sensu lato)*, D = *B. randi*, E = *E. marioni*.

Table 2. Generalised linear model (GLZ) results for the effects of sex, island (including an interaction between sex and island), mean annual temperature of the year preceding weevil collection (averaged into year groups) and altitude on the body size of weevils on Marion Island and Prince Edward Island. Altitude was excluded from models for the coastal species *P. eatoni* and *B. randi*. A Gaussian distribution with an identity link function was used. AIC_c values and weights (AIC_w) for the full models are given, as well as for optimal models sought by sequential deletion of non-significant terms (indicated in brackets). Deviance explained (DE) values for full models are also indicated. Significant terms in the optimal models were the same as those found in the full models.

Species and variable	df	Estimate	s.e.	x^2	p
P. eatoni (*n* = 929)					
AIC_c = 649.63 (652.02), AIC_w = 0.77 (0.23)					
DE = 32.42%					
Intercept		5.728	0.225		
Sex (m)	1	-0.499	0.030	390.35	< 0.0001
Island (PEI)	1	-0.011	0.035	2.63	0.1051
Mean annual temperature	1, 928	-0.161	0.037	19.11	< 0.0001
Sex (m) * Island (PEI)	1	0.093	0.048	3.76	0.0526
B. elongatus (*n* = 1135)					
AIC_c = -4.5057 (-8.4183), AIC_w = 0.12 (0.88)					
DE = 19.76%					
Intercept		3.030	0.134		
Sex (m)	1	-0.231	0.020	228.39	< 0.0001
Island (PEI)	1	-0.026	0.023	2.30	0.1294
Altitude	1	0.000*	0.000*	21.78	< 0.0001
Mean annual temperature	1, 1134	-0.001	0.021	0.00	0.9766
Sex (m) * Island (PEI)	1	0.001	0.031	0.01	0.9199
B. parvulus (sensu lato) (*n* = 1708)					
AIC_c = 1791.5 (1790.9), AIC_w = 0.43 (0.57)					
DE = 16.53%					
Intercept		4.248	0.183		
Sex (m)	1	-0.287	0.028	203.28	< 0.0001
Island (PEI)	1	0.150	0.032	26.67	< 0.0001
Altitude	1	0.000*	0.000*	66.51	< 0.0001
Mean annual temperature	1, 1707	-0.032	0.030	1.17	0.2796
Sex (m) * Island (PEI)	1	-0.065	0.045	2.10	0.1473

Table 2 continued

Species and variable	df	Estimate	s.e.	X^2	p
B. randi (*n* = 919)					
AIC$_c$ = 1330.3 (1329.6), AIC$_w$ = 0.41 (0.59)					
DE = 47.42%					
Intercept		6.479	0.360		
Sex (m)	1	-0.991	0.044	762.04	< 0.0001
Island (PEI)	1	0.289	0.059	67.83	< 0.0001
Mean annual temperature	1, 918	0.100	0.058	2.95	0.0857
Sex (m) * Island (PEI)	1	0.027	0.074	0.14	0.7123
E. marioni (*n* = 6057)					
AIC$_c$ = 16901 (16907), AIC$_w$ = 0.95 (0.05)					
DE = 14.21%					
Intercept		5.865	0.312		
Sex (m)	1	-0.324	0.032	120.66	< 0.0001
Island (PEI)	1	0.954	0.048	770.60	< 0.0001
Altitude	1	0.000*	0.000*	6.17	0.013
Mean annual temperature	1, 6056	0.006	0.050	0.01	0.9047
Sex (m) * Island (PEI)	1	0.073	0.068	1.17	0.2796

s.e. = standard error, m = male, PEI = Prince Edward Island, * = non-zero but small effect

Table 3. Generalised linear model (GLZ) results for the effects of sex, mean annual temperature of the year preceding weevil collection (averaged into year groups) and altitude on the body size of weevils on Prince Edward Island. Altitude was excluded from models for the coastal species *P. eatoni* and *B. randi*. A Gaussian distribution with an identity link function was used. AIC_c values and weights (AIC_w) for the full models are given, as well as for optimal models sought by sequential deletion of non-significant terms (indicated in brackets). Deviance explained (DE) values for full models are also indicated. Significant terms in the optimal models were the same as those found in the full models.

Species and variable	df	Estimate	s.e.	X^2	p
P. eatoni (*n* = 342)					
AIC_c = 300.11					
DE = 25.07%					
Intercept		5.893	0.402		
Sex (m)	1	-0.404	0.040	99.85	< 0.0001
Mean annual temperature	1, 341	-0.191	0.067	8.07	0.0045
B. elongatus (*n* = 426)					
AIC_c = -160.31					
DE = 27.85%					
Intercept		3.530	0.204		
Sex (m)	1	-0.232	0.019	142.27	< 0.0001
Altitude	1	0.000*	0.000*	6.84	0.0089
Mean annual temperature	1, 425	-0.092	0.031	8.82	0.0030
B. parvulus (sensu lato) (*n* = 648)					
AIC_c = 442.74					
DE = 24.8%					
Intercept		5.257	0.272		
Sex (m)	1	-0.348	0.029	147.77	< 0.0001
Altitude	1	0.000*	0.000*	23.65	< 0.0001
Mean annual temperature	1, 647	-0.173	0.045	14.94	0.0001
B. randi (*n* = 335)					
AIC_c = 393.84 (394.89), AIC_w = 0.63 (0.37)					
DE = 53.12%					
Intercept		8.167	0.458		
Sex (m)	1	-0.950	0.051	351.89	< 0.0001
Mean annual temperature	1, 334	-0.134	0.077	3.04	0.0814
E. marioni (*n* = 1869)					
AIC_c = 3652.7 (5569.2), AIC_w = 1.0 (0.0)					
DE = 5.62%					
Intercept		10.660	0.520		
Sex (m)	1	-0.271	0.058	22.07	< 0.0001
Altitude	1	0.000*	0.000*	1.00	0.3179
Mean annual temperature	1, 1868	-0.640	0.086	55.92	< 0.0001

s.e. = standard error, m = male, * = non-zero but small effect

Table 4. Generalised linear model (GLZ) results for the effects of sex, mean annual temperature of the year preceding weevil collection (averaged into year groups) and altitude on the body size of weevils on Marion Island. Altitude was excluded from models for the coastal species *P. eatoni* and *B. randi*. A Gaussian distribution with an identity link function was used. AIC_c values and weights (AIC_w) for the full models are given, as well as for optimal models sought by sequential deletion of non-significant terms (indicated in brackets). Deviance explained (DE) values for full models are also indicated. Significant terms in the optimal models were the same as those found in the full models.

Species and variable	df	Estimate	s.e.	X^2	p
P. eatoni (*n* = 587)					
AIC_c = 347.33					
DE = 36.57%					
Intercept		5.633	0.265		
Sex (m)	1	-0.499	0.029	305.35	< 0.0001
Mean annual temperature	1, 586	-0.145	0.043	11.20	0.0008
B. elongatus (*n* = 709)					
AIC_c = 111.47 (112.35), AIC_w = 0.61 (0.39)					
DE = 17.57%					
Intercept		2.738	0.180		
Sex (m)	1	-0.229	0.022	108.73	< 0.0001
Altitude	1	0.000*	0.000*	12.69	0.0004
Mean annual temperature	1, 708	0.049	0.029	2.86	0.0908
B. parvulus (sensu lato) (*n* = 1111)					
AIC_c = 1280.5 (1278.9), AIC_w = 0.31 (0.69)					
DE = 12.55%					
Intercept		3.894	0.238		
Sex (m)	1	-0.284	0.031	85.28	< 0.0001
Altitude	1	0.000	0.000	39.91	< 0.0001
Mean annual temperature	1, 1110	0.025	0.039	0.40	0.5263
B. randi (*n* = 582)					
AIC_c = 899.78					
DE = 45.06%					
Intercept		5.548	0.501		
Sex (m)	1	-0.990	0.048	430.49	< 0.0001
Mean annual temperature	1, 581	0.251	0.081	9.55	0.0020
E. marioni (*n* = 4793)					
AIC_c = 13179 (13180), AIC_w = 0.62 (0.38)					
DE = 2.99%					
Intercept		3.996	0.380		
Sex (m)	1	-0.326	0.032	104.79	< 0.0001
Altitude	1	0.000*	0.000*	2.87	0.0902
Mean annual temperature	1, 4792	0.311	0.061	25.58	< 0.0001

s.e. = standard error, m = male, * = non-zero but small effect

A. *E. marioni* (on *A. selago*)

1986-1988, MI, m (*n* = 566)

1986-1988, MI, f (*n* = 586)

2008-2010, MI, m (*n* = 310)

2008-2010, MI, f (*n* = 378)

1986-1988, PEI, m (*n* = 189)

1986-1988, PEI, f (*n* = 217)

2008-2010, PEI, m (*n* = 169)

2008-2010, PEI, f (*n* = 200)
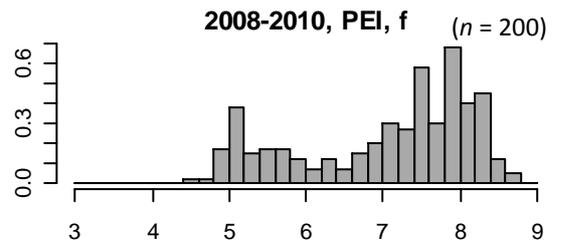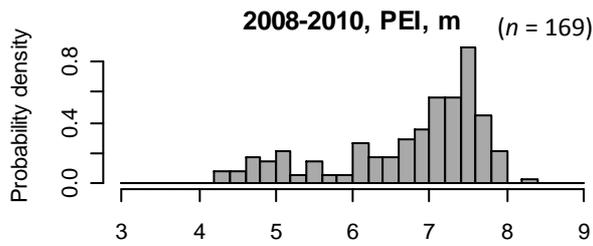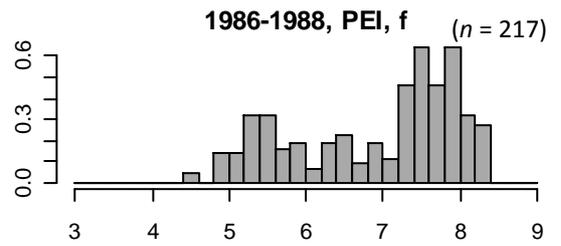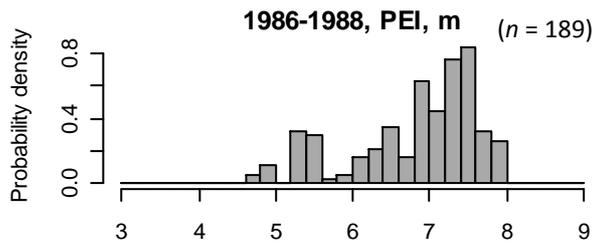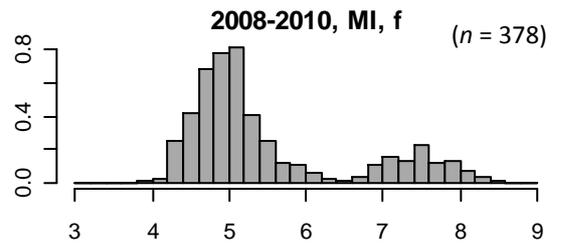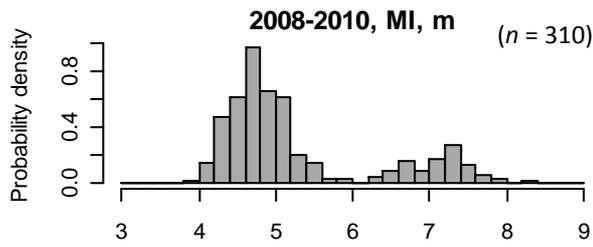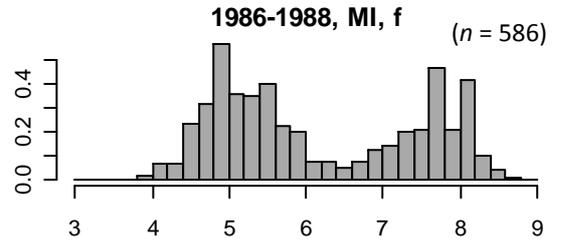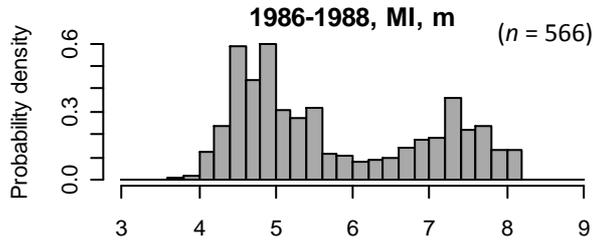
Body length (mm)

Body length (mm)

133
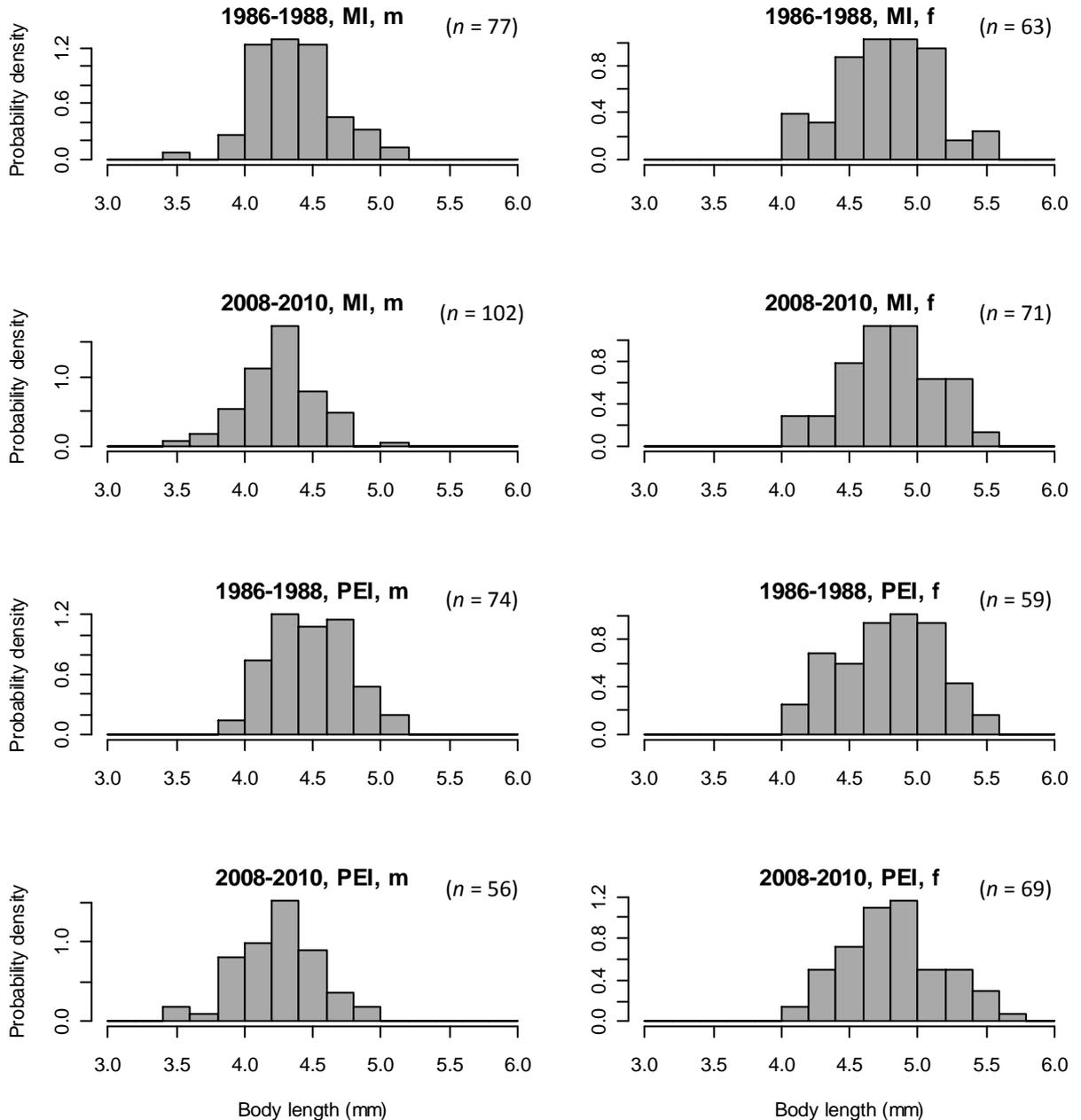
B. *P. eatoni*

Figure 3. Body size frequency distributions for (A) a preferred prey species, *E. marioni* (on *A. selago*), and (B) a non-preferred species, *P. eatoni*, for the year groups 1986-1988 and 2008-2010 (MI = Marion Island, PEI = Prince Edward Island, m = males, f = females). The histograms have been normalised to display relative frequencies (i.e. proportions or probability densities).

Discussion

The results from the present study indicate that environmental changes are causing a morphological response in weevils on the Prince Edward Islands. Most significantly, the body size of all weevil species on PEI show a decline with increasing mean annual temperature. Unlike the situation on PEI however, a negative relationship between mean annual temperature and body size was found only for *P. eatoni* on MI. This supralittoral species occurs on seaward-facing rock faces in the splash zone and into the intertidal (Chown 1989; Chown & van Drimmelen 1992), habitats that are not used by mice given their substantial exposure to wave action (Gleeson & van Rensburg 1982; Matthewson *et al.* 1994). Thus, no evidence has been found that mice consume *P. eatoni*. In all of the other species, size either showed no relationship or a positive relationship with temperature. Given that no difference among islands was found for *P. eatoni*, but a marked difference among the islands in the other species, it seems likely that the among-island variation is a consequence not of some intrinsic physical characteristic of the islands, but some other difference among them. The most likely explanation is the presence of invasive alien house mice on MI, but not on PEI, given that mice not only feed on weevils, but appear to have switched their diets over time to preferential selection of these animals (Gleeson & van Rensburg 1982; Crafford & Scholtz 1987; Chown & Smith 1993; Smith *et al.* 2002). Nevertheless, the potential mechanism underlying the positive relationships between body size and mean annual temperature on MI necessitates further discussion.

One explanation is that metabolic demands of endotherms can be influenced by temperature. Given that large individuals of all of the weevil species tend to be less common, any increase in predation coupled with a preference for larger sizes, which the mice clearly show (Chown & Smith 1993), would lead to a decline in the mean size of the species. If colder years impose a higher metabolic demand on mice than warmer ones, higher feeding rates should be required to sustain basal and field metabolic rates. This should lead to greater predation and a decrease in size of the weevils, especially if the size variation is largely phenotypic, rather than with a pronounced genotypic underpinning. A largely phenotypic response seems likely for the weevils given their year-long life cycle (Chown & Scholtz 1989; Chown 1990; Barendse & Chown 2000), and much evidence from other studies that responses tend to be largely phenotypic (Teplitsky *et al.* 2008). Whether

body size is a heritable trait in these weevils, as has been found for other insects (e.g. Bitner-Mathé & Klaczko 1999), is also not clear. Moreover, it is clear from studies of mice from Porto Santo, Madeira, from which the Marion Island population likely originated (Jansen van Vuuren & Chown 2007), that their thermoneutral zone's lower limit is ~24°C (Mathias *et al.* 2004). Basal metabolic rates and the lower limit to the thermoneutral zone are also almost identical to House Mice investigated over a very wide range of temperatures by Oufara *et al.* (1987). Indeed, it is widely appreciated that mice show limited ability to alter their thermoneutral zone limits (van Aarde & Jackson 2007). At temperatures below the thermoneutral zone, basal metabolic rates rise rapidly, and between 10°C and 5°C the rate increases from *c*. 23 to 28 mW.g^{-1} (Oufara *et al.* 1987). Thus, metabolic demand would certainly have increased between the lower and higher temperatures experienced by mice over the period of our study, and perhaps more so, given much lower microhabitat temperatures (see Deere *et al.* 2006) than those obtained from the Stevenson Screen values which were the only long-term data available to us. Field metabolic rates have been calculated specifically for the mice on MI (Rowe-Rowe *et al.* 1989), and using these data and the temperature-related variation in basal metabolic rate provided by Mathias *et al.* (2004) it can be estimated that the energy demand by mice between cold and warm years might change by 0.40 kJ.g^{-1}.day^{-1}. Given that the average energy value for a weevil is 5.3 kJ.g^{-1} (wet mass) (Rowe-Rowe *et al.* 1989) and the average wet mass of a weevil is 0.013 ± 0.002 g (Chown & Scholtz 1989; Chown 1993a), that would amount to an additional 0.5 weevils per day per mouse (as weevils (larvae & adults) make up about 8.5% volume to gut contents of mice (Smith *et al.* 2002)). Assuming mouse densities of 86 mice.ha^{-1} (Avenant & Smith 2004) that might amount to an extra predation pressure of 43 weevils per hectare per day. If the surface area on MI below 400 m is considered (Meiklejohn & Smith 2008), this would amount to 3.E+08 extra weevils per year. These estimates are liberal given that an accurate energy budget model for mice would also take into account opportunities for thermoregulation and daily temperature variation (see e.g. Porter 1989). High predation levels could easily mean that larger individuals would be removed rapidly from the population, resulting in the interannual variation in body size on MI and the complete contrast with PEI. Other biological differences occur between the islands, largely to do with the presence and absence of invasive species (Chown & Froneman 2008). However, the consistency of the results among

weevils from very different habitats, all of which are affected by mice, and the similarity of the trends among islands in *P. eatoni*, in a habitat not affected by mice, lends credence to the interpretation that mice are ultimately responsible for the among island differences.

The effects of predators on the body size of indigenous species has been documented in both aquatic (e.g. Brooks & Dodson 1965; Galbraith 1967; Reznick *et al.* 1997; Cousyn *et al.* 2001; Fisk *et al.* 2007) and terrestrial (e.g. Phillips & Shine 2004; Phillips *et al.* 2009) systems. Typically, the large individuals are lost from an assemblage, the consequences of which are similar to those of overexploitation where the loss of larger organisms of a species causes the 'erosion of large areas of phenotypic space' (Chown & Gaston 2008, pp. 1473). By losing the larger individuals, species may not be as well adapted to cope with changing environmental conditions over the short- and long-term (Chown & Gaston 2008). Larger individuals also tend to have greater survival, fecundity and mating success (Kingsolver 2009). Furthermore, losing the larger individuals within a population of a species can lead to trophic restructuring of a system (Chown & Gaston 2008), as large and small individuals play different functional roles in food webs (Cohen *et al.* 2005).

The decline in weevil body size with increasing temperature on PEI strengthens Daufresne *et al.*'s (2009) suggestion that reduced body size is one of the most universal ecological responses to warming, and this study forms one of very few that have addressed this for ectotherms. The changes recorded on PEI thus provide support for what has become known as the temperature-size rule (TSR), that is animals reaching larger sizes at lower developmental temperatures (see Atkinson 1994; Angiletta 2009). That weevil body size is influenced by temperature is also evident from increases in this trait with altitude (particularly on MI above *c.* 600 m) (Appendix B) and that temperature decreases with altitude on the islands (reviewed in le Roux 2008). Such increases in body size with altitude have previously been recorded for MI weevils (Chown & Klok 2003). While a number of studies have reported correlative evidence for a decline of body size in response to warming (e.g. Smith & Betancourt 2006; Gienapp *et al.* 2008; Teplitsky *et al.* 2008; Gardner *et al.* 2009), the causal nature of the link between the TSR and the response in terms of body size changes is not clear (Angilletta *et al.* 2004). Moreover, contention exists as to whether the changes are phenotypic or genetic (see review in Gienapp *et al.* 2008). Distinguishing between plasticity and adaptation is difficult and direct evidence for genetic responses to warming are rare, although there is strong evidence in some insects (Gienapp

et al. 2008). Body size responses of the weevils on the Prince Edward Islands are likely to be phenotypic rather than adaptive (see above). These weevils do display phenotypic plasticity in other traits, including locomotion performance (see Chapter 5 of this thesis) and critical thermal minima (Klok & Chown 2003). Nevertheless, regardless of whether the body size changes are genetically based or not, the life history consequences are potentially profound. As body size is a fundamental trait that scales with many ecological properties, such as metabolic rate, fecundity, population growth rate, competitive interactions, and longevity (Peters 1983; Calder 1984; Schmidt-Nielsen 1984; Gillooly *et al.* 2001), changes to body size could potentially have significant consequences for an animal's life history and ecology, as well as on its ability to cope with changing environmental conditions (Chown & Gaston 2008). Moreover, as it has long been known that metabolic rate scales allometrically in predictable ways with body size and temperature, and that metabolism provides the energy and materials link between organisms and the environment (Gillooly *et al.* 2001), changes to animal body size can potentially have severe consequences for ecosystem functioning. This is certainly likely to be the case on the Prince Edward Islands where the weevils are crucial to nutrient cycling (Chown & Scholtz 1989; Smith & Steenkamp 1992, 1993).

While the results provide support for the TSR, some might argue that decreases in body size may be plastic responses to changes in a range of other environmental factors, such as changes in competition or nutrition (Gardner *et al.* 2009; Yom-Tov & Geffen 2011). Competition is unlikely to be responsible for the intraspecific variation in body size over time displayed by the weevils. While it has been suggested that interspecific competition may be important in structuring some weevil assemblages on the Prince Edward Islands (Chown 1992; Chown 1993b), intraspecific competition has not been reported. Nutrition is a significant factor controlling insect body size, with both diet quality and quantity affecting growth rate (Chown & Gaston 2010). As Chown (1990) noted diet-associated differences in the body size of weevils on the Prince Edward Islands (also see Chown 1989; Chown & Scholtz 1989), it could be argued that nutrition might be responsible for the observed weevil body size changes and, in particular, for the differences in these changes between the two islands. However, this is unlikely to be the case. Extensive impacts due to the Domestic Cat (*Felis catus* L.) on MI, removed from the island by 1991 (Bester *et al.* 2000), included the removal of thousands of seabirds (van Aarde 1980), the populations of which

are slowly recovering (Ryan & Bester 2008). A consequence of this was a marked reduction in nutrient inputs to the island (Burger *et al.* 1978). It could therefore be assumed that a poorer nutrient content on MI could lead to reduced food quality, with potential consequences to the body size of organisms on that island. However, evidence against this and further support for temperature effects is that the body sizes of weevils were observed to increase with altitude (see above). Not only does vegetation biomass decrease with altitude (Gremmen & Smith 2008), but nutrient input decreases with distance from the coast (Smith 2008). At the higher elevations, the polar desert habitat complex is nutrient poor, dominated by lichens and mosses (Gremmen & Smith 2008) on which the weevils found at these altitudes feed (Chown 1989). Thus, were the nutrition argument true, high altitude weevils would be expected to be smaller in size than individuals at the coast. This is clearly not the case (Appendix B). The opposite is true on Heard Island, where the body size of weevils has been observed to decrease with altitude, an effect that is ascribed to the shorter growing season on this island compared to the Prince Edward Island group (Chown & Klok 2001). Weevils have also been reported as being important prey items for the endemic Lesser Sheathbill (Huyser *et al.* 2000). However, Sheathbills are found on both PEI and MI and predation by them is therefore unlikely to account for the differences observed in weevil body size between the two islands.

The temperature-related size effects are also in keeping with other studies (e.g. Sweeney *et al.* 1986; Hogg & Williams 1996). In summary, the body size changes of the weevils on the Prince Edward Islands indicate a strong effect of both warming and biological invasions, although the form of the interaction among these impacts on MI is complex. These findings emphasise the importance of considering synergistic impacts among environmental change drivers (Brook *et al.* 2008), particularly in the sub-Antarctic where such interactions are predicted to significantly influence terrestrial systems (Chown & Convey 2007) as work on MI has already indicated (Chown *et al.* 2007; Janion *et al.* 2010). As the mouse population is increasing on MI probably due to warming (Smith 2002), this study concurs with several suggestions that synergistic impacts between climate change and invasions will likely increase the impacts of invasive species (e.g. Dukes & Mooney 1999; Theoharides & Dukes 2007; Brook 2008; Brook *et al.* 2008). The results also highlight the importance of long-term records for understanding organism responses to environmental changes.

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Appendices

Appendix A. Descriptive statistics for the body size of each weevil species on Marion Island (MI) and Prince Edward Island (PEI) for all year groups, showing sexes separately.

Year	Island	Sex	n	Mean	Median	s.e.
P. eatoni						
1986-1988	MI	male	77	4.373	4.340	0.035
1992	MI	male	24	4.279	4.275	0.063
1999-2003	MI	male	105	4.135	4.150	0.030
2008-2010	MI	male	102	4.278	4.300	0.026
1986-1988	PEI	male	74	4.498	4.475	0.032
1992	PEI	male	19	4.324	4.310	0.080
1999-2003	PEI	male	27	4.057	4.080	0.076
2008-2010	PEI	male	56	4.278	4.265	0.041
1986-1988	MI	female	63	4.776	4.750	0.044
1992	MI	female	23	4.665	4.650	0.071
1999-2003	MI	female	82	4.686	4.690	0.043
2008-2010	MI	female	71	4.825	4.810	0.042
1986-1988	PEI	female	59	4.813	4.810	0.045
1992	PEI	female	12	4.772	4.845	0.074
1999-2003	PEI	female	26	4.367	4.310	0.092
2008-2010	PEI	female	69	4.850	4.850	0.044
B. elongatus						
1986-1988	MI	male	31	2.974	2.940	0.050
1992	MI	male	-	-	-	-
1999-2003	MI	male	202	3.004	3.000	0.019
2008-2010	MI	male	450	2.980	2.940	0.014
1986-1988	PEI	male	60	2.961	2.960	0.018
1992	PEI	male	-	-	-	-
1999-2003	PEI	male	31	2.767	2.770	0.035
2008-2010	PEI	male	140	2.895	2.910	0.015
1986-1988	MI	female	30	3.274	3.240	0.063
1992	MI	female	-	-	-	-
1999-2003	MI	female	183	3.232	3.230	0.025
2008-2010	MI	female	363	3.260	3.240	0.017
1986-1988	PEI	female	51	3.164	3.160	0.031
1992	PEI	female	-	-	-	-
1999-2003	PEI	female	39	2.953	2.850	0.036
2008-2010	PEI	female	105	3.170	3.190	0.019

Appendix A continued

Year	Island	Sex	*n*	Mean	Median	s.e.
B. parvulus (sensu lato)						
1986-1988	MI	male	32	3.854	3.810	0.064
1992	MI	male	28	3.511	3.550	0.041
1999-2003	MI	male	276	3.940	3.920	0.028
2008-2010	MI	male	351	3.931	3.880	0.026
1986-1988	PEI	male	109	3.998	4.000	0.031
1992	PEI	male	29	3.713	3.750	0.050
1999-2003	PEI	male	72	3.737	3.770	0.041
2008-2010	PEI	male	138	4.017	4.000	0.029
1986-1988	MI	female	49	4.109	4.100	0.049
1992	MI	female	29	3.869	3.850	0.058
1999-2003	MI	female	293	4.271	4.310	0.030
2008-2010	MI	female	335	4.237	4.200	0.030
1986-1988	PEI	female	110	4.349	4.275	0.037
1992	PEI	female	11	4.195	4.130	0.087
1999-2003	PEI	female	54	4.084	4.080	0.041
2008-2010	PEI	female	125	4.339	4.350	0.033
B. randi						
1986-1988	MI	male	59	6.159	6.070	0.053
1992	MI	male	40	6.009	5.900	0.115
1999-2003	MI	male	135	6.169	6.150	0.034
2008-2010	MI	male	57	5.956	6.060	0.055
1986-1988	PEI	male	84	6.435	6.435	0.036
1992	PEI	male	28	6.544	6.580	0.063
1999-2003	PEI	male	43	6.267	6.230	0.062
2008-2010	PEI	male	65	6.408	6.400	0.040
1986-1988	MI	female	66	7.080	7.010	0.073
1992	MI	female	21	6.659	6.580	0.106
1999-2003	MI	female	115	7.198	7.310	0.066
2008-2010	MI	female	48	7.056	7.120	0.078
1986-1988	PEI	female	25	7.595	7.580	0.095
1992	PEI	female	6	7.180	7.165	0.275
1999-2003	PEI	female	25	7.118	7.080	0.132
2008-2010	PEI	female	60	7.381	7.320	0.067
E. marioni						
1986-1988	MI	male	1244	5.645	5.410	0.028
1992	MI	male	152	5.444	5.040	0.086
1999-2003	MI	male	563	5.486	5.230	0.044

Appendix A continued

Year	Island	Sex	*n*	Mean	Median	s.e.
2008-2010	MI	male	464	5.364	5.050	0.044
1986-1988	PEI	male	269	6.609	6.670	0.050
1992	PEI	male	158	5.916	6.170	0.098
1999-2003	PEI	male	135	6.044	6.380	0.090
2008-2010	PEI	male	312	6.740	7.040	0.053
1986-1988	MI	female	1272	5.969	5.660	0.030
1992	MI	female	188	5.798	5.500	0.086
1999-2003	MI	female	648	5.899	5.540	0.048
2008-2010	MI	female	540	5.596	5.250	0.043
1986-1988	PEI	female	339	6.664	6.580	0.053
1992	PEI	female	167	6.271	6.580	0.098
1999-2003	PEI	female	166	6.561	6.920	0.093
2008-2010	PEI	female	323	7.011	7.360	0.062

s.e. = standard error

Appendix B. Body size changes with altitude for males and females on Marion Island (MI) and Prince Edward Island (PEI) for (A) *B. elongatus* and (B) *B. parvulus (sensu lato).*

A. *B. elongatus*
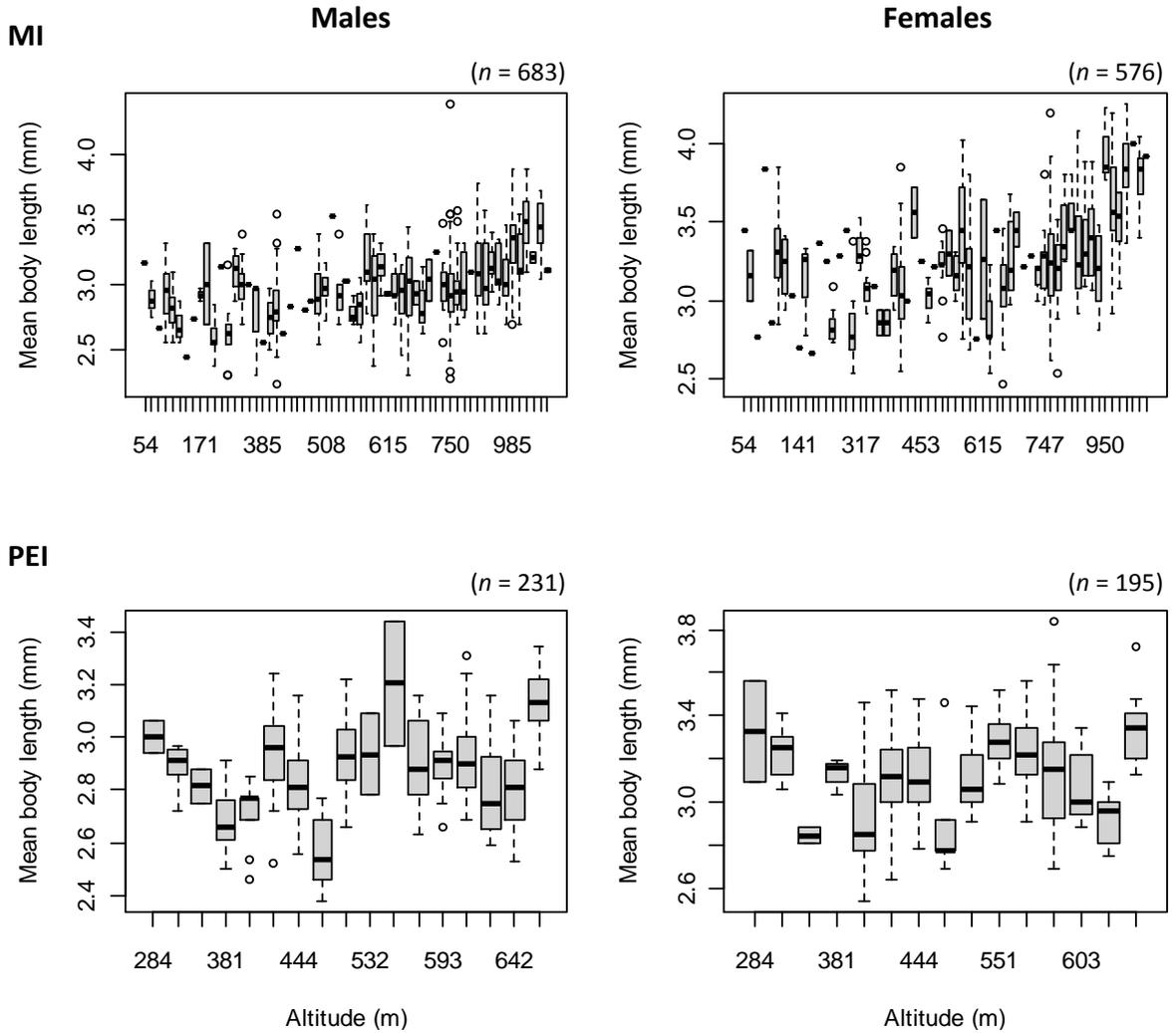
B. *B. parvulus (sensu lato)*
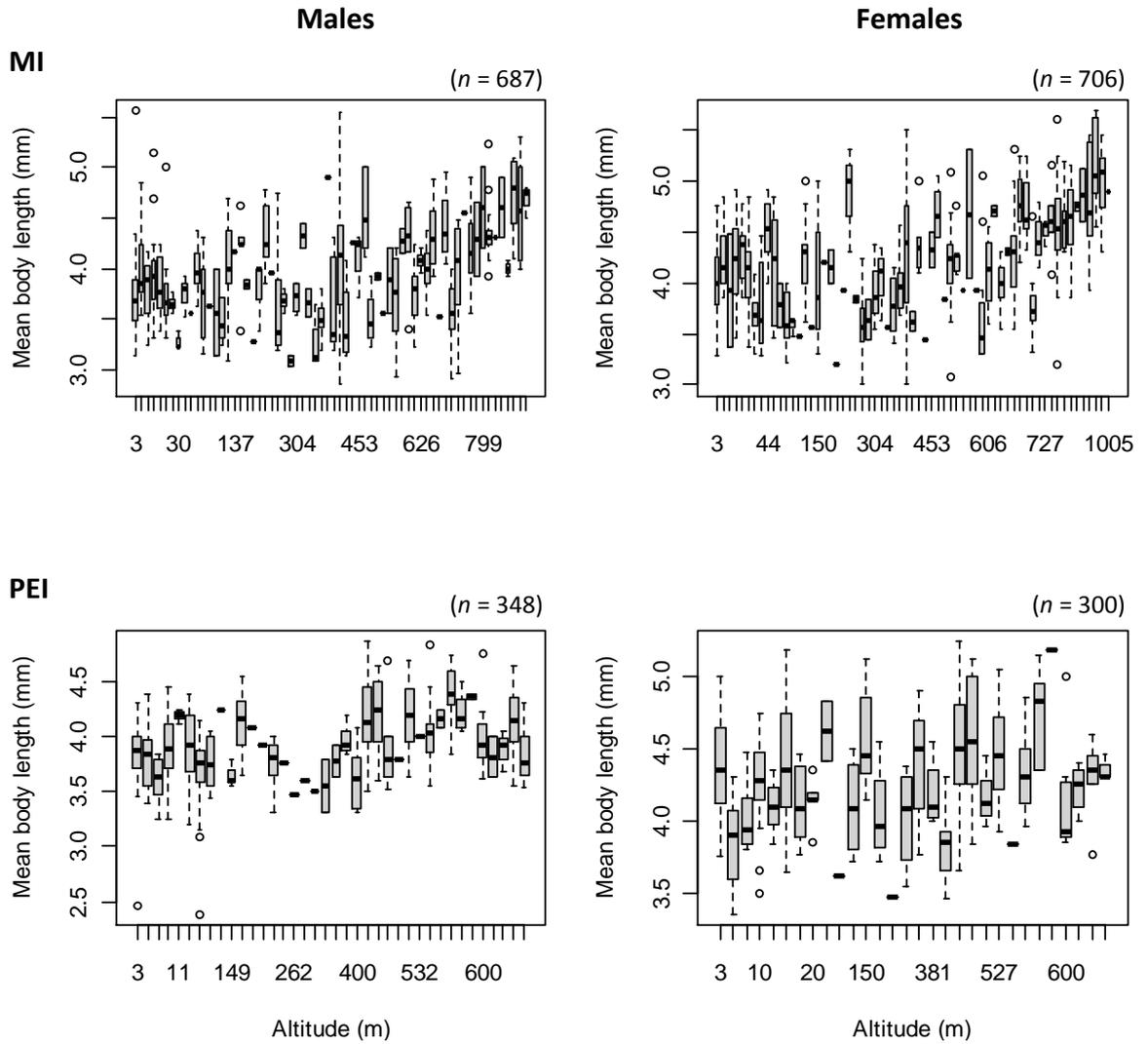
Chapter 5 – Phenotypic plasticity and thermoregulatory behaviour

of a monophyletic group of weevils: a test of the hypotheses[1]

Ectemnorhinus marioni (Jeannel 1940), indigenous to the Prince Edward

Island group

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Introduction

It is well known that the thermal environment experienced by organisms has a direct effect on survival, growth and reproduction (Chown & Nicolson 2004; Angilletta 2009). This is especially significant for ectothermic animals as environmental temperature directly affects body temperature and therefore physiological processes (Huey & Stevenson 1979; Huey & Berrigan 1996; Huey & Kingsolver 1989). These relationships between organisms and their thermal environments have long fascinated physiologists, an interest which has increased in recent years due to rapidly changing modern climates and the potential responses of organisms to these changes (Sinclair *et al.* 2003). One way organisms may respond to variable environmental conditions is by altering their phenotypes (Kingsolver & Huey 1998), a response that can take place over a range of time scales (Chown & Terblanche 2007). Termed phenotypic plasticity, this flexibility in the expression of different phenotypes depending on environmental conditions ultimately alters an organism's fitness (Kingsolver & Huey 1998). Thus such phenotypic change has long been seen as beneficial (Deere & Chown 2006), a view formally known as the beneficial acclimation hypothesis (BAH), defined by Leroi *et al.* (1994) as 'acclimation to a particular environment gives an organism a performance advantage in that environment over another organism that has not had the opportunity to acclimate to that particular environment'.

However, researchers of optimal models of acclimation have largely abandoned the BAH as being too naïve, largely due to the assumption made that acclimation imposes no cost to an organism (Angiletta 2009). Not surprisingly, support for the BAH is mixed (see Huey *et al.* 1999; Angiletta 2009 for reviews) and some empirical studies have found poor evidence for the hypothesis (e.g. Leroi *et al.* 1994; Bennet & Lenski 1997; Gilchrist & Huey 2001; Deere & Chown 2006; but see Marais & Chown 2008). In addition, some studies into the BAH have been criticised for not considering changing environmental conditions (Deere & Chown 2006). In fact, many studies that have included investigations of alternatives have not been able to reject one or more of them (Chown & Terblanche 2007). Therefore, explicit tests for alternative hypotheses, such as optimal temperatures or deleterious acclimation, should always be undertaken (Huey & Berrigan 1996; Huey *et al.* 1999; Wilson & Franklin 2002).

An acclimation response that has recently been receiving renewed interest is whether warmer conditions enhance performance, i.e. is hotter better (Huey & Kingsolver 1989; Angilletta *et al.* 2010). This hypothesis, which predicts that organisms acclimated to a high temperature environment will always have a performance advantage over another organism held at a lower temperature (Deere & Chown 2006), has received strong support (Angilletta *et al.* 2010), particularly for insects (e.g. Frazier *et al.* 2006). However, while many studies find that hotter is better, the environmental context must be considered, particularly if that environment is variable. For example, if an environment is highly variable, it is possible that phenotypic plasticity would not be able to keep up with the change. Acclimation would not be expected in such circumstances, as a lack of response to acclimation indicates no plasticity (Deere & Chown 2006). Moreover, recent work has shown that in environments that are unpredictably variable, organisms are less likely to respond to changing environments via acclimation (i.e. show less phenotypic plasticity) than organisms in environments with predictable variation (Deere & Chown 2006; Deere *et al.* 2006).

Another way that animals can respond to variation in environmental temperature is through behaviour (Huey & Bennett 1987; Angilletta *et al.* 2002a; Angilletta *et al.* 2002b). Such thermoregulatory behaviour appears to be governed by distinct thermal preferences (Angilletta *et al.* 2002a). Indeed, an assumption often made is that the optimum temperature of animals is where they prefer to be, in other words that the preferred body temperature of an organism should match the thermal optimum for performance (Angilletta *et al.* 2006). Physiological ecologists have long assumed that thermal preferences coincide with temperatures that maximise Darwinian fitness (Martin & Huey 2008) and many studies document thermal preferences that match or are close to temperatures that maximise performance (e.g. Angilletta *et al.* 2002b). The reason behind this concordance is thought to be that if fitness is positively related to performance, by selecting body temperatures that optimise performance, animals should enjoy greater fitness than those that select other body temperatures (Angilletta *et al.* 2002a). Over many generations, this should lead to coadaptation of thermoregulatory behaviour and thermal physiology and the coadaptation hypothesis predicts that an animal's preferred body temperature will correspond to its optimum temperature of performance (Bauwens *et al.* 1995; Angilletta *et al.* 2002a; Angilletta 2009).

However, the coadaptation hypothesis has been challenged and mismatches between thermal preference and thermal optima can occur (Asbury & Angilletta 2010). For example, a model developed by Martin & Huey (2008) predicted that 'suboptimal is optimal', i.e. animals should select temperatures that are lower than the temperature at which performance (or fitness) is maximal. Further evidence against coadaptation was provided by Huey & Bennett (1987) and Garland *et al.* (1991); however to date such studies have involved a limited range of taxa.

Therefore, in this study, I examine the above hypotheses on a monophyletic group of five weevil species on a sub-Antarctic island. Weevils are used as the exemplar group due to their key importance as primary consumers in the island's relatively simple food webs (Chown 1989; Crafford & Chown 1991; Smith & Steenkamp 1992). The climate on the island is changing rapidly and thus determinations of the physiological responses of key organisms to the environment are paramount. Specifically, I first explore the extent to which variation in locomotion performance and thermal preference among species and populations can be ascribed to acclimation ability. In particular, acclimation effects on preferred temperature (T_{pref}) and on the responses of three key traits of thermal performance curves: optimum speed ($\approx$ maximum speed, U_{max}), temperature of the optimum speed (optimum temperature, T_{opt}) and performance breadth (T_{br}) (Huey & Stevenson 1979; Gilchrist 1996), are examined. To do this, I adopt a strong inference approach and use ordered factorial ANOVAs with orthogonal polynomial contrasts (Huey *et al.* 1999). Competing hypotheses can be tested by examining the form and sign of the linear and quadratic effects (see Deere & Chown 2006 for a rationale). Next, I explore the extent to which the preferred temperatures of the organisms match the thermal optima for performance.

Materials and methods

Study area and species

Sub-Antarctic Marion Island (46°54'S, 37°45'E) is part of the Prince Edward Island group, which lies approximately 2300 km southeast of Cape Town, South Africa. The island is highly oceanic with a mean annual coastal temperature of *c*. 6.5°C, total annual precipitation of *c*. 1900 mm (as measured at the meteorological station on the north

eastern coast), generally high humidity, and strong winds. The climate has shown substantial change over the last 50 years, including an increase in mean annual temperature of more than 1°C and a decline in precipitation of more than 500 mm per annum (Smith 2002; le Roux & McGeoch 2008). The island has two major biomes: tundra, which predominates in lowland areas, and polar desert, restricted to high elevations (Smith & Mucina 2006; Gremmen & Smith 2008). Species richness of plants and invertebrates is low and no indigenous terrestrial vertebrates are found except for the Lesser Sheathbill (*Chionis minor marionensis* Reichenow) (see Chown & Froneman 2008 for an overview). House Mice (*Mus musculus* L.) were accidentally introduced in the 1800s. Six indigenous weevil species (Coleoptera: Curculionidae) of the *Ectemnorhinus* group of genera occur on Marion Island, of which three are restricted to the coast with the remainder occupying most of the altitudinal range of the island (Table 1) (see Chown 1989 for detailed information on diet and habitat distribution; Grobler *et al.* 2011). Two of these are cryptic species (*Bothrometopus parvulus* and the recently discovered *Bothrometopus huntleyi* (Grobler *et al.* 2011)). *B. huntleyi* is the more common of the two and while very few individuals were identified post-experimentation as *B. parvulus*, this was considered of no consequence to the results due to the considerable similarities between the two species (Grobler *et al.* 2011). The *Ectemnorhinus* group is a monophyletic unit of weevils that is restricted to South Indian Ocean Province Islands in the Southern Ocean, on which they constitute an important part of the terrestrial arthropod fauna (Kuschel & Chown 1995; Grobler *et al.* 2011).

Collection

Weevils of each species were collected by hand from the locations and altitudes shown in Table 1 during September to November 2008 and April to May 2010 for the performance and thermal preference trials respectively. Individuals were kept at low density in 100 ml plastic jars with moist plaster of Paris substrates and small pieces of their preferred food of either algae, bryophytes, lichens, or *Azorella selago* Hook. f. (Apiaceae), depending on the species (Chown 1989), and returned to the laboratory within two hours. In the laboratory, individuals were sorted for acclimation treatments, each being placed in an individual 65 ml

Table 1. The indigenous weevil species on Marion Island and their altitudinal ranges (see Chapter 4 of this thesis), showing collection locations, altitudes, acclimation treatments and sample sizes for locomotion performance and thermal preference experiments.

Species	Altitudinal range (m)	Collection locations	Collection altitude (m)	Acclimation treatments (°C) (*n* in brackets)
Palirhoeus eatoni	3 - 10	Trypot; Archway	4-6	0 (10); 0-15 (10); 15 (10)
Bothrometopus randi	3 - 36	Trypot; Archway	4-6	0 (10); 0-15 (10); 15 (10)
Bothrometopus huntleyi	3 - 1005	Trypot	4-6	0 (10); 0-15 (10); 15 (10)
		Tafelberg	400	0 (10); 0-15 (10); 15 (10)
		Katedraal	750	0 (10); 0-15 (10); 15 (10)
Bothrometopus elongatus	54 - 1261	Tafelberg	400	0 (10); 0-15 (10); 15 (10)
		Katedraal	750	0 (10); 0-15 (10); 15 (10)
Ectemnorhinus marioni - small black[1]	3 - 1026	Tafelberg	400	0 (10); 0-15 (10); 15 (10)
Ectemnorhinus marioni - large brown[1]	3 - 1026	Tafelberg	400	0 (10); 0-15 (10); 15 (10)

[1] The species *E. marioni* has two distinct size morphs, both of which were included in this study separately.

plastic container with moist plaster of Paris and preferred food as above. The animals were kept in climate chambers (LABCON, Johannesburg, South Africa, accurate to ± 1°C) at the temperatures shown in Table 1 with a photoperiod set to match summer environmental day light cycles (12 L : 12 D). The acclimation temperatures were based on microclimate temperatures (see Deere & Chown 2006 for a rationale). Temperature within the climate chambers was measured with I-button Thermochron dataloggers (Models DS 1921G and DS 1922H; accurate to ± 0.5°C, Dallas Semiconductors, Dallas, TX, USA). A period of seven days was selected for acclimation following Hoffmann & Watson (1993). Previous studies on arthropods on both Marion (e.g. Klok & Chown 2003; Terblanche *et al.* 2007; Marais & Chown 2008) and elsewhere (e.g. Terblanche *et al.* 2006) suggest this is sufficient time for phenotypic responses to temperature that could alter performance (Angilletta 2009) to equilibrate (Terblanche *et al.* 2007). Shelf effects in the climate chamber were unlikely to

have had any influence on the temperature as the containers took up little space on a single shelf.

Microclimate temperatures have been logged on an hourly basis at *c.* 100 m intervals from sea level to 750 m (including the collection sites of the present study) across the eastern slope of Marion Island since 2002 using I-button Thermochron dataloggers (Deere *et al.* 2006). Data from 2002 to 2009 were processed in R2.12.0 (R Development Core Team 2010) to obtain mean daily minimum, maximum, mean and range for the three collection sites.

Locomotion performance

A temperature controlled walking stage was attached to a Grant LTC 12 water bath which was set to regulate stage temperature. Stage temperature was monitored at each end using Type T 20-gauge thermocouples connected to CHY 507 Digital Thermometers (CHY Firemate Co, Taiwan). A single weevil was introduced to the stage at a time using tweezers. Weevils were given two minutes to equilibrate to the set temperature by placing a small round metal lid (radius 2 cm x height 0.5 cm) over them. The path that the individuals then ran in 10 seconds was traced using a non-permanent marker and this line was measured using string. The fastest speed from three repetitions was used for each individual (for rationale, see Garland & Losos 1994; Angilletta *et al.* 2002b) at eight temperatures (in this order: 2°C, 7°C, 14°C, 21°C, 24°C, 28°C, 32°C and 35°C). Individuals were returned to their acclimation treatments in the climate chambers in-between temperature runs for at least 1-2 hours. Locomotion data were used to construct performance curves, of which the key traits are optimum speed (≈ maximum speed, U_{max}), temperature of the optimum speed (optimum temperature, T_{opt}) and performance breadth (the index of the breadth of the curve, T_{br}) (Huey & Stevenson 1979; Gilchrist 1996). Values of U_{max} and T_{opt} were obtained from the experimental data and were then used to calculate performance breadth (T_{br}) using Gilchrist's (1996) formula:

$$T_{br} = \sqrt{\sum \left[\frac{u_i (T_i - T_{opt})}{u_{max}} \right]^2}$$

(1)

where T_{opt} is the temperature at which an individual ran the maximum speed, U_{max} is the maximum speed at T_{opt}, and U_i is the speed at T_i.

Thermal preference

A temperature gradient was generated along an aluminium floor of a perspex arena of 76 cm x 30 cm, which was longitudinally split into ten channels of 2.5 cm x 2.0 cm x 76 cm each by perspex divisions. The floor of the arena was covered with rough paper to provide a non-slippery surface on which the weevils could walk. The paper was replaced after each trial. The arena also had a perspex cover to prevent the weevils from escaping and to maintain a stable temperature gradient without exchange with external air currents. A Peltier module attached to one end of the aluminium floor was used for heating and the other end was inserted into a cold Grant LTC 12 water bath in order to generate a temperature gradient ranging from about -7°C to ~32°C. These temperatures were chosen to incorporate the range of performance temperatures found in locomotion trials. Thermal preference data was collected by placing one weevil per channel (i.e. ten weevils at a time) into the arena, giving three hours of settling time with no light, and determining the temperature preference of each individual using temperatures of the gradient as measured by Type T 36-gauge thermocouples and logged every 10 min by an eight channel SQ800 Squirrel data logger.

Data analysis

A performance curve was constructed for each individual on the basis of the fastest speed recorded at each temperature (Angilletta 2006). Ordered factorial ANOVAs with orthogonal polynomial contrasts (Huey *et al.* 1999) were used to test the acclimation hypotheses and implemented in R2.12.0. To adhere to the strong inference approach and the necessity of examining covariation of the three aspects of thermal performance curves, predictions were made for the form and sign of the linear and quadratic effects for T_{opt}, U_{max} and T_{br} for each of the hypotheses (Table 2). The ordered-factor approach requires strict adherence to the assumptions of ANOVA, in particular normally distributed residuals, a balanced design and homogeneity of variance (Huey *et al.* 1999). Therefore, normality of the data was checked with the Shapiro-Wilks test using the shapiro.test function in R2.12.0. Some

instances of non-normality were found; however these were generally not significant after tabulated data were subjected to sequential Bonferroni correction using the p.adjust function in R2.12.0.

Table 2. Predictions of the form and sign of the linear and quadratic effects of acclimation temperature on optimum temperature (T_{opt}), maximum speed at the optimum temperature (U_{max}) and performance breadth (T_{br}) for each of the major acclimation hypotheses (adapted from Deere & Chown 2006).

Hypothesis	U_{max}	T_{opt}	T_{br}
Beneficial acclimation (BAH)	L^+	L^+	
Colder is better (CIB)	L^-	n.s.	L^-
Hotter is better (HIB)	L^+	n.s.	L^+
Deleterious acclimation (DAH)	$L^{+/-}$, Q^-	$L^{+/-}$, Q^-	
No plasticity	n.s.	n.s.	n.s.

L^+ = positive linear, L^- = negative linear, Q^- = negative quadratic, and n.s. = not significant

Results

Acclimation was not significant for any of the key traits for the coastal species *P. eatoni* (Appendix A; Fig. 1). Conversely, acclimation effects on all three of the key traits for the larger coastal species, *B. randi*, were significant with individuals acclimated to 0°C showing much lower values than those acclimated to fluctuating or higher temperatures (Fig. 1; Table 3). In fact in the case of U_{max}, significant effects of acclimation were found for all species except *P. eatoni*, with individuals acclimated to 0°C showing much lower values than the other two acclimation treatments (Appendix A; Fig. 1; Table 3). Responses of T_{opt} to acclimation treatment varied between species. For some, coastal *B. huntleyi*, *B. randi*, *B. elongatus* at 400 m.a.s.l. and small *E. marioni*, T_{opt} was significantly affected by acclimation, while the other species and population groups showed no significant effects of acclimation

on this trait (Appendix A). Apart from *B. randi*, T_{br} also showed little variation associated with acclimation temperature (Appendix A).

A. *P. eatoni* (0 m.a.s.l.)
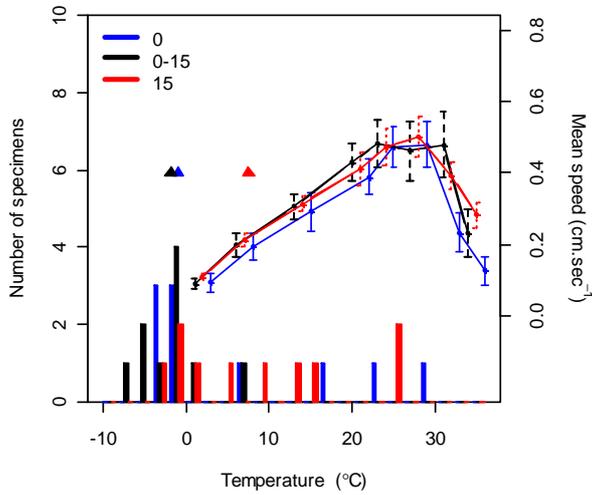
B. *B. randi* (0 m.a.s.l.)
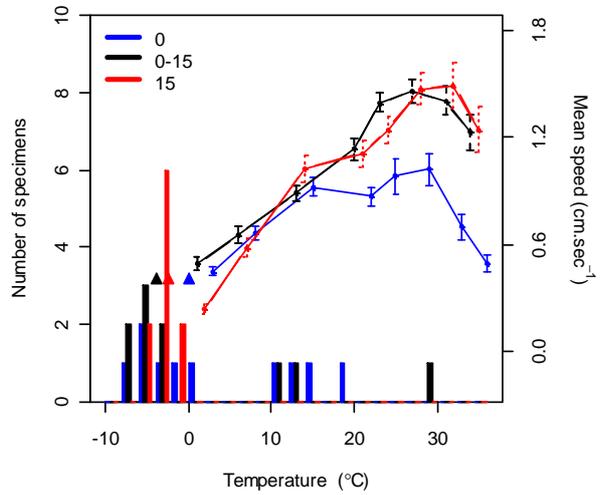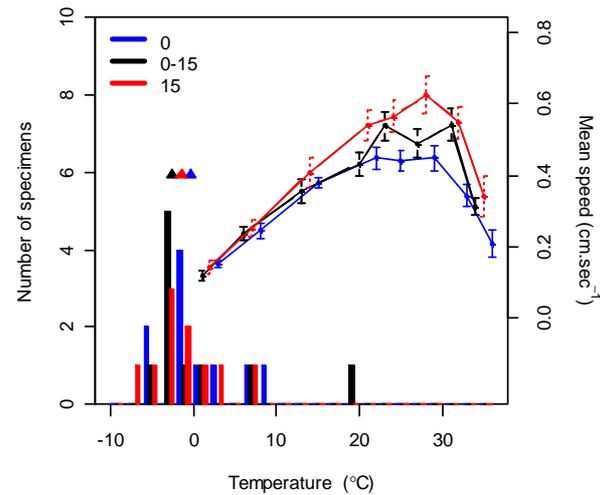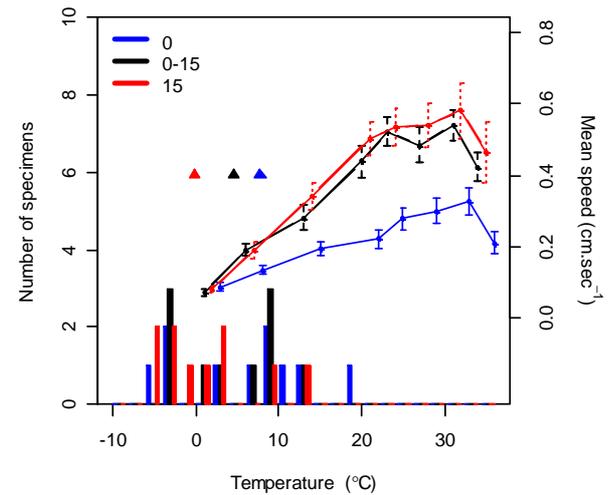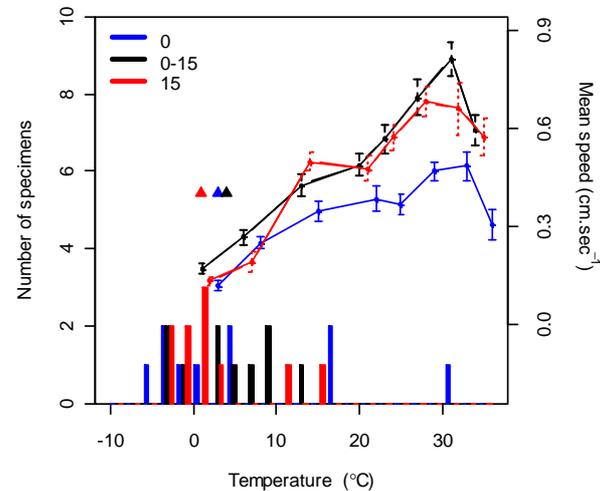
C. *B. huntleyi* (0 m.a.s.l.)

D. *B. huntleyi* (400 m.a.s.l.)

E. *B. huntleyi* (750 m.a.s.l.)

F. *B. elongatus* (400 m.a.s.l.)
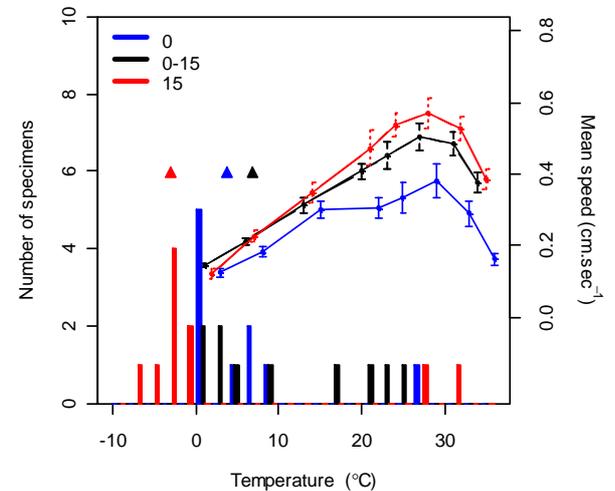
G. *B. elongatus* (750 m.a.s.l.)
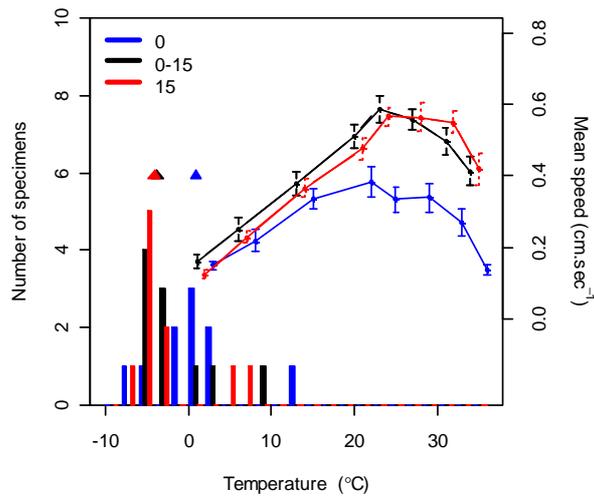
H. *E. marioni*, small (400 m.a.s.l.)
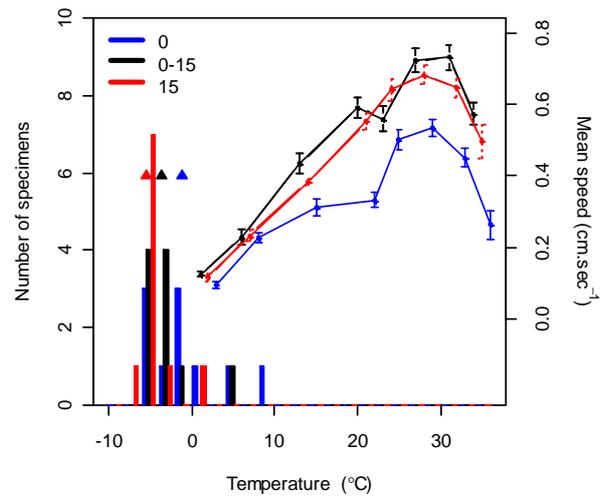
I. *E. marioni*, large (400 m.a.s.l.)
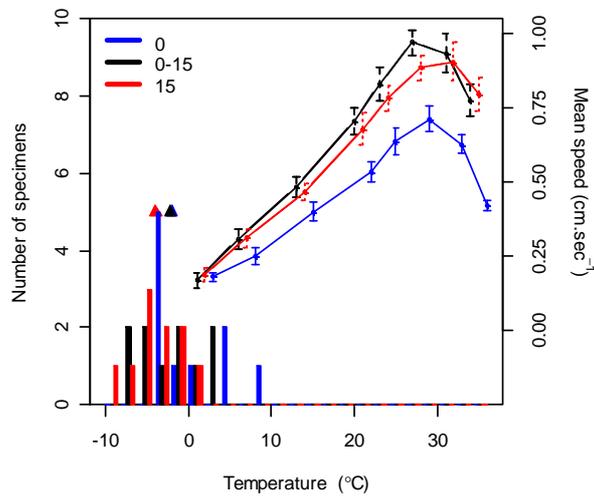
Figure 1. Results of locomotion performance and thermal preference experiments for nine populations of weevils. A = *P. eatoni* (0 m.a.s.l.), B = *B. randi* (0 m.a.s.l.), C = *B. huntleyi* (0 m.a.s.l.), D = *B. huntleyi* (400 m.a.s.l.), E = *B. huntleyi* (750 m.a.s.l.), F = *B. elongatus* (400 m.a.s.l.), G = *B. elongatus* (750 m.a.s.l.), H = *E. marioni*, small (400 m.a.s.l.), I = *E. marioni*, large (400 m.a.s.l.) for three acclimation treatments (0°C, 0°C - 15°C and 15°C). The line charts show the performance curves (as mean speed in cm.sec^{-1}) with standard errors indicated by the vertical lines. Bar graphs indicate thermal preference (as number of specimens). The triangles indicate median thermal preference values. (m.a.s.l. = metres above sea level).

Table 3. Summary statistics for the performance curve traits maximum rate of performance (U_{max}), optimum temperature (T_{opt}), and performance breadth (T_{br}), as well as thermal preference (T_{pref}) for each weevil species population.

Species (altitude m.a.s.l.)	Mean U_{max}	U_{max} (s.e.)	Mean T_{opt}	T_{opt} (s.e.)	Mean T_{br}	T_{br} (s.e.)	Mean T_{pref}	T_{pref} (s.e.)
0°C acclimation								
P. eatoni (4-6)	0.548	0.040	25.0	0.894	11.722	1.585	6.448	3.873
B. randi (4-6)	1.127	0.071	24.0	1.764	20.289	1.053	3.922	3.096
B. huntleyi (4-6)	0.479	0.034	22.3	1.654	18.648	1.022	0.962	1.450
B. huntleyi (400)	0.363	0.035	28.4	1.108	16.411	0.867	5.942	2.416
B. huntleyi (750)	0.547	0.032	27.8	2.037	18.130	0.816	6.533	3.683
B. elongatus (400)	0.416	0.039	24.1	1.841	17.137	1.177	6.106	2.556
B. elongatus (750)	0.425	0.038	23.1	2.008	18.894	1.759	1.048	1.731
E. marioni small (400)	0.571	0.020	27.2	0.800	14.353	0.746	-0.698	1.460
E. marioni large (400)	0.720	0.043	28.4	0.718	16.241	0.898	0.384	1.375
0°C - 15°C acclimation								
P. eatoni (4-6)	0.603	0.071	27.0	1.366	12.872	0.919	-1.708	1.199
B. randi (4-6)	1.576	0.049	29.1	1.140	18.913	1.175	1.759	3.775
B. huntleyi (4-6)	0.567	0.044	27.2	1.162	16.777	0.645	0.393	2.242
B. huntleyi (400)	0.583	0.039	28.3	1.334	15.161	0.895	4.342	1.781
B. huntleyi (750)	0.855	0.040	31.1	0.737	15.726	0.789	4.145	1.702
B. elongatus (400)	0.527	0.036	28.8	0.998	18.579	1.253	10.642	3.026
B. elongatus (750)	0.608	0.033	25.7	0.817	16.425	0.851	-1.493	1.450
E. marioni small (400)	0.770	0.033	30.3	0.817	16.408	1.068	-2.931	0.993
E. marioni large (400)	1.034	0.049	28.1	1.120	14.523	0.694	-2.225	1.067
15°C acclimation								
P. eatoni (4-6)	0.544	0.047	24.3	1.383	14.753	0.930	8.908	3.648
B. randi (4-6)	1.577	0.102	28.8	0.998	16.676	0.487	-2.676	0.393
B. huntleyi (4-6)	0.645	0.046	27.7	1.033	16.710	1.172	-0.868	1.341
B. huntleyi (400)	0.653	0.068	29.6	1.368	16.230	1.169	1.441	1.877
B. huntleyi (750)	0.739	0.054	26.7	2.329	17.977	1.418	2.393	1.875
B. elongatus (400)	0.636	0.037	28.5	1.186	15.659	0.665	3.066	4.333
B. elongatus (750)	0.625	0.026	26.5	1.167	15.666	0.694	-2.512	1.496
E. marioni small (400)	0.714	0.023	26.5	1.003	14.842	0.470	-4.615	0.593
E. marioni large (400)	0.994	0.036	30.3	1.174	16.180	0.669	-3.806	0.880

m.a.s.l. = metres above sea level; s.e. = standard error

The orthogonal polynomial contrast approach revealed support for two hypotheses (Table 4). For most species, the hotter is better (HIB) hypothesis was supported, specifically *B. huntleyi* at 400 m.a.s.l. and 750 m.a.s.l., *B. elongatus* at 750 m.a.s.l. and *E. marioni*, both small and large. By contrast, in *B. randi*, coastal *B. huntleyi* and *B. elongatus* at 400 m.a.s.l., evidence for the beneficial acclimation hypothesis (BAH) was found. For the coastal species, *P. eatoni*, the hypothesis of no plasticity could not be rejected.

Preferred body temperatures differed substantially from T_{opt} for all species from all acclimation treatments, with temperatures much lower than those which maximise performance being selected in all cases (Fig. 1). Acclimation effects on thermal preference were not significant for all but one species population group (large *E. marioni*) (Appendix B).

Table 4. Summarised outcomes of the ordered-factor ANOVAs with orthogonal polynomial contrasts for the effects of acclimation on maximum rate of performance (U_{max}), optimum temperature (T_{opt}), and performance breadth (T_{br}), for each weevil species population.

Species	Altitude (m.a.s.l.)	U_{max}	T_{opt}	Hypothesis	T_{br}	Hypothesis for T_{br}
P. eatoni	0	n.s.	n.s.	n.p.	n.s.	n.p.
B. randi	0	L^+, Q^-	L^+	BAH	L^-	
B. huntleyi	0	L^+	L^+	BAH	n.s.	n.p.
B. huntleyi	400	L^+	n.s.	HIB	n.s.	n.p.
B. huntleyi	750	L^+, Q^-	n.s.	HIB	n.s.	n.p.
B. elongatus	400	L^+	L^+	BAH	n.s.	n.p.
B. elongatus	750	L^+, Q^-	n.s.	HIB	n.s.	n.p.
E. marioni (small)	400	L^+, Q^-	n.s.	HIB	n.s.	n.p.
E. marioni (large)	400	L^+, Q^-	n.s.	HIB	n.s.	n.p.

m.a.s.l. = metres above sea level, L^+ = positive linear, L^- = negative linear, Q^- = negative quadratic, n.s. = not significant, n.p. = no plasticity, BAH = beneficial acclimation hypothesis, HIB = hotter is better. The full analytical outcomes are given in the supporting information (Appendix A).

Discussion

The primary goals of this study were to evaluate whether variation in locomotion performance and thermal preference among species and populations of Marion Island weevils can be ascribed to acclimation ability (thus providing support for one or more acclimation hypotheses), and secondly to determine the extent to which the organisms' preferred temperatures match the thermal optima for performance. The analyses show that different forms of plasticity are present for the performance curve traits investigated and preferred temperatures are much lower than thermal optima, implying that the patterns and predictions for plasticity and acclimation responses are complex.

Of particular interest is that the current results provide little support for the hypothesis that in unpredictably variable environments, organisms are less likely to respond to environmental change via acclimation (i.e. show less phenotypic plasticity) than organisms in environments with predictable variation (Deere & Chown 2006; Deere *et al.* 2006; Chown & Terblanche 2007). The climate on Marion Island is considered one of the least variable globally (Gremmen 1981), although more importantly this variability in temperature is unpredictable (see Deere & Chown 2006). Thus, many traits do not show acclimation responses, such as supercooling points (SCPs) in a flightless moth (Klok & Chown 1998), SCPs and lower lethal temperatures (Deere *et al.* 2006) and locomotion performance traits (Deere & Chown 2006) in mites, and upper and lower lethal limits in kelp fly larvae and adults (Marais *et al.* 2009). However, other traits do show acclimation responses, such as SCPs and critical thermal minima in a different species of flightless moth (Klok & Chown 1997), critical thermal minima in weevils (Klok & Chown 2003), SCPs of a psocopteran (Slabber & Chown 2004), several thermal tolerance traits (Slabber *et al.* 2007) and desiccation resistance (Chown *et al.* 2007) in springtails, chill coma recovery in kelp flies (although the form of the response differs between adults and larvae) (Marais & Chown 2008), and critical thermal minima and maxima in spiders (although responses are decoupled) (Jumbam *et al.* 2008). Unfortunately, it is not yet known why traits vary in their response to acclimation; however, this variation needs more future consideration (Chown & Convey 2007).

The three key traits of thermal performance curves of weevils investigated in this study did show acclimation responses. The strong inference approach revealed support for

the HIB hypothesis for five of the nine weevil populations, while an effect of beneficial acclimation cannot be rejected for three of the populations (Table 4). This is not surprising, as the BAH has been severely criticised as being too simplistic (Angiletta 2009) and the HIB has received strong support as an alternative (Huey *et al.* 1999; Frazier *et al.* 2006; Kingsolver & Huey 2008). Nevertheless, why the weevils on Marion should show plasticity despite the predictions originating from unpredictable environments (although this has been found for other species and traits - see above) necessitates discussion.

One reason for the apparent dichotomy could be due to the form of the environmental variation on the island. Environmental variation is not stable, but varies both spatially and temporally. Historical views held that selection on the performance curve is determined by the typical environmental conditions of an area (Huey & Kingsolver 1989). This view has been challenged and it has been argued that responses of components of thermal performance curves might rather be determined by rare extreme events (e.g. brief exposure to high temperatures) (Kingsolver & Watt 1983; Huey & Kingsolver 1989). The principal reason for this is that the consequences of experiencing body temperatures above the optimum are much greater than those of experiencing temperatures below the optimum (Kingsolver & Huey 1998). On Marion, higher altitudes experience a wider range of temperatures than lower ones (Table 5) and temperatures of 30.0°C have been recorded on rock faces at high elevations (Boelhouwers *et al.* 2003), a habitat of high altitude weevils. As the ability of organisms to remain active across an appropriate range of environmental temperatures is a significant component of fitness (Kristensen *et al.* 2007; Loeschcke & Hoffmann 2007), it would therefore be advantageous for the performance curve to incorporate high temperatures experienced in the environment. This way, the weevils avoid being negatively affected by extreme temperatures which could be damaging and potentially lethal. Continued exposure to these upper temperatures is lethal (observations from present study and critical thermal maximum values of the weevils (Klok & Chown 2003) are close to the optima found in this study); however, brief exposures (i.e. rare extreme events) could be responsible for selection on the performance curve. This could explain why it only seems to be the mid to high altitude weevil species or populations that tend to show HIB (Table 4).

Moreover, it not only seems to be plasticity that is important, but the form that this plasticity takes, as has been found for other Marion organisms (e.g. Chown *et al.* 2007).

Even within species, it is only the high altitude populations that show HIB (*B. elongatus* at 750 m.a.s.l and *B. huntleyi* at 400 m.a.s.l. and 750 m.a.s.l.), while the lower altitude populations of these species do not (*B. elongatus* at 400 m.a.s.l and *B. huntleyi* at 0 m.a.s.l.) (Table 4). This pattern of plasticity has also been found in two other traits in this group of weevils, critical thermal minimum and maximum (Klok & Chown 2003). Such adaptation of physiological traits to different microhabitats (see Chown 1989 for a description of habitat use of the weevils) has also previously been discussed as a survival strategy against water loss in these weevils (Chown 1993). Thus, differences in the abiotic conditions experienced by these organisms seem to be mirrored by differences in their physiology, as suggested by Chown & van Drimmelen (1992).

Table 5. Descriptive statistics of soil temperatures (°C) across an altitudinal transect on the eastern side of Marion Island from 2002 to 2009 (P. le Roux, unpublished data).

Altitude (m.a.s.l.)	n	Mean	s.d.	Maximum	Minimum	Range
4-6	54899	6.1	2.7	22.5	-1.1	23.5
400	53471	3.7	3.3	19.9	-8.1	28.0
750	53992	2.1	3.8	24.5	-11.2	35.7

m.a.s.l. = metres above sea level, s.d. = standard deviation

Of course it may be argued that as different physiological traits have different thermal sensitivities (Martin & Huey 2008), then even though the three key performance curve traits investigated in this study gave evidence of plasticity, it is possible that other traits might not, as has been found for other Marion organisms (Marais & Chown 2008; Marais *et al.* 2009). Even within the HIB hypothesis, some aspects of performance show stronger support than others (Kingsolver 2009; Angilletta *et al.* 2010). Furthermore, evidence of different acclimation hypotheses has been found for other organisms on Marion, such as the 'colder is better' hypothesis supported by springtails (Chown *et al.* 2007). However, a reason for the difference in this instance could be that the springtails

investigated by Chown *et al.* (2007) are found predominantly within soil and vegetation, a much more stable habitat with a smaller range of temperatures than exposed areas such as rock faces or above ground on vegetation where weevils tend to be found.

By contrast to the species and populations showing plasticity, for one species the hypothesis of no plasticity could not be rejected (Table 4). This species, *P. eatoni*, is restricted to the supralittoral zone where it can be found in clumps of algae, which are often splashed by sea water (Chown 1989). Conditions keep changing for this species, where they can be splashed by sea water one day, but not the next. Thus, in this highly unpredictably variable environment, this species could be predicted to show no phenotypic plasticity. Whether such an absence of phenotypic plasticity can be considered adaptive is not clear. Several conditions would have to be satisfied to show this, including evidence of consistent variation among individuals, a genetic basis for the traits, and a relationship between variation in locomotor performance and fitness (Deere & Chown 2006). Moreover, *P. eatoni* is moderately chill-tolerant and avoids freezing (van der Merwe *et al.* 1997), which is in keeping with the generally high subzero microclimate temperatures which the species is likely to encounter.

The actual body temperature an animal achieves depends largely on its behaviour (Huey 1991). Behaviour forms one of the major links between the environment and an organismal response (Huey 1991; Huey *et al.* 2003), and should therefore have a major influence on both the extent of and the form that plasticity takes (Marais & Chown 2008). For this reason, it has long been considered a driver of evolutionary change (Bogert 1949). For the weevils on Marion, while the thermal optima are high, the preferred temperatures of the animals are low (Fig. 1). While this mismatch could initially be surprising, it could also have been predicted. It in fact makes sense when the HIB is supported, as fitness may be maximized when optimal temperature is above the environmental temperature (Frazier *et al.* 2006; Kingsolver & Huey 2008; Kingsolver 2009). Importantly, environmental temperatures much higher than body temperatures could impose a severe cost to an organism. As weevils are exposed to brief periods of high temperatures (see above), it would be important to be physiologically equipped to cope with such events.

These mechanisms and predictions do not just apply to those species and populations which show HIB, as in fact thermal optima exceeding preferred temperatures have been predicted for high latitude temperate species (Deutsch *et al.* 2008). Deutsch *et*

al. (2008) reported that temperate ectotherms perform best at body temperatures far above mean environmental air temperature. Two metrics used to explain this observation, the safety margin and warming tolerance (see Deutsch *et al.* 2008), tend to increase with increasing latitude. This suggests that temperate ectotherms might actually be at an advantage under changing climate conditions compared to tropical ectotherms in that warming would increase mean fitness (Martin & Huey 2008; Kingsolver 2009). On Marion, where the climate is warming and mean annual temperature has already increased by ~1°C over the last 50 years (le Roux & McGeoch 2008), the weevils could be well equipped to cope with the change. Although this might not be the case if rare extreme events increase as predicted (le Roux & McGeoch 2008).

Thermoregulatory behaviour has also been demonstrated to have a direct link to nutritional state (Coggan *et al.* 2011). At low temperatures, some insects experience slower growth rates than they would at higher temperatures, but nutrient utilisation and thus efficiency increase (Miller *et al.* 2009; Coggan *et al.* 2011). Miller *et al.* (2009) found that locusts at low temperatures had higher nutrient assimilation efficiency and grew more slowly than locusts at higher temperatures. These insects have even been shown to adjust their thermoregulatory behaviour when faced with nutrient poor diets, selecting lower temperatures at which assimilation efficiency was maximal (Coggan *et al.* 2011). As the weevils on Marion survive on low quality diets (Lawrey 1987; Chown 1989; Crafford & Chown 1991), it is plausible that the selection of low temperatures by these animals might be linked to nutrient utilisation efficiency. This idea is one that should be explored in future work.

In conclusion, the threat of rapidly changing climates has increased the significance of studies of thermal physiology. However, this study has shown that the patterns and predictions for plasticity and acclimation responses are complex, particularly if these predictions are to be made for changing environmental conditions. In particular, the results have reinforced the view that rather than merely invoking a single hypothesis, competing hypotheses should also be tested. The potential ecological and evolutionary consequences of patterns, the HIB in particular, are largely unexplored (Kingsolver & Huey 2008; Kingsolver 2009). However, the observed complexity and dynamics of such patterns highlight that predicting evolutionary responses of physiological phenotypes will be very difficult (Gibert *et al.* 2001).

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Appendices

Appendix A. Outputs of the ordered-factor ANOVAs with orthogonal polynomial contrasts on the effects of acclimation on maximum rate of performance (U_{max}), optimum temperature (T_{opt}), and performance breadth (T_{br}), for each weevil species population. In each case the main effect and the linear and quadratic contrasts as well as the sign and value of their estimates is shown.

P. eatoni, 0 m.a.s.l.

U_{max}

Source	df	Type III SS	MS	F	p
Acclim	2	0.0217	0.0109	0.36	0.6980
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	0.0001	0.0001	0.003	0.9591
Acclim quadratic	1	0.0217	0.0217	0.73	0.4016
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		-0.0028	0.0546	-0.06	0.959
Acclim quadratic		-0.0465	0.0546	-0.85	0.402

T_{opt}

Source	df	Type III SS	MS	F	p
Acclim	2	39.27	19.633	1.29	0.2927
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	2.45	2.450	0.16	0.6918
Acclim quadratic	1	36.82	36.817	2.41	0.1320
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		-0.4950	1.2354	-0.40	0.692
Acclim quadratic		-1.9188	1.2354	-1.55	0.132

T_{br}

Source	df	Type III SS	MS	F	p
Acclim	2	46.83	23.413	1.66	0.2084
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	45.93	45.935	3.26	0.0820
Acclim quadratic	1	0.89	0.891	0.06	0.8033
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		2.1432	1.1864	1.81	0.082
Acclim quadratic		0.2984	1.1864	0.25	0.803

B. randi, 0 m.a.s.l.

U_{max}

Source	df	Type III SS	MS	F	p
Acclim	2	1.3470	0.6735	11.29	0.0003
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	1.0125	1.0125	16.98	0.0003
Acclim quadratic	1	0.3345	0.3345	5.61	0.0253
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		0.3182	0.0772	4.12	0.0003
Acclim quadratic		-0.1829	0.0772	-2.37	0.0253

T_{opt}

Source	df	Type III SS	MS	F	p
Acclim	2	163.8	81.900	4.55	0.0199
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	115.2	115.200	6.39	0.0176
Acclim quadratic	1	48.6	48.600	2.70	0.1121
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		3.394	1.342	2.53	0.0176
Acclim quadratic		-2.204	1.342	-1.64	0.1121

T_{br}

Source	df	Type III SS	MS	F	p
Acclim	2	66.504	33.252	3.66	0.0393
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	65.269	65.269	7.18	0.0124
Acclim quadratic	1	1.236	1.236	0.14	0.7153
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		-2.5548	0.9534	-2.68	0.0124
Acclim quadratic		-0.3515	0.9534	-0.37	0.7152

B. huntleyi, 0 m.a.s.l.

U_{max}

Source	df	Type III SS	MS	F	p
Acclim	2	0.1380	0.0690	4.04	0.0292
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	0.1378	0.1378	8.07	0.0085
Acclim quadratic	1	0.0002	0.0002	0.01	0.9220
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		0.1174	0.0413	2.84	0.0085
Acclim quadratic		-0.0041	0.0413	-0.10	0.9220

T_{opt}

Source	df	Type III SS	MS	F	p
Acclim	2	178.07	89.033	5.18	0.0124
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	145.80	145.800	8.49	0.0071
Acclim quadratic	1	32.27	32.267	1.88	0.1818
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		3.8184	1.3106	2.91	0.0071
Acclim quadratic		-1.7963	1.3106	-1.37	0.1818

T_{br}

Source	df	Type III SS	MS	F	p
Acclim	2	24.203	12.1016	1.28	0.2942
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	18.779	18.7792	1.99	0.1700
Acclim quadratic	1	5.424	5.4240	0.57	0.4552
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		-1.3704	0.9721	-1.41	0.170
Acclim quadratic		0.7365	0.9721	0.76	0.455

B. huntleyi, 400 m.a.s.l.

U_{max}

Source	df	Type III SS	MS	F	p
Acclim	2	0.4580	0.2290	9.35	0.0008
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	0.4205	0.4205	17.17	0.0003
Acclim quadratic	1	0.0375	0.0375	1.53	0.2266
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		0.2051	0.0495	4.14	0.0003
Acclim quadratic		-0.0612	0.0495	-1.24	0.2266

T_{opt}

Source	df	Type III SS	MS	F	p
Acclim	2	10.47	5.2333	0.32	0.7275
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	7.20	7.2000	0.44	0.5114
Acclim quadratic	1	3.27	3.2667	0.20	0.6575
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		0.8485	1.2750	0.67	0.511
Acclim quadratic		0.5715	1.2750	0.45	0.658

T_{br}

Source	df	Type III SS	MS	F	p
Acclim	2	9.127	4.5634	0.47	0.6306

Contrast	df	Contrast SS	MS	F	p
Acclim linear	1	0.164	0.1638	0.02	0.8977
Acclim quadratic	1	8.963	8.9629	0.92	0.3456

Parameter		Estimate	s.e.	t	p
Acclim linear		-0.1280	0.9863	-0.13	0.898
Acclim quadratic		0.9467	0.9863	0.96	0.346

B. huntleyi, 750 m.a.s.l.

U_{max}

Source	df	Type III SS	MS	F	p
Acclim	2	0.4840	0.2420	13.13	0.0001

Contrast	df	Contrast SS	MS	F	p
Acclim linear	1	0.1843	0.1843	1.00	0.0038
Acclim quadratic	1	0.2996	0.2996	16.25	0.0004

Parameter		Estimate	s.e.	t	p
Acclim linear		0.1358	0.0429	3.16	0.0039
Acclim quadratic		-0.1731	0.0429	-4.03	0.0004

T_{opt}

Source	df	Type III SS	MS	F	p
Acclim	2	104.87	52.433	1.56	0.2296

Contrast	df	Contrast SS	MS	F	P
Acclim linear	1	6.05	6.050	0.18	0.6753
Acclim quadratic	1	98.82	98.817	2.93	0.0984

Parameter		Estimate	s.e.	t	p
Acclim linear		-0.7778	1.8365	-0.42	0.6753
Acclim quadratic		-3.1435	1.8365	-1.71	0.0984

T_{br}

Source	df	Type III SS	MS	F	p
Acclim	2	36.232	18.116	1.65	0.2113

Contrast	df	Contrast SS	MS	F	p
Acclim linear	1	0.117	0.117	0.01	0.9186
Acclim quadratic	1	36.115	36.115	3.29	0.0811

Parameter		Estimate	s.e.	t	p
Acclim linear		-0.1082	1.0486	-0.10	0.919
Acclim quadratic		1.9004	1.0486	1.81	0.081

B. elongatus, 400 m.a.s.l.

U_max

Source	df	Type III SS	MS	F	p
Acclim	2	0.2420	0.1210	8.61	0.0013
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	0.2420	0.2420	17.23	0.0003
Acclim quadratic	1	0.00001	0.000007	0.0005	0.9828
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		0.1556	0.0375	4.15	0.0003
Acclim quadratic		-0.0008	0.0375	-0.02	0.9828

T_opt

Source	df	Type III SS	MS	F	p
Acclim	2	138.47	69.233	3.59	0.0415
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	96.80	96.800	5.02	0.0335
Acclim quadratic	1	41.67	41.667	2.16	0.1533
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		3.111	1.389	2.24	0.0335
Acclim quadratic		-2.041	1.389	-1.47	0.1533

T_br

Source	df	Type III SS	MS	F	p
Acclim	2	42.634	21.317	1.88	0.1718
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	10.922	10.922	0.96	0.3349
Acclim quadratic	1	31.712	31.712	2.80	0.1059
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		-1.0451	1.0644	-0.98	0.335
Acclim quadratic		-1.7808	1.0644	-1.67	0.106

B. elongatus, 750 m.a.s.l.

U_max

Source	df	Type III SS	MS	F	p
Acclim	2	0.2459	0.1230	11.61	0.0002
Contrast	**df**	**Contrast SS**	**MS**	**F**	**P**
Acclim linear	1	0.2000	0.2000	18.88	0.0002
Acclim quadratic	1	0.0459	0.0459	4.34	0.0470
Parameter		**Estimate**	**s.e.**	**t**	**P**
Acclim linear		0.1414	0.0326	4.35	0.0002
Acclim quadratic		-0.0678	0.0326	-2.08	0.0470

T_{opt}

Source	df	Type III SS	MS	F	p
Acclim	2	63.2	31.600	1.56	0.2277
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	57.8	57.800	2.86	0.1023
Acclim quadratic	1	5.4	5.400	0.27	0.6094
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		2.4042	1.4214	1.69	0.102
Acclim quadratic		-0.7348	1.4214	-0.52	0.609

T_{br}

Source	df	Type III SS	MS	F	p
Acclim	2	56.97	28.487	1.99	0.1566
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	52.10	52.100	3.63	0.0673
Acclim quadratic	1	4.87	4.873	0.34	0.5647
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		-2.2825	1.1973	-1.91	0.0673
Acclim quadratic		0.6981	1.1973	0.58	0.5647

E. marioni (small) 400 m.a.s.l.

U_{max}

Source	df	Type III SS	MS	F	p
Acclim	2	0.2106	0.1053	15.77	< 0.0001
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	0.1023	0.1023	15.31	0.0006
Acclim quadratic	1	0.1084	0.1084	16.23	0.0004
Parameter		**Estimate**	**s.e.**	**t**	**P**
Acclim linear		0.1011	0.0258	3.91	0.0006
Acclim quadratic		-0.1041	0.0258	-4.03	0.0004

T_{opt}

Source	df	Type III SS	MS	F	P
Acclim	2	81.80	40.900	5.30	0.0114
Contrast	**df**	**Contrast SS**	**MS**	**F**	**P**
Acclim linear	1	2.45	2.450	0.32	0.5776
Acclim quadratic	1	79.35	79.350	10.29	0.0034
Parameter		**Estimate**	**s.e.**	**t**	**P**
Acclim linear		-0.4950	0.8781	-0.56	0.5776
Acclim quadratic		-2.8169	0.8781	-3.21	0.0034

T_{br}

Source	df	Type III SS	MS	F	P
Acclim	2	23.048	11.5242	1.80	0.1841
Contrast	**df**	**Contrast SS**	**MS**	**F**	**P**
Acclim linear	1	1.196	1.1956	0.19	0.6688
Acclim quadratic	1	21.853	21.8527	3.42	0.0754
Parameter		**Estimate**	**s.e.**	**t**	**P**
Acclim linear		0.3458	0.7994	0.43	0.6688
Acclim quadratic		-1.4783	0.7994	-1.85	0.0754

$E.$ $marioni$ (large) 400 m.a.s.l.

U_{max}

Source	df	Type III SS	MS	F	p
Acclim	2	0.5842	0.2921	15.95	< 0.0001
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	0.3754	0.3754	20.50	0.0001
Acclim quadratic	1	0.2089	0.2089	11.40	0.0022
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		0.1938	0.0428	4.53	0.0001
Acclim quadratic		-0.1445	0.0428	-3.38	0.0022

T_{opt}

Source	df	Type III SS	MS	F	p
Acclim	2	28.467	14.233	1.36	0.2747
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	18.050	18.050	1.72	0.2008
Acclim quadratic	1	10.417	10.417	0.99	0.3280
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		1.3435	1.0245	1.31	0.201
Acclim quadratic		1.0206	1.0245	1.00	0.328

T_{br}

Source	df	Type III SS	MS	F	p
Acclim	2	19.003	9.5015	1.64	0.2120
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	0.019	0.0186	0.003	0.9552
Acclim quadratic	1	18.984	18.9844	3.28	0.0811
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		-0.0431	0.7603	-0.06	0.9552
Acclim quadratic		1.3778	0.7603	1.81	0.0811

m.a.s.l. = metres above sea level, MS = mean squares, SS = sums of squares, s.e. = standard error

Appendix B. Outputs of the ordered-factor ANOVAs with orthogonal polynomial contrasts on the effects of acclimation on preferred temperature (T_{pref}) for each weevil species population. In each case the main effect and the linear and quadratic contrasts as well as the sign and value of their estimates is shown.

P. eatoni, 0 m.a.s.l.

T_{pref}

Source	df	Type III SS	MS	F	p
Acclim	2	597.99	298.99	3.19	0.0577
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	21.28	21.28	0.23	0.6377
Acclim quadratic	1	576.70	576.70	6.15	0.0199
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		1.740	3.146	0.55	0.5850
Acclim quadratic		7.664	3.090	2.48	0.0199

B. randi, 0 m.a.s.l.

T_{pref}

Source	df	Type III SS	MS	F	p
Acclim	2	226.31	113.157	1.42	0.2603
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	217.71	217.710	2.73	0.1105
Acclim quadratic	1	8.60	8.604	0.11	0.7454
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		-4.6659	2.8278	-1.65	0.111
Acclim quadratic		-0.9276	2.8278	-0.33	0.745

B. huntleyi, 0 m.a.s.l.

T_{pref}

Source	df	Type III SS	MS	F	p
Acclim	2	17.54	8.7711	0.30	0.7471
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	16.74	16.7434	0.56	0.4597
Acclim quadratic	1	0.80	0.7988	0.03	0.8711
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		-1.2940	1.7251	-0.75	0.460
Acclim quadratic		-0.2826	1.7251	-0.16	0.871

***B. huntleyi,* 400 m.a.s.l.**

T_{pref}

Source	df	Type III SS	MS	F	p
Acclim	2	104.11	52.054	1.25	0.3037
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	101.29	101.293	2.42	0.1311
Acclim quadratic	1	2.81	2.815	0.07	0.7972
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		-3.1827	2.0441	-1.56	0.1311
Acclim quadratic		-0.5305	2.0441	-0.26	0.7972

***B. huntleyi,* 750 m.a.s.l.**

T_{pref}

Source	df	Type III SS	MS	F	p
Acclim	2	86.35	43.177	0.65	0.5308
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	85.68	85.681	1.29	0.2666
Acclim quadratic	1	0.67	0.674	0.01	0.9206
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		-2.9271	2.5802	-1.13	0.2666
Acclim quadratic		0.2596	2.5802	0.10	0.9206

***B. elongatus,* 400 m.a.s.l.**

T_{pref}

Source	df	Type III SS	MS	F	p
Acclim	2	290.71	145.353	1.27	0.2984
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	46.19	46.195	0.40	0.5314
Acclim quadratic	1	244.51	244.511	2.13	0.1561
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		-2.149	3.390	-0.63	0.5314
Acclim quadratic		-4.945	3.390	-1.46	0.1562

B. elongatus, 750 m.a.s.l.

T_{pref}

Source	df	Type III SS	MS	F	p
Acclim	2	67.23	33.613	1.38	0.2700
Contrast	**df**	**Contrast SS**	**MS**	**F**	**P**
Acclim linear	1	63.37	63.365	2.59	0.1191
Acclim quadratic	1	3.86	3.861	0.16	0.6942
Parameter		**Estimate**	**s.e.**	**t**	**P**
Acclim linear		-2.5172	1.5636	-1.61	0.119
Acclim quadratic		0.6213	1.5636	0.40	0.694

E. marioni (small) 400 m.a.s.l.

T_{pref}

Source	df	Type III SS	MS	F	p
Acclim	2	77.204	38.602	3.34	0.0507
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	76.700	76.700	6.63	0.0158
Acclim quadratic	1	0.504	0.504	0.04	0.8362
Parameter		**Estimate**	**s.e.**	**t**	**P**
Acclim linear		-2.7695	1.0755	-2.58	0.0158
Acclim quadratic		0.2245	1.0755	0.21	0.8362

E. marioni (large) 400 m.a.s.l.

T_{pref}

Source	df	Type III SS	MS	F	p
Acclim	2	89.54	44.772	3.53	0.0435
Contrast	**df**	**Contrast SS**	**MS**	**F**	**p**
Acclim linear	1	87.78	87.782	6.92	0.0139
Acclim quadratic	1	1.76	1.762	0.14	0.7123
Parameter		**Estimate**	**s.e.**	**t**	**p**
Acclim linear		-2.9628	1.1264	-2.63	0.0139
Acclim quadratic		0.4198	1.1264	0.37	0.7123

m.a.s.l. = metres above sea level, MS = mean squares, SS = sums of squares, s.e. = standard error

Chapter 6 – Conclusion

View of Prince Edward Island from Marion Island

Climate change and biological invasions have been recognised as major threats to biodiversity (Millennium Ecosystem Assessment 2005) and their effects have been recorded around the world (see Parmesan 2006; Mainka & Howard 2010). In particular, these threats are predicted to influence terrestrial systems of the sub-Antarctic (Chown & Convey 2007), a region facing rapid climate change and an increase in the rate of introduction of non-indigenous species (Kennedy 1995; Convey 1997; Bergstrom & Chown 1999; Frenot *et al.* 2005; Chown & Lee 2009). The consequences of both climate change and biological invasions for the sub-Antarctic are also widely reported, and impacts on species and communities have been recorded (e.g. Crafford & Scholtz 1986; Chown & Smith 1993; Kennedy 1995; Gremmen *et al.* 1998; Bergstrom & Chown 1999; Frenot *et al.* 2001, 2005; Smith *et al.* 2002; Lee *et al.* 2007, 2009; Wanless *et al.* 2007). There has therefore been much interest in the region, particularly as it is of considerable conservation importance due to the large number of indigenous and endemic species found there (Gressitt 1970; Chown *et al.* 1998; Bergstrom & Chown 1999; Chown *et al.* 2001).

The Prince Edward Island group provides an ideal system in which to explore these threats. Both islands in the group, Marion Island (MI) and Prince Edward Island (PEI), are experiencing rapid warming (le Roux & McGeoch 2008), yet have different invasive assemblages and in consequence are experiencing different impacts (Crafford *et al.* 1986; Crafford & Scholtz 1987; Gremmen & Smith 1999; Chown *et al.* 2002a). In this study, I examined the impacts of climate change and invasive species using weevils and springtails as exemplar taxa. These organisms were chosen due to their known importance in the islands' food webs and in ecosystem functioning (Smith 1977; Burger 1985; Smith & Steenkamp 1992a, b; Hopkin 1997; Wardle *et al.* 2004; Smith 2008). Four key themes were addressed, namely species-energy relationships, abundance structure and range limits, synergistic effects of climate change and biological invasions, and physiological responses to temperature.

Species-energy relationships

Variation in the patterns of invasive species richness and abundance and their underlying causes are matters of considerable ecological and conservation significance. A positive relationship between indigenous and alien species richness has been recorded at relatively

large spatial scales for a variety of taxa (e.g. Stohlgren *et al.* 1999, 2008; Fridley *et al.* 2004, 2007; Herben *et al.* 2004; Chown *et al.* 2005; Evans *et al.* 2005a; Borges *et al.* 2006; Stark *et al.* 2006; Lilleskov *et al.* 2008; Souza *et al.* 2011; Terauds *et al.* 2011). At these larger spatial scales, the positive relationship is a consequence of variation in thermal energy availability, spatial heterogeneity and/or metacommunity dynamics (Levine & D'Antonio 1999; Stohlgren *et al.* 1999; Evans *et al.* 2005b; Melbourne *et al.* 2007; Souza *et al.* 2011). These influences of thermal energy availability and community dynamics suggest that the same mechanisms responsible for positive species-energy relationships in indigenous species at the macro-scale, result in positive relationships in alien species. However, examination of the mechanisms underlying these relationships remains uncommon. Moreover, these mechanisms are rarely examined for alien species (Marini *et al.* 2009), and where investigations of positive alien species-energy relationships or relationships between alien and indigenous richness are undertaken these typically focus on plants or birds (Herben *et al.* 2004; Hurlbert 2004; Evans *et al.* 2005c; Pyšek & Richardson 2006; Lambdon *et al.* 2008; Marini *et al.* 2009), and less commonly on arthropods (Terauds *et al.* 2011).

In Chapter 2, I therefore examined the form of the species-energy relationship and the mechanisms underlying the patterns for indigenous and invasive springtails along two altitudinal gradients on MI. Specifically, the nine mechanisms identified by Evans *et al.* (2005b) were explored, and the extent to which geometric constraints can account for richness variation was examined, as were the effects of area. Richness of both indigenous and invasive species was found to decline with elevation and a mid-domain effect (MDE) of species richness does not occur, thus concurring with arguments that geometric constraints on species' ranges do not provide a general explanation for richness gradients (Zapata *et al.* 2005). The most significant finding was that the same mechanisms responsible for positive species-energy relationships in indigenous species result in positive relationships in invasive species. It is therefore not surprising that the relationships among the indigenous and invasive species richness and abundance were largely positive. This provides support for the possibility that a likely reason behind the 'rich get richer' hypothesis (Stohlgren *et al.* 2003) is that the same species-energy mechanisms underlie both indigenous and invasive diversity patterns.

The results showed that disturbance thresholds and stressful temperatures are more important than increased population sizes in determining the variation in springtail

species richness on MI, indicating that the species-energy relationships for both indigenous and invasive species are determined by the physiological tolerances of the species. This could be the case across the whole of the sub-Antarctic, and certainly seems to be so on Macquarie Island where positive relationships between diversity and environmental favourability have been found (Terauds *et al.* 2011). Because of this, a warming climate could have far-reaching consequences for these organisms. In particular, invasive species are predicted to be at an advantage relative to indigenous species under warming conditions, as has previously been suggested for most parts of the world (Walther *et al.* 2009). On MI, one species where this seems especially likely, given its physiological responses to experimental warming and drying, is the large invasive tomocerid, *Pogonognathellus flavescens* (Chown *et al.* 2007; Leinaas *et al.* 2009). Determining whether this will be the case however depends on understanding the factors underlying its range limits and abundance structure, which was addressed in Chapter 3.

Abundance structure and range limits

Understanding spatial variation in abundance is essential for forecasts of and conservation responses to environmental change impacts on diversity (Channel & Lomolino 2000; Gaston 2003; Thuiller 2007; Chown & Gaston 2008; Gallien *et al.* 2010; Dawson *et al.* 2011). Despite this, few studies have sought to distinguish causal components of abundance structure. This issue was addressed in Chapter 3 using an invasive springtail species, *P. flavescens*, on MI. Specifically, the BAM framework developed by Soberón (2007) to explain the causal basis of abundance structure and range limits was tested explicitly. In Soberón's (2007) BAM framework, the area where a population may persist is defined as the intersection of those: where it can cope with competition and other interactions (B), where it has intrinsic abilities to survive, grow and reproduce under given environmental conditions (A), and to which individuals can readily disperse to found the population (M).

Given the physiological tolerances of this habitat generalist species (Chown *et al.* 2007; Slabber *et al.* 2007; Janion *et al.* 2009, 2010; Leinaas *et al.* 2009), survival should be possible across a range of low elevation habitats below 200 m. However, the springtail is restricted to indigenous *Poa cookii* tussock grassland habitats in the southeast of the island. Preference for this habitat seems to be associated with the presence of vertebrates and

low Ca and pH, the reasons for which are not clear, but may have to do with a founder effect. Irrespective however, *P. flavescens* is absent from many other areas of suitable habitat around the island. The results showed that the current range limits are set by dispersal limitation (i.e. contingent absences are most significant) and that the inability of individuals to traverse other habitat types between tussock grassland patches seems likely to be associated with a strong preference for tussock grasslands and their soil characteristics, rather than mortality in other habitat types. The latter is a significant illustration of the need to consider more carefully edaphic variables, rather than climatic ones only, when modelling the distributions of soil invertebrates.

Given the low genetic diversity in *P. flavescens* (Myburgh *et al.* 2007), the results also provide support for the recommendation that the introduction of additional individuals into a population should be avoided because of evidence that in some cases this can lead to increased genetic variation and invasion of previously unoccupied sites (Dlugosch & Parker 2008). However, over time, the widening distribution of *P. cookii*, as a consequence of a major management intervention (the eradication of feral cats), may enable it to increase its range across the island. Such interactions among alien species, mediated in some cases by management interventions (see also Bergstrom *et al.* 2009), make the case for the prevention of introductions all the more compelling. Spread may also be driven by jump dispersal events aided by humans, which reinforces the necessity for management interventions to reduce the spread of invasive species around the island. For example, visitors to MI should be aware of and strictly adhere to preventative measures to reduce such spread while walking on the island (see recommendations below).

Synergistic effects of climate change and biological invasions

While much evidence exists for the impacts of both climate change and biological invasions on species and their life history characteristics, the form of the interactions among these impacts are poorly understood. Surprisingly few studies have examined this issue and calls have been made that a wider range and extent of work is required to determine the extent to which synergies among environmental change drivers might be realised and might already be taking place (Brook *et al.* 2008). This question was therefore addressed in Chapter 4.

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A prediction of the temperature-size rule (see Atkinson 1994) is that with warming, body size of the weevil species on both Marion and Prince Edward Islands should decline. However, predation by mice of the weevils on Marion Island (MI) should fundamentally affect the pattern of such change, causing it to differ from neighbouring Prince Edward Island (PEI). To test these predictions, a 24-year time series was used to explore the relative effects of warming and mouse predation on body size distributions of the six indigenous weevil (Curculionidae) species that occur on the Prince Edward Island group. Body size is one of the most obvious features of animals and is of considerable ecological and physiological significance (Peters 1983; Brown *et al.* 2004). Both temperature (through the temperature-size rule (Atkinson 1994)) and invasive species (by influencing mortality and production rates (Chown & Smith 1993; Phillips & Shine 2004; Fisk *et al.* 2007)) can influence body size. The potential synergistic impacts of these threats were tested here, as both islands are experiencing rapid warming, and weevils are predated on by mice on MI, which are not present on PEI.

The results indicated that environmental changes are causing a morphological response in weevils on the Prince Edward Island group. Most significantly, the body size of all weevil species on PEI showed a decline with increasing mean annual temperature. Unlike the situation on PEI however, a negative relationship between mean annual temperature and body size was found only for *P. eatoni* on MI, a species not eaten by mice. In all of the other species on MI, size either showed no relationship or a positive relationship with mean annual temperature. Given that no difference among islands was found in the trends shown by *P. eatoni*, but a marked difference among the islands in the other species, it seems likely that the among-island variation is a consequence not of some intrinsic physical characteristic of the islands, but because of the presence of invasive house mice on MI. A possible explanation for the positive relationships between body size and mean annual temperature could be due to metabolic demands associated with temperature for endothermic species. Given that large individuals of all of the weevil species tend to be less common, any increase in predation coupled with a preference for larger sizes, which the mice clearly show (Chown & Smith 1993), would lead to a decline in the mean size of the species. If colder years impose a higher metabolic demand on mice than warmer ones, higher feeding rates should be required to sustain basal and field metabolic rates. This should lead to greater predation and a decrease in size of the weevils,

especially if the size variation is largely phenotypic, rather than with a pronounced genotypic underpinning. This is in keeping with the outcomes of other studies (e.g. Brooks & Dodson 1965; Galbraith 1967; Reznick *et al.* 1997; Cousyn *et al.* 2001; Phillips & Shine 2004; Fisk *et al.* 2007; Phillips *et al.* 2009) where typically the large individuals are lost from an assemblage. The consequences of this are similar to those of overexploitation where the loss of larger organisms of a species causes the 'erosion of large areas of phenotypic space' (Chown & Gaston 2008, pp. 1473). By losing the larger individuals, species may not be as well adapted to cope with changing environmental conditions over the short- and long- term (Chown & Gaston 2008).

The decline in weevil body size with increasing mean annual temperature on PEI strengthens Daufresne *et al.*'s (2009) suggestion that reduced body size is one of the most universal ecological responses to warming, and this study forms one of very few that have addressed this for ectotherms. The interactions between warming and biological invasions on MI appear complex. Nevertheless, as the indigenous weevil species on the Prince Edward Island group form an important component of the islands' relatively simple food webs (Burger 1985; Smith & Steenkamp 1992b), and because body size relates directly to metabolic function (Chown *et al.* 2002b; Brown *et al.* 2004), changes to the body sizes of these weevil species could have serious impacts on the islands' functioning.

These findings emphasise the importance of considering synergistic impacts among environmental change drivers (Brook *et al.* 2008), particularly in the sub-Antarctic where such interactions are predicted to significantly influence terrestrial systems (Chown & Convey 2007) as work on MI has already indicated (Chown *et al.* 2007; Janion *et al.* 2010). As the mouse population is increasing on MI probably due to warming (Smith 2002), this study concurs with several suggestions that synergistic impacts between climate change and invasions will likely increase the impacts of invasive species (e.g. Dukes & Mooney 1999; Theoharides & Dukes 2007; Brook 2008; Brook *et al.* 2008). The results also highlight the importance of long term records for understanding organism responses to environmental changes.

Physiological responses to temperature

In Chapter 5, physiological responses to temperature were explored in more detail. It is well known that the thermal environment experienced by organisms has a direct effect on survival, growth and reproduction (Chown & Nicolson 2004). The physiological response of organisms to rapidly changing climates is therefore a primary concern. One way that organisms may respond to variable environmental conditions is by altering their phenotypes, otherwise known as phenotypic plasticity (Kingsolver & Huey 1998). Various hypotheses have been proposed to explain how individuals might respond to changing environmental conditions, such as the beneficial acclimation hypothesis (BAH). However, the BAH has been criticised as being too simplistic (Angiletta 2009) and alternative hypotheses such as the 'Hotter is Better' hypothesis (HIB) have received renewed interest (Huey & Kingsolver 1989; Angilletta *et al.* 2010). However, studies seldom include investigations of alternative hypotheses (but see Deere & Chown 2006). This was done in Chapter 5, where acclimation responses of locomotion performance were tested within a strong inference framework on weevils along an altitudinal gradient on MI. Moreover, an assumption often made is that the optimum temperature of animals is where they prefer to be, in other words that the preferred body temperature of an organism should match the thermal optimum for performance (Angilletta *et al.* 2006). Therefore, the thermal preference of the weevil populations was also determined to explore the extent to which the preferred temperatures of the organisms matched the thermal optima for performance.

The strong inference approach revealed support for the HIB hypothesis for five of the nine weevil populations, while an effect of beneficial acclimation could not be rejected for three of the populations. However, the results indicated that the patterns and predictions for plasticity and acclimation responses are complex. Of particular interest was that the weevils did not provide support for the idea that in unpredictably variable environments, organisms are less likely to respond to environmental change via acclimation. One explanation for this is the occurrence of rare extreme temperature events (Kingsolver & Watt 1983; Huey & Kingsolver 1989) and the advantage therefore for the performance curves to incorporate high temperatures experienced in the environment. This could also explain why only the mid to high altitude populations tended to show HIB.

Thus, as has been found for other MI organisms (e.g. Chown *et al.* 2007), it not only seems to be plasticity *per se* that is important, but the form that this plasticity takes. Acclimation responses of weevils differed among species and among populations of the same species. Thus, differences in the abiotic conditions experienced by these organisms seem to be mirrored by differences in their physiology, as suggested by Chown & van Drimmelen (1992). Only one species, *Palirhoeus eatoni*, did not display phenotypic plasticity, which could have been predicted based on the microclimate conditions in the habitat in which this species lives. In addition, a mismatch between thermal optimum and preferred temperature was found for all species. This could be expected when the HIB is supported, and has also been predicted for high latitude temperate species (Deutsch *et al.* 2008). That such a mismatch exists could mean that the weevils on MI are well equipped to cope with warming conditions. However, this might not be the case if the prediction of an increase of rare extreme events such as extreme temperatures is realised (le Roux & McGeoch 2008). Furthermore, changes to the body size of weevils (see above) could have implications for how these organisms can cope with changing environmental conditions.

This study has shown that the patterns and predictions for plasticity and acclimation responses are complex, particularly if these predictions are to be made for changing environmental conditions. In particular, the results have reinforced the view that rather than merely invoking a single hypothesis, competing hypotheses should also be tested. The potential ecological and evolutionary consequences of patterns, the HIB in particular, are largely unexplored (Kingsolver & Huey 2008; Kingsolver 2009). However, the observed complexity and dynamics of such patterns highlight that predicting evolutionary responses of physiological phenotypes will be very difficult (Gibert *et al.* 2001).

Conclusion and recommendations

Rapidly changing climates and an increase in the introduction of non-indigenous species are major conservation concerns. This has increased the significance of studies on the impacts of these threats. However, this thesis has shown that to understand such processes, it is essential that an integration of disciplines be undertaken. This thesis thus adopted a multidisciplinary approach and highlighted key issues associated with both climate change and biological invasions. Whilst it is beyond the scope of any one

organisation or country to tackle the issue of climate change, if isolated and rare systems such as the Prince Edward Island group are to be conserved, the issue must be addressed. Studies such as this one which clearly demonstrate the impacts of climate change help to highlight and raise awareness of the threats.

As rising temperatures are likely to facilitate both the establishment and subsequent spread of invasive species, understanding how this might influence patterns of indigenous and invasive species richness and abundance is of considerable ecological significance. However, predicting species responses and range shifts is problematic, especially as multiple factors will change concurrently and how these factors might change is unclear (le Roux & McGeoch 2008). Nevertheless, it can be expected that changing environmental conditions will alter patterns of species richness and abundance of indigenous and invasive species. In particular, invasive species are predicted to be at an advantage relative to indigenous species under warming conditions. However, to properly determine whether this will be the case depends on understanding the causal basis of range limits and abundance structure of these species, which few studies have sought to do.

Organisms are likely to show both morphological and physiological responses to changing environmental conditions. This highlights the importance of long-term records for understanding organism responses to such changes. Impacts to indigenous species are likely to be exacerbated by the predicted increase in the rate of introductions with climate change. This makes the case for managing the prevention of invasive species reaching new areas all the more important. Management interventions should also prevent the spread of invasive species that have already established in new areas. For example, on MI, a predicted widening distribution of *P. cookii* or jump dispersal events aided by humans could provide the conditions required for the invasive springtail, *P. flavescens*, to spread around the island. This highlights the need for walkers on the island to be aware of and strictly adhere to measures to prevent such events. For example, mud and vegetation should be cleaned from boots, and field gear should be brushed free of vegetation in between field trips and before leaving a field hut. The small brooms in the huts can be used for this purpose. Furthermore, *P. flavescens* does not occur on PEI. The helicopters used during the handover periods to transfer passengers and cargo between the ship and islands frequently make contact with ground vegetation and soil during field operations. Thus, if

the helicopters are to be used on PEI after having landed on MI, they should be properly washed first, with particular attention being paid to the landing skids.

Future work

Studies of exemplar taxa, particularly those that are crucial to nutrient cycling and ecosystem functioning (as those organisms studied here), are useful for estimating the effects of perturbations. This study has both set a baseline of information on which future projects or continued monitoring should be based, and it has also continued the monitoring in one instance by extending the weevil body size time series to 24 years.

Suggestions for future work

- It would be beneficial to repeat the altitudinal springtail study every few years to monitor changes in indigenous and invasive springtail species richness and abundance patterns with warming. This is especially important due to the threat of increased introductions and how a potential increase in invasive species richness might influence such patterns.

- *P. flavescens* should continue to be monitored for potential range shifts on MI, particularly if *P. cookii* habitat spreads.

- The results here indicate that environmental changes are causing body size responses in weevils. This work highlights the importance of a long-term record in understanding biotic responses to environmental changes. Therefore the 24-year time series for the weevil body size database should be extended into the future to assess the effects of environmental changes on this important trait, and thus on the functioning of the islands' ecosystems.

- Investigations into the potential ecological and evolutionary consequences of the 'Hotter is Better' hypothesis should be made, as these are largely unexplored.

- It would be useful to determine if the selection of low temperatures by the weevils is linked to nutrient utilisation efficiency as the animals on the islands survive on low quality diets.

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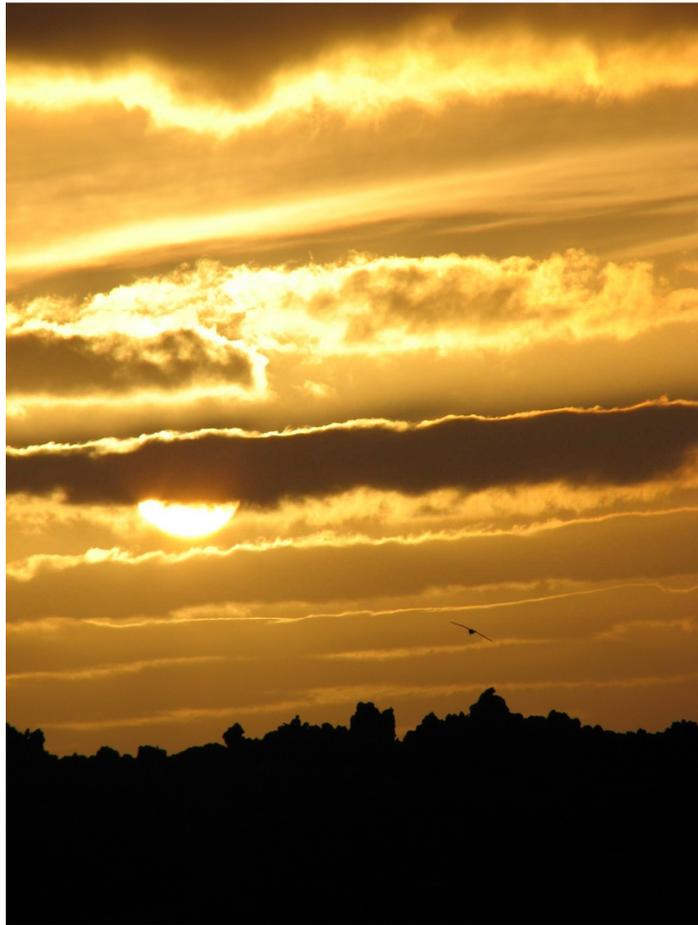
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Cryptic species, biogeographic complexity and the evolutionary history of the *Ectemnorhinus* group in the sub-Antarctic, including a description of *Bothrometopus huntleyi*, n. sp.

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Abstract: The biogeography of the South Indian Ocean Province (SIP) biotas has long been controversial. Much of the discussion has been based on interpretation of species distributions, based on morphological or anatomical delimitations. However, molecular phylogenetic approaches elsewhere have recently shown that interpretations based solely on morphological data may be misleading. Nonetheless, few studies have employed molecular phylogenetic approaches to understand the biogeography of the SIP biotas. We do so here for the *Ectemnorhinus* group of genera, a monophyletic unit of weevils endemic to the region. We use mitochondrial cytochrome oxidase I DNA sequence data to reconstruct relationships among 13 species and 22 populations in the genera *Palirhoeus*, *Bothrometopus* and *Ectemnorhinus*. On the basis of this analysis we find little support for separating the genus *Palirhoeus* from *Bothrometopus*, and little support for the morphologically-based species groups currently recognized within *Bothrometopus*. Using a molecular clock we show that dispersal among islands probably took place against the prevailing wind direction. These data also support a previous hypothesis of radiation of the epilithic genera *Bothrometopus* and *Palirhoeus* during the Pliocene/early Pleistocene, but reject the hypothesis that the genus *Ectemnorhinus* radiated following the last glacial maximum. We show that *Bothrometopus parvulus* (C.O. Waterhouse) on the Prince Edward Islands comprises two species that are not sister taxa. We name the second species *Bothrometopus huntleyi* n. sp. and provide a description thereof.

Introduction

The evolutionary history and biogeography of the sub-Antarctic islands have long been the topics of both interest and controversy. Since the biotas of the region were first described in the 1800s, many hypotheses have been proposed concerning the origins thereof and the biogeographic relationships among the various islands in the region (e.g. Gressitt 1970, Chown 1990, 1994, Michaux & Leschen 2005, Van der Putten *et al.* 2010). More generally, the geological history of the Kerguelen Plateau and the role it might have played in influencing distributions among the continents has also featured prominently in debates about the biogeographic history of the Southern Hemisphere.

Much of the discussion of sub-Antarctic biogeography has, to date, centred on assessments of species distributions based primarily on either phylogenetic analyses or presence/absence data using morphological or anatomical species delimitations (e.g. Gressitt 1970, Kuschel & Chown 1995). Indeed, even the most recent assessments, though clearly providing modern geological interpretations and contexts (e.g. Craig *et al.* 2003,

Michaux & Leschen 2005, Van der Putten *et al.* 2010) still rely heavily on such approaches. Whilst these works have provided a range of important insights (Chown *et al.* 1998, Craig *et al.* 2003) they are also limited, and modern, molecular approaches have shown how misleading interpretations, founded solely on morphologically-based distributional data, may be. In particular, they have demonstrated that dispersal across the Southern Hemisphere has been much more common than previously thought (e.g. De Queiroz 2005). In addition to providing a means for dating significant biogeographic events, molecular studies also bring additional data to bear on hypotheses of relationships among taxa and areas. Such information is particularly useful where analyses of morphological variation might be confounded by cryptic species or substantial environmental influences (see De Wever *et al.* 2009, Torricelli *et al.* 2010).

Despite the benefits that molecular approaches bring to investigations of biogeography and evolutionary history of any region and its biota, few such investigations have focussed on terrestrial taxa. The most common investigations are those of relationships among marine species and

populations across the region (Thornhill *et al.* 2008, Fraser *et al.* 2009, Wilson *et al.* 2009), and for terrestrial groups among plant taxa from New Zealand and its sub-Antarctic islands (see Michaux & Leschen 2005). Several studies have also sought to explore the phylogeography of particular species typically on a single island or archipelago (Grobler *et al.* 2006, Myburgh *et al.* 2007, McGaughran *et al.* 2010a) or relationships among populations or species on the Antarctic Peninsula and Scotia Arc islands (Allegrucci *et al.* 2006, McGaughran *et al.* 2010b). By contrast, investigations of terrestrial taxa across one or more sub-Antarctic archipelagos are limited to springtails (Stevens *et al.* 2006), ameronothroid mites (Mortimer *et al.* 2010), and the Antarctic hair grass (Van de Wouw *et al.* 2007). This situation is particularly concerning given the considerable change in perspective on the evolution and biogeography of both Antarctic and sub-Antarctic groups that has resulted from molecular approaches (reviewed in Chown & Convey 2007), and the controversy surrounding the origins of many of the groups endemic to the sub-Antarctic islands (Jeannel 1964, Chown 1994, Van der Putten *et al.* 2010).

Such controversy about origins and species relationships has been a feature of investigations of the *Ectemnorhinus* group of genera, a monophyletic unit of weevils (Kuschel & Chown 1995) restricted to the South Indian Ocean Province (or Kerguelen Biogeographic Province) of the sub-Antarctic (reviewed in Chown 1992, 1994). Although the group is small by comparison with other taxa in the Curculionidae, it is one of the most speciose monophyletic taxa in the South Indian Ocean Province (Chown 1989), providing an ideal group with which to investigate biogeographic hypotheses in the region. Thus, we provide an analysis of phylogenetic relationships among species from the genera *Palirhoeus*, *Bothrometopus* and *Ectemnorhinus*, based on the material available from Heard Island in the east to the Prince Edward Islands in the west. Whilst this study does not comprise a complete analysis of the six genera and 36 species of the group (= Ectemnorhinini (Kuschel & Chown 1995, Alonso-Zarazaga & Lyal 1999, Grobler *et al.* 2006)), it does provide a strong argument for reconsideration of the species in the group and its evolution, and, as a consequence the need for additional molecular-based investigations of taxa endemic to the sub-Antarctic.

Materials and methods

Study animals and sites

The *Ectemnorhinus* group of genera (Kuschel & Chown 1995) is confined to the South Indian Ocean Province Islands, and is thought to be most closely related to the genera *Oclandius* and *Heterexis* from the New Zealand sub-Antarctic islands (Kuschel & Chown 1995). The systematics of the group has been controversial, especially the status of species within the genera, the genera that are valid, and the evolutionary and biogeographic relationships among these taxa (Kuschel 1971, Dreux & Voisin 1987, 1989, Kuschel & Chown 1995). All of this work has been based on morphological assignments of individuals to species and subsequent assessments of the ecological characteristics and geographic distributions of these species (reviewed in Chown 1994). However, the systematic complexity of the group given its morphological variability suggests that interpretations of the systematic, biogeography and evolutionary history of the group would benefit considerably from, and likely be substantially altered by, the inclusion of molecular data.

One recent approach of this kind has shown that this is indeed the case, demonstrating that the genus *Ectemnorhinus* on the Prince Edward Islands does indeed comprise two species, though not as originally envisaged (cf. Kuschel 1971). *Ectemnorhinus similis* (= *E. marioni* junior synonym) is found on both islands, whereas *E. kuscheli* Grobler *et al.* is found on Prince Edward Island only (Grobler *et al.* 2006). Such complexity is perhaps not unexpected given the extent of variation within the genus *Ectemnorhinus*, and the intricacy of the ecological situation on the Prince Edward Islands, where individuals of the genus *Ectemnorhinus* are a preferred prey item of introduced house mice present on Marion, but not on Prince Edward Island (Chown & Smith 1993). However, both a revision of the *Bothrometopus* species on Possession Island (Chown & Kuschel 1994) and a recent assessment of the phylogeography of the species found on the Prince Edward Islands (Grobler *et al.* 2006, 2011) suggested that cryptic species and complicated evolutionary relationships may also be a feature of other genera in the *Ectemnorhinus* group. We explore this question here.

The geological and glacial histories of the South Indian Ocean Province islands have been summarized (e.g. Hall 2002, Boelhouwers *et al.* 2008, Van der Putten *et al.* 2010) and their contemporary climatic characteristics (generally cool and oceanic) and nature of their ecosystems have also been reviewed in a range of studies (e.g. Chown *et al.* 1998). The islands vary in age from 0.5 million years (m.y.) for Marion Island to *c.* 40 m.y. for the Kerguelen archipelago, with substantial variation within archipelagos in terms of age, history and extent of glaciation. Perhaps the most enigmatic of the groups in terms of its biogeography is the Crozet archipelago (Jeannel 1964, Chown 1994, Van der Putten *et al.* 2010), owing to a complex geological history.

Taxon sampling, genetic characterization and phylogenetic analysis

For this study we focussed on the genera *Palirhoeus* Kuschel, *Bothrometopus* Jeannel, and *Ectemnorhinus* G.R. Waterhouse. Whilst material of the genera *Canonopsis* C.O. Waterhouse and *Christensenia* Brinck were available, we were unable to obtain DNA in condition that was suitable for sequencing. We obtained sequence data from approximately half of the total number of species in the three

Table I. Summary of the sampling localities from which the genetically characterized specimens included in this study were collected.

Species	Sampling locality (a.s.l.)	Geographic coordinates		Number of specimens per locality
B. parvulus	Ship's Cove MI (0 m)	46°51'41"S	37°50'66"E	5
	Trypot Beach MI (0 m)	46°53'05.2"S	37°52'06"E	1
	Goodhope Bay MI (0 m)	46°57'55.9"S	37°42'04.4"E	2
	Cape Davis MI (0 m)	46°49'41.2"S	37°41'83.3"E	3
	Kildalkey Bay MI (0 m)	46°57'38.3"S	37°51'22.2"E	3
	McAll Coast PEI (0 m)	NA		3
B. randi	Ship's Cove MI (0 m)	46°51'41"S	37°50'66"E	1
	Trypot Beach MI (0 m)	46°53'05.2"S	37°52'06"E	1
	Water Tunnel MI (0 m)	46°57'49.2"S	37°44'50.44"E	1
	Goodhope Bay MI (0 m)	46°57'55.9"S	37°42'04.4"E	1
	Long Ridge South MI (450 m)	46°52'45"S	37°47'00"E	1
	Katedraalkrans MI (800 m)	46°53'89.6"S	37°46'48.2"E	1
	Tafelberg MI (250 m)	46°53'03.5"S	37°48'20.1"E	1
	Feldmark Plateau MI (600 m)	46°56'35"S	37°46'10"E	1
	Pyroxene Kop MI (600 m)	46°56'43.4"S	37°41'40.5"E	2
	Cave Bay PEI (0 m)	46°38'75.2"S	37°59'78"E	1
B. huntleyi	Ship's Cove MI (0 m)	46°51'41"S	37°50'66"E	2
	Kildalkey Bay MI (0 m)	46°57'38.3"S	37°51'22.2"E	1
	Trypot Beach MI (0 m)	46°53'05.2"S	37°52'06"E	1
	First Red Hill MI (400 m)	46°53'41.2"S	37°48'21"E	2
	Junior's Kop MI (200 m)	46°52'79.4"S	37°50'08.3"E	1
	Feldmark Plateau MI (600 m)	46°56'35"S	37°46'10"E	1
	Cave Bay PEI (0 m)	46°38'75.2"S	37°59'78"E	4
	PEI (400 m)	46°38'21.1"S	37°57'48.2"E	1
	Top of VZB PEI (672 m)	46°37'59"S	37°55'89.1"E	1
B. elongatus	Tafelberg MI (250 m)	46°53'03.5"S	37°48'20.1"E	1
	Stony Ridge MI (150 m)	46°54'88.1"S	37°51'48.4"E	1
	Long Ridge South MI (450 m)	46°52'45"S	37°47'00"E	1
	PEI (600 m)	46°37'53.3"S	37°55'98.5"E	2
B. fasciatus	Possession Island*	46°25'33.9"S	51°51'38.2"E	2
B. gracilipes	Heard Island*	53°01'09.4"S	73°23'30.5"E	2
B. angusticollis	Kerguelen Island*	49°21'05.7"S	70°13'09.4"E	2
B. sulcatus	Kerguelen Island*	49°21'05.7"S	70°13'09.4"E	5
B. brevis	Kerguelen Island*	49°21'05.7"S	70°13'09.4"E	7
	Heard Island*	53°01'09.4"S	73°23'30.5"E	2
E. similis	Junior's Kop MI (200 m)	46°52'79.4"S	37°50'08.3"E	1
	Ice Plateau MI (1000 m)	46°54'29"S	37°45'37.5"E	1
	Cave Bay PEI (0 m)	46°38'75.2"S	37°59'78"E	2
E. kuscheli	Cave Bay PEI (0 m)	46°38'75.2"S	37°59'78"E	1
	PEI (400 m)	46°38'21.1"S	37°57'48.2"E	1
E. viridis	Heard Island*	53°01'09.4"S	73°23'30.5"E	2
	Kerguelen Island*	49°21'05.7"S	70°13'09.4"E	1
P. eatoni	Ship's Cove MI (0 m)	46°51'41"S	37°50'66"E	3
	Kildalkey Bay MI (0 m)	46°57'38.3"S	37°51'22.2"E	1
	Trypot Beach MI (0 m)	46°53'05.2"S	37°52'06"E	1
	Cave Bay PEI (0 m)	46°38'75.2"S	37°59'78"E	2
	Heard Island*	53°01'09.4"S	73°23'30.5"E	1

VZB = Van Zinderen Bakker Peak, * = geographic coordinates given for the scientific stations on Kerguelen and Possession Islands, and for Atlas Cove on Heard Island.

genera and what we thought initially was 12 species and 20 populations representing all of the major archipelagos, but which following analysis turned out to be 13 species from 22 populations (Table I). The most comprehensive sampling was undertaken on the most readily accessible Prince Edward Islands (see also Grobler *et al*. 2006, 2011). For an outgroup, we used two COI gene sequences from *Sitona discoideus* (Curculionidae: Etiminae; Genbank accession numbers EF118292 and EF118299) from Norfolk Island, Australia (Vink & Phillips 2007).

DNA from each individual was extracted from a leg which, following removal from ethanol was washed and rehydrated in distilled water for ten minutes prior to being frozen in liquid nitrogen and ground in individual Eppendorf tubes using an Eppendorf pestle. DNA was extracted using the High Pure PCR Template Preparation
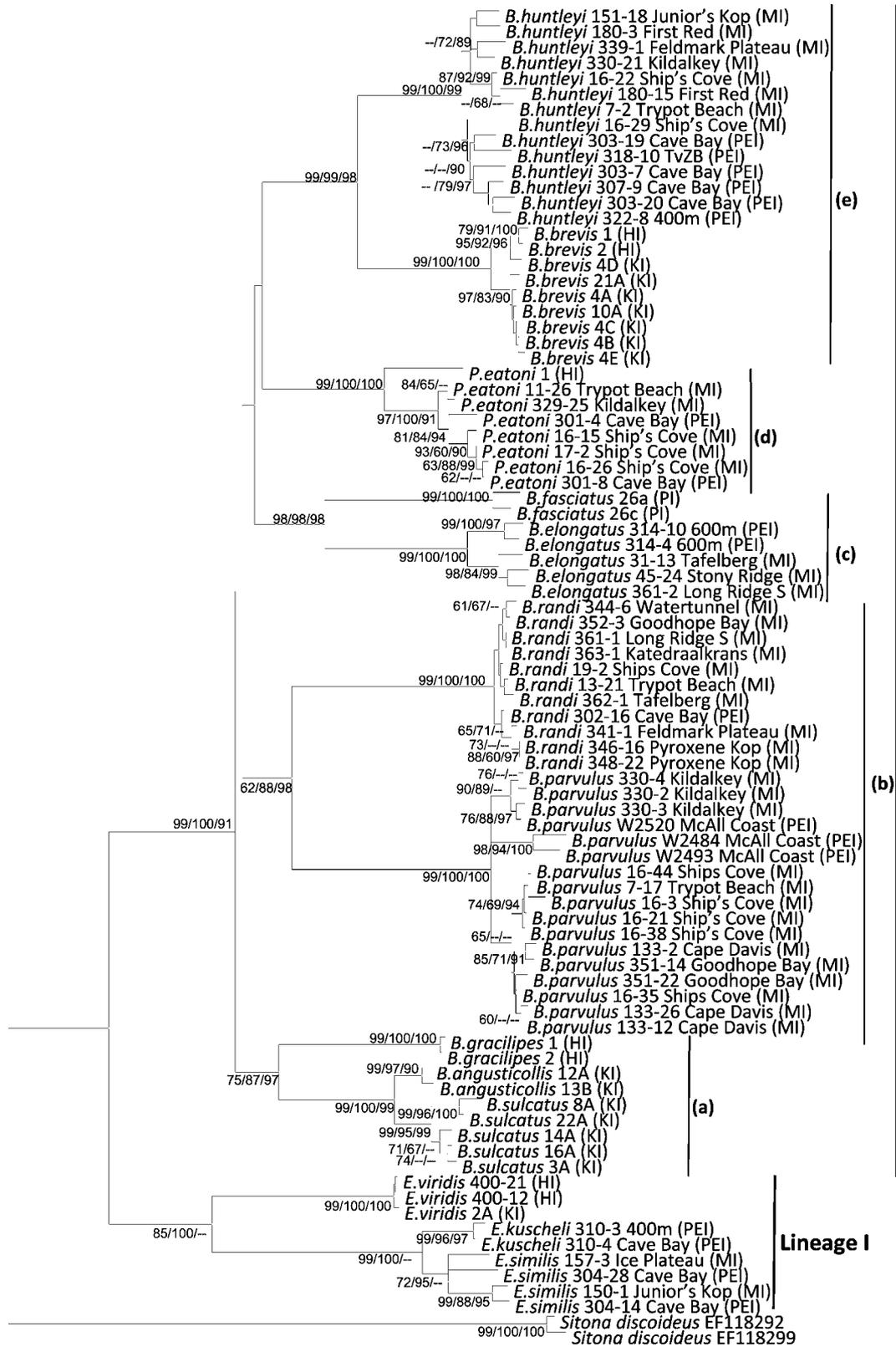
Kit (Roche Applied Science) using the supplier's procedure for isolation of nucleic acids from mammalian tissue with modification to the proteinase K tissue lysis incubation step which was performed for 24 h instead of the recommended 1 h for mammalian tissue.

Taxon-specific COI primers, GF5-1940 and GR5-2935 (Grobler *et al.* 2006), were used to amplify a 996 bp PCR product under previously described reaction conditions (Grobler *et al.* 2006) using a thermal cycling profile comprising an initial denaturation step at 94°C for 90 s, followed by 40 cycles of 94°C for 22 s, 46°C for 30 s and 72°C for 1 min and concluding with a final extension step of 1 min at 72°C. PCR products of the correct size were purified directly from the tube using a Roche High Pure PCR Product Purification Kit. DNA sequences were determined by automated cycle sequencing reactions run on an ABI PRISM™ 3100 Analyser and generated using the ABI PRISM Big Dye™ Terminator V3.0 sequencing standard (Applied Biosystems). The sequences were viewed, edited and aligned using the alignment explorer function incorporated within the MEGA4 programme (Tamura *et al.* 2007).

Neighbour-Joining (NJ) and Minimum Evolution (ME) algorithms in MEGA4 (Tamura *et al.* 2007) were used to construct distance trees. Bayesian inference (BI) using MrBayes version 3.1.2 (Ronquist & Huelsenbeck 2003) was performed with the model and parameters estimated in jModelTest 0.1.1 (Guindon & Gascuel 2003, Posada 2008) under the Akaike Information Criterion (AIC). The analysis was initiated with random starting trees with four parallel runs for 10 000 000 generations using one cold and three heated Markov chains using the default heating setting. The Markov chains were sampled every 1000 generations. Tracer plots were visually inspected and tracer diagnostics (standard deviation of split frequencies, effective sample size), as implemented in MrBayes and Tracer v1.4 (Drummond & Rambaut 2007) were checked to ensure that the Markov chain had reached stationarity. Of the 10 000 trees obtained 2000 were discarded as "burn-in" and the trees were summarized using an 'all-compatible' consensus. Maximum parsimony (MP) analyses were performed in PAUP* (Swofford 2003). Starting trees were obtained by closest stepwise addition and heuristic searches were performed using the tree-bisection reconnection (TBR) branch swapping algorithm. Characters were unordered and assigned equal weights in the initial analysis, and subsequently reweighted using the rescaled consistency (RC) index as detailed

previously by Farris (1969). Nodal support was assessed by 100 bootstrap replicates.

Haplotype (h) and nucleotide diversities (π) were estimated in DNASP 5.00.07 (Librado & Rozas 2009). To obtain more accurate divergence estimates for the older splits, the standard 2.3% nucleotide sequence divergence per million years estimate (Brower 1994) was used in combination with a model of sequence evolution that corrects for multiple hits and accounts for rate heterogeneity (Papadopoulou *et al.* 2010). We therefore retained and imposed the original 2.3% estimate as it was shown to correspond well with the mean mtDNA divergence rate obtained for Aegean tenebrionids (2.23% and 2.39% m.y^{-1}) when using the GTR+Γ+I model under a strict and relaxed clock, respectively (Papadopoulou *et al.* 2010). BEAST 1.5.3 (Drummond & Rambaut 2007) was used to obtain an ultrametric tree using Bayesian MCMC analysis orientated towards rooted, time-measured phylogenetics. Well supported nodes identified following NJ, ME, MP and BI analyses were constrained to be monophyletic and the HKY+I+Γ model identified in jModelTest 0.1.1 (Posada 2008, Guindon & Gascuel 2003) under the AIC was enforced using a strict molecular clock model. The results of two independent runs were merged and analysed with Tracer v1.4 and TreeAnnotator v1.4.7 (Drummond & Rambaut 2007).

Results

Genetic characterization and phylogenetic analyses

All sequences used in our final dataset were 885 bp in length and correspond to nucleotide positions 514 to 1399 of the COI gene. All novel sequences have been deposited in the Genbank database under accession numbers: GQ856478-80, GQ856482-8, GQ856490-1, GQ856493–GQ856500 and GU947664–GU947703, and were complemented with nucleotide sequence entries from two other studies, *viz.* AY762278, AY762285, AY762298-9, AY762317-20 (Grobler *et al.* 2006) GQ131943, GQ131946, GQ131952, GQ131954-5, GQ131961, GQ131967, GQ131979, GQ131997, GQ131999, GQ132004, GQ132006, GQ132009, GQ132012-4 (Grobler *et al.* 2011).

Of the 885 sequenced sites 592 were conserved across all 86 specimens in the dataset. Of the 293 variable sites 277 sites were parsimony informative and 159 of the latter were assigned weights other than one after rescaled consistency index (RCI) character reweighting. Parsimony

Fig. 1. Minimum Evolution (ME) tree of 13 species from the *Ectemnorhinus* group of genera based on 885 nucleotides of the mitochondrial cytochrome oxidase I (COI) gene. Each taxon label contains the species designation, sample number, sampling locality, and island of origin. Nodal support values obtained from 10 000 bootstrap replications (ME), 100 bootstrap replications from Maximum Parsimony (MP) and posterior support from Bayesian Inference (BI) analyses, expressed as percentages and denoted ME/MP/BI on each node. '- -' indicates support values < 65 (for ME and MP) and < 90 (for BI). The scale indicates the number of nucleotide substitutions. Islands are abbreviated as follows: Marion Island (MI), Prince Edward Island (PEI), The Prince Edward Island Archipelago (PEIA), Heard Island (HI), Kerguelen Island (KI) and Possession Island (PI).
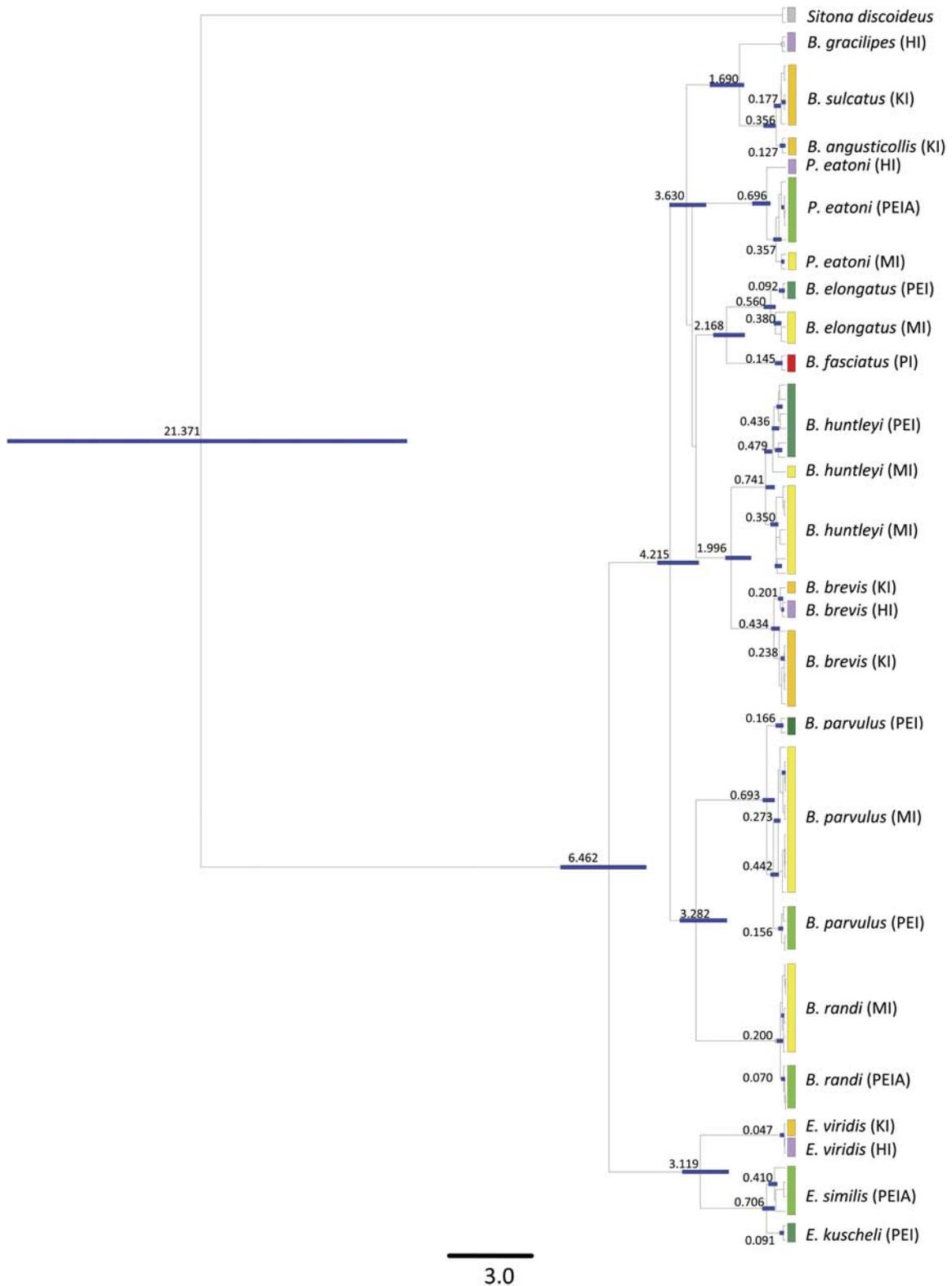
Fig. 2. Ultrametric tree obtained with BEAST with a clock rate of 2.3% sequence divergence per million years. The topology was constrained to retain monophyletic lineages recovered across all methods of inference (i.e. NJ, MP and BI). The numbers in the nodes correspond to the estimated age in million years, and the blue bars to the 95% confidence interval. The scale indicates change in million years. Islands are abbreviated as follows: Marion Island (MI), Prince Edward Island (PEI), The Prince Edward Island Archipelago (PEIA), Heard Island (HI), Kerguelen Island (KI) and Possession Island (PI).

analyses with equal weighted characters recovered 92 trees with a length of 779 and homoplasy indexes of: CI = 0.485, RI = 0.898 and RCI = 0.435. The analysis in which characters were RCI reweighted also recovered 92 trees, all 342.97 in length, with homoplasy indexes of: CI = 0.672, RI = 0.935 and RCI = 0.629.

The HKY+I+Γ model of sequence evolution selected under the AIC in jModelTest 0.1.1 (Guindon & Gascuel 2003, Posada 2008) recovered a transition transversion ratio of 4.4317, a gamma distribution shape parameter (Γ) of 1.000, proportion of invariable sites (I) = 0.6020 and base frequencies of A = 0.3462, C = 0.1528, G = 0.1012 and T = 0.3998 (% AT = 74.60%). The molecular phylogenies obtained with the different inference methods were topologically similar and recovered two main evolutionary lineages (denoted I and II in Fig. 1) for the *Ectemnorhinus* group of genera. Pairwise uncorrected p-distance comparisons of each monophyletic lineage/species within these lineages revealed mean inter-specific sequence divergence values of between 1.8 and 13.1%, and mean intra-specific diversity values ranging from 0.1 to 1.2% (see supplementary table S1 at www.journals.cambridge.org/jid_ANS). Lineage I (85% bootstrap support from ME and 100% from MP) which contains all of the *Ectemnorhinus* species characterized in this study is basal to the lineage II (99% and 100% bootstrap support from ME and MP, respectively) containing representatives of the genera *Palirhoeus* and *Bothrometopus*. Of the three *Ectemnorhinus* species characterized, *E. viridis* is basal to *E. similis* and *E. kuscheli* and intra-specific divergence for this species is low despite the fact that the *E. viridis* individuals are from different (Heard and Kerguelen) islands. According to the age estimates in Fig. 2, *E. viridis* last shared a common ancestor with the *Ectemnorhinus* species from the Prince Edward Archipelago approximately 3.12 million years ago (m.y.a.). *Ectemnorhinus kuscheli* from Prince Edward Island is basal to *E. similis* that occurs on both Marion Island and Prince Edward Island, and they shared their last common ancestor *c.* 0.71 m.y.a. (Fig. 2).

Lineage II comprises five monophyletic lineages (labelled a–e in Fig. 1) that coalesced approximately 4.22 m.y.a. These clades contain all nine *Bothrometopus* species characterized in this study as well as *Palirhoeus eatoni* (Fig. 1, clade d) suggesting that the monotypic genus *Palirhoeus* should be synonymized with *Bothrometopus* pending confirmation from nuclear gene analyses. Within the *Palirhoeus* lineage, which is estimated to have arisen *c.* 0.696 m.y.a., the *P. eatoni* specimen from eastern Heard Island, is basal to the western Prince Edward Islands' specimens. *Bothrometopus gracilipes*, *B. angusticollis* and *B. sulcatus* group together in a monophyletic clade (Fig. 1, clade a) with 75–91% nodal support. The Heard Island *B. gracilipes* lineage is estimated to have diverged from the remaining species approximately 1.69 m.y.a. The sister taxa *B. angusticollis* and *B. sulcatus*, represented by specimens from Ile Kergeulen, diverged *c.* 0.356 m.y.a.
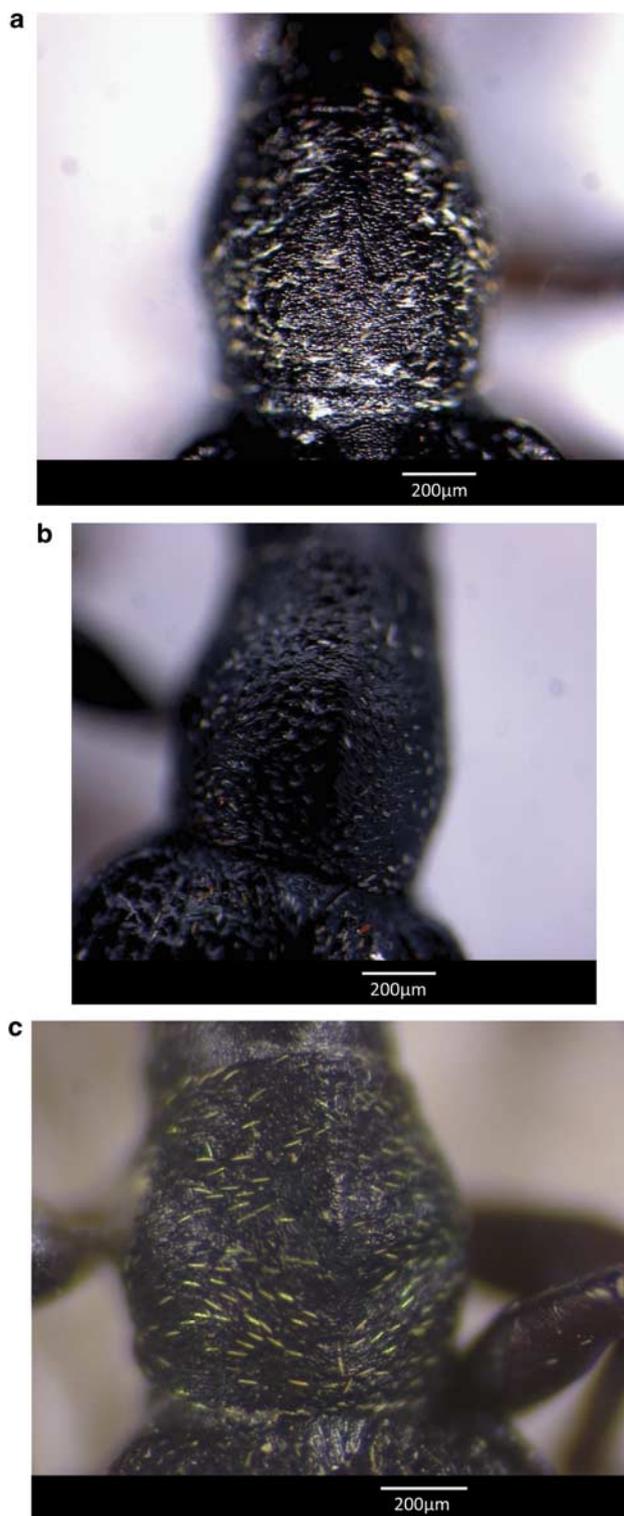

Fig. 3. Light micrographs of the pronota of **a.** *B. parvulus*, **b.** *B. huntleyi*, and **c.** *B. parvulus* type specimen from the National History Museum, London. Both the type specimen and *B. parvulus* show granular microsculpture on the pronotum. The pronotum of *B. huntleyi* is smoother in appearance.

Bothrometopus fasciatus from Possession Island, groups with, and is basal to, *B. elongatus* from the Prince Edward Islands (Fig. 1, clade c). The estimated time to *B. fasciatus* and *B. elongatus* lineage coalescence is *c*. 2.2 m.y.a. Individuals of *B. elongatus* from Prince Edward Island are distinct from those from Marion Island, diverging *c*. 0.56 m.y.a. Additional *B. elongatus* specimens would need to be examined to determine the extent of gene flow between Prince Edward and Marion Islands.

When examining the remaining two clades (Fig. 1b & e) it became clear that both clades contain individuals from the Prince Edward Islands archipelago, identified morphologically as *B. parvulus*, but which are not sister taxa. One of

these clades is sister to *B. randi* from the Prince Edward Islands, having diverged from this sister taxon approximately 3.3 m.y.a., whilst the other morphologically similar counterpart, groups with *B. brevis* from the Kerguelen and Heard islands, constituting a lineage which is estimated to have arisen *c*. 2.0 m.y.a. (Fig. 2).

Detailed external morphological examination of these two species, and comparison with images of the holotype of *B. parvulus* held by the Natural History Museum, London, revealed considerable similarity, with the exception of the microsculpture of the pronotum, which provides a reliable means of distinguishing between them (and also between some species on Possession Island, see Chown & Kuschel 1994). In the case of the holotype of *B. parvulus*, and indeed all material henceforth assigned to that species, the pronotal microsculpture appears pointillistic under a light microscope with granular microsculpture (Fig. 3a & c), and alutaceous when examined using scanning electron microscopy (Fig. 4). By contrast, the other species, which we describe formally below, has a smoother appearance under both light (Fig. 3b) and electron microscopy (Fig. 4), with distinct large punctations. No other completely reliable means exist to distinguish morphologically between these two species, but the characters are 100% reliable, as assessed via two independent approaches. First, morphology-based, in which one of us (SLC) with no advance knowledge of specimen identity, visually matched all specimens to the sequence data determinations with 100% congruence. Second, based on morphology, additional material from Prince Edward
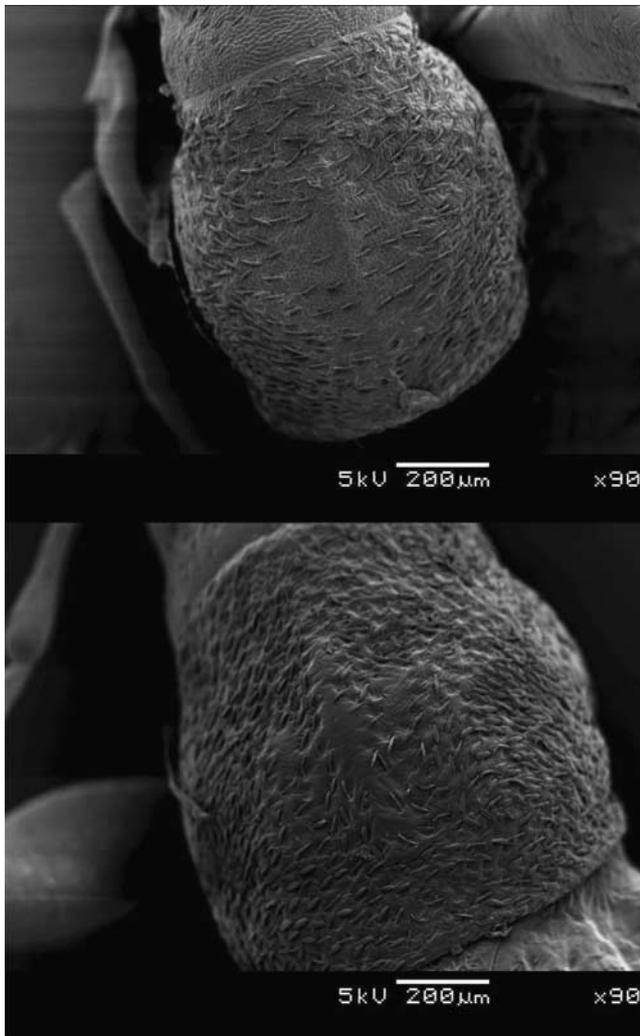

Fig. 4. Scanning electron microscopic comparison of the thorax of *B. parvulus* (top) and that of *B. huntleyi* (bottom) using scanning electron microscopy (SEM). No distinct setal patterning can be discerned, however the *B. parvulus* specimen appears to have a more granular surface and fewer scales than *B. huntleyi*. This feature can be observed with a standard, light microscope and can be used to readily distinguish *B. parvulus* from *B. huntleyi*.

Fig. 5. Dorsal habitus of *B. huntleyi* n. sp. male (length from anterior of eyes to posterior of elytra = 4.7 mm).
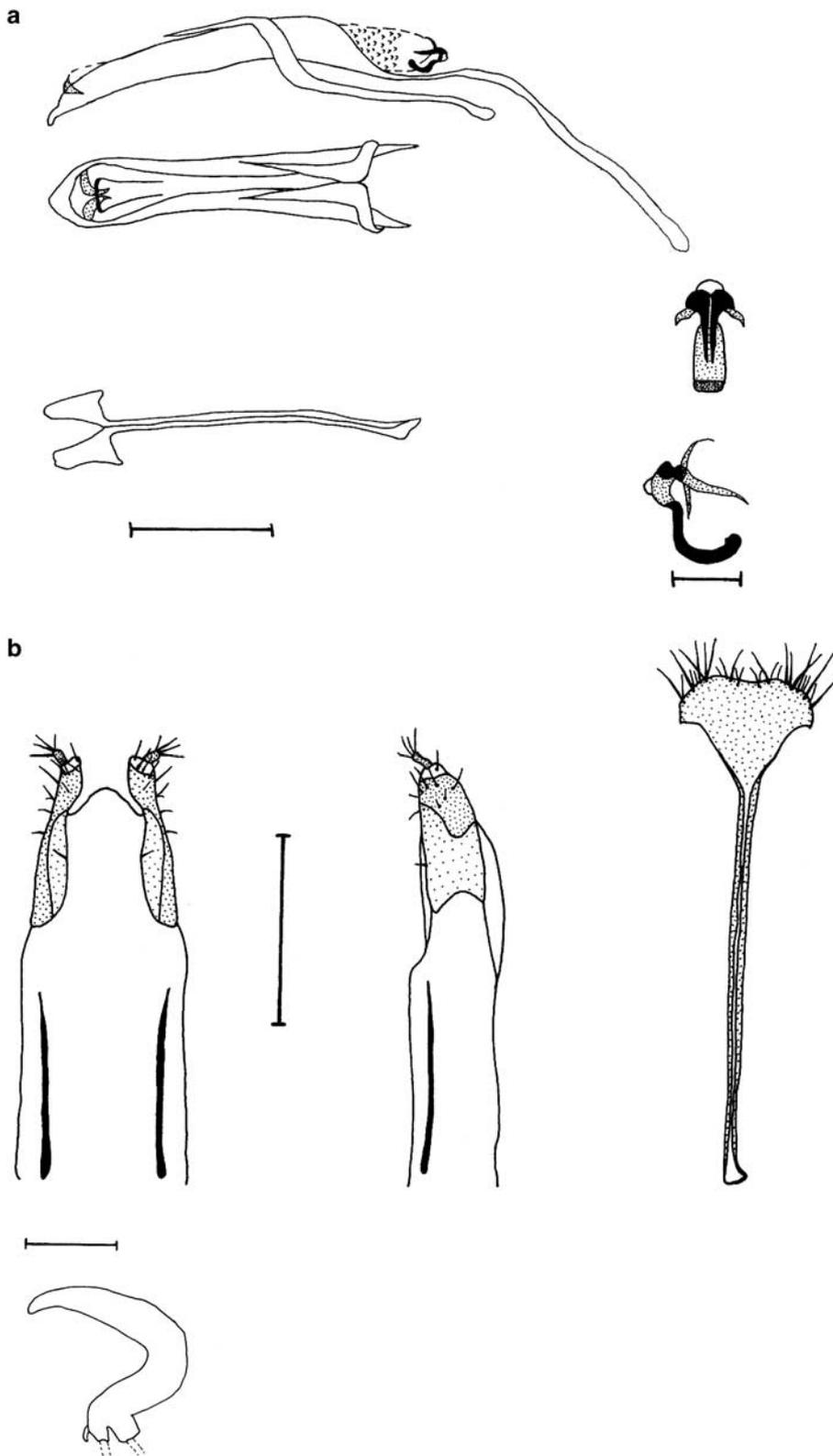
Fig. 6. *Bothrometopus huntleyi* n. sp. **a.** Male genitalia with the aedeagus in lateral and dorsal views (scale bar = 0.5 mm) with the genital armature to the right (scale bar = 0.1 mm). **b.** Female genitalia in dorsal and lateral view (scale bar = 0.5 mm). The spermatheca is shown below (scale bar = 0.2 mm).

Island was identified by one of us (AMT) and then provided to another author (GCG) who sequenced the material without prior knowledge of morphological assignment.

The assignment match was 100%. We also noted that the individuals that correspond to *B. parvulus* appear to be restricted to coastal regions whereas the new species is

distributed island-wide. The new cryptic species, *Bothrometopus huntleyi*, initially identified as *B. parvulus* based on morphology, is formally described below and compared to *B. parvulus*.

Bothrometopus huntleyi n. sp.

Description: Length (anterior of eyes to posterior of elytra): Overall: 3.1–5.5 mm; males: mean ± S.E. = 4.1 ± 0.03 mm ($n = 156$); females: 4.4 ± 0.04 mm ($n = 136$). Body dark brown to black with a variable covering of green to blue scales on the dorsal surface; the ventral surface is black (Fig. 5). The density of scales is highest on the elytra, most variable on the prothorax and sparse on the head and femora. The tibiae and tarsi lack scales, with the former having stiff, spine-like setae. On the elytra the scales occasionally form an anchor-shaped pattern, or two spots, one on each of the elytra. Where the scale density is high the scales are not imbricate. Occasionally, on the lateral margins of the elytra, small, fine and transparent to golden-brown to green erect hair-like scales may be present. These do not resemble the stiff, marked erect spines found on the elytra of species in the genus *Ectemnorhinus*. Antennae with light-brown to reddish-brown scape, reddish-brown funicle and dark-brown to almost black club. The first three funicle segments typically have the ratio 0.94:1:0.61 (Fs1:Fs2:Fs3) ($n = 10$). Epistome symmetric, sometimes with pronounced lobes, but also with a straight margin. Mandibles reddish-brown, each one asymmetric, with the dorsal tooth more pronounced than the ventral tooth, except after substantial wear. Labial palps three-segmented. Ommatidia coarse. Prothorax with an indistinct to distinct dorsal carina which can occasionally be entirely absent; where present it tends not to run the full length of the prothorax. Dorsal surface of the prothorax with pronounced punctations with an otherwise smooth surface between them. No granular microsculpturing is present. Elytra obovate each with a humeral carina which is moderately to well developed. Striations are pronounced as a consequence of deep punctations that are virtually contiguous. Legs reddish-brown to black with lighter colouration towards the base of the femora. Third tarsal segment with a ventral surface of densely packed white setae forming a brush. Tarsal claw segment shorter than the other three segments combined. Aedeagus as in Fig. 6a with a unique basal sclerite. Female genitalia as in Fig. 6b.

Etymology: This new species is named in honour of the youngest biologist on the first biological and geological expedition (1965/1966) to the Prince Edward Islands: Brian John Huntley.

Remarks: Bothrometopus huntleyi is a medium-sized *Bothrometopus* species – the genus varies from *c.* 2–10 mm in length (Chown & Kuschel 1994, Kuschel & Chown 1995). It is morphologically very similar to *B. parvulus* (C.O. Waterhouse) from the Prince Edward Islands and *B. brevis* (C.O. Waterhouse) from Kerguelen and Heard islands. Adults of *B. huntleyi* can be separated from *B. parvulus* based on the former species' deep punctations and lack of granular microsculpture on the prothorax, dorsal carina which does not stretch from end to end of the prothorax, and typically lighter funicle segments of the antennae by comparison with the general body colouration. The most reliable distinguishing feature is the difference in microsculpture on the prothorax of the two species (as described above and shown in Figs 3 & 4). No characters have yet been found to distinguish the larvae.

Distribution: Island-wide (coastal rocks and inland areas, see Chown 1989, 1992) on both Marion Island and Prince Edward Island. This contrasts with *B. parvulus*, which thus far has only been found on coastal rocks at both Marion Island and Prince Edward Island. The phylogeography of this new species is discussed in detail in Grobler *et al.* (2011).

Material examined

Holotype:
♂, South Africa, Marion Island, 400 m a.s.l., First Red Hill, 46°53.412'S, 37°48.21'E, Genbank no. GQ131999, voucher no. 180-15, collected April 2001, collector G.C. Grobler. Deposited in the Iziko South African Museum, Cape Town, South Africa.

Paratypes:
♀, South Africa, Marion Island, 0 m a.s.l., 'Ship's Cove', 46°51'41"S, 37°50'66"E, Genbank no. GQ132012, voucher no. 16-22, collected April 2001, collector G.C. Grobler. Deposited in the Iziko South African Museum, Cape Town, South Africa.
♂, South Africa, Marion Island, 200 m a.s.l., Junior's Kop, 46°52.794'S, 37°50.083'E, Genbank no. GQ131946, voucher no. 151-18, collected April 2001, collector G.C. Grobler. Deposited in the Iziko South African Museum, Cape Town, South Africa.
♀, South Africa, Marion Island, 600 m a.s.l., Feldmark Plateau, 46°56'35"S, 37°46'10"E, Genbank no. GQ131952, voucher no. 339-1, collected April 2002, collector G.C. Grobler. Deposited in the Iziko South African Museum, Cape Town, South Africa.
♂, South Africa, Marion Island, 400 m a.s.l., First Red Hill, 46°53.412'S, 37°48.21'E, Genbank no. GQ131967, voucher no. 180-3, collected April 2001, collector G.C. Grobler. Deposited in the Natural History Museum, London, United Kingdom.
♀, South Africa, Marion Island, 0 m a.s.l., 'Ship's Cove', 46°51'41"S, 37°50'66"E, Genbank no. GQ131943, voucher no. 16-29, collected April 2001, collector G.C. Grobler. Deposited in the Natural History Museum, London, United Kingdom.
♂, South Africa, Prince Edward Island, 0 m a.s.l., Cave Bay, 46°38.752'S, 37°59.780'E, Genbank no. GQ131954, voucher no. 303-19, collected April 2003, collector G.C. Grobler. Deposited in the Iziko South African Museum, Cape Town, South Africa.

Table II. Summary of the 43 nucleotide sites in the COI gene region characterized in this study, that are consistently different between *B. parvulus* and *B. huntleyi*.

Nucleotide site	Base position	*B. parvulus*	*B. huntleyi*
9	3rd	T	A
33	3rd	T	C
39	3rd	T	A
42	3rd	A	G
63	3rd	T	A
64	1st	C	T
82	1st	C	T
84	3rd	T	G
87	3rd	A	T
141	3rd	T	C
153	3rd	T	A
195	3rd	A	T
285	3rd	T	A
288	3rd	T	C
321	3rd	A	C
333	3rd	C	T
348	3rd	T	C
360	3rd	A	G
366	3rd	T	C
444	3rd	A	T
468	3rd	T	A
482	2nd	C	A
486	3rd	A	T
492	3rd	C	T
493	1st	C	T
507	3rd	T	A
543	3rd	C	A
547	1st	G	A
585	3rd	T	C
615	3rd	C	T
618	3rd	T	C
648	3rd	A	T
651	3rd	A	T
669	3rd	T	C
672	3rd	C	A
699	3rd	C	T
706	1st	T	C
712	1st	G	A
714	3rd	C	T
750	3rd	G	A
765	3rd	T	C
780	3rd	C	T
876	3rd	C	T

♀, South Africa, Prince Edward Island, 672 m a.s.l., Top of van Zinderen Bakker, 46°37.590'S, 37°55.891'E, Genbank no. GQ131961, voucher no. 318-10, collected April 2003, collector G.C. Grobler. Deposited in the Iziko South African Museum, Cape Town, South Africa.

♂, South Africa, Prince Edward Island, 0 m a.s.l., Cave Bay, 46°38.752'S, 37°59.780'E, Genbank no. GQ131955, voucher no. 303-20, collected April 2003, collector G.C. Grobler. Deposited in the Iziko South African Museum, Cape Town, South Africa.

♀, South Africa, Prince Edward Island, 400 m a.s.l., 46°38.211'S, 37°57.482'E, Genbank no. GQ132006, voucher no. 307-9, collected April 2003, collector G.C. Grobler.

Deposited in the Iziko South African Museum, Cape Town, South Africa.

♂, South Africa, Prince Edward Island, 400 m a.s.l., 46°38.211'S, 37°57.482'E, Genbank no. GQ132004, voucher no. 322-8, collected April 2003, collector G.C. Grobler. Deposited in the Natural History Museum, London, United Kingdom.

♀, South Africa, Prince Edward Island, 0 m a.s.l., Cave Bay, 46°38.752'S, 37°59.780'E, Genbank no. GQ131997, voucher no. 303-7, collected April 2003, collector G.C. Grobler. Deposited in the Natural History Museum, London, United Kingdom.

Additional material was examined for the morphometric analysis on which the length measurements used in the description are based (A. Treasure and S.L. Chown, unpublished data).

Molecular comment

DNA barcoding, its recognized flaws notwithstanding (Rubinoff 2006), was considered here as a complementary tool for the unequivocal differentiation of *B. parvulus* from *B. huntleyi*. The 43 nucleotide sites that are conserved within species, and consistently different between the two morphologically indistinct species occurring on PEIA are summarized in Table II. When comparing the partial amino acid COI gene sequences of the thirteen species of the *Ectemnorhinus* group of genera generated in this study, 17 non-synonymous amino acid substitutions were observed in the 84 ingroup taxon dataset. These non-synonymous amino acid substitutions revealed several consistent and therefore possibly diagnostic differences between species and include the following positions in our dataset: Codon 7 (I in *B. elongatus* and M in all other species, except for two *B. sulcatus* specimens which have a V at this position); Codon 19 (V in *E. viridis* and I in all other species); Codon 41 (all species within the genus *Ectemnorinus* have an I at this position, whereas a V is present in all species of the genera *Bothrometopus* and *Palirhoeus*); Codon 161 (T in *B. parvulus* and N in all other species); Codon 183 (V in *B. parvulus* and I in all other species); Codon 241 (M in *B. gracilipes*, and either a V or a L in all other species). As some of the species in this study are only represented by two specimens, additional data will need to be generated to determine the consistency and species-exclusivity of some of these characters.

Discussion

The phylogenetic analyses revealed three major points. First, the monotypic genus *Palirhoeus* is not readily distinguishable, on a mtCOI sequence basis, from the genus *Bothrometopus*, thus questioning the retention of the species *P. eatoni* in a separate genus, *Palirhoeus*, created by Kuschel (1971), and its position in Kuschel & Chown's (1995) phylogeny as basal to the genera *Bothrometopus* and *Ectemnorhinus*. Nonetheless, limited taxon and gene

sampling means that we refrain from proposing formal generic synonymy. Second, the two species groups in the genus *Bothrometopus* (*fasciatus* group and *gracilipes* group), identified on the basis of absence or presence of dorsal wall vaginal spicules, by Kuschel & Chown (1995) are not supported by the COI gene phylogeny. *Bothrometopus gracilipes* and *B. angusticollis* fall into the *gracilipes* group of *Bothrometopus* species (Kuschel & Chown 1995) while *B. sulcatus* falls in the *fasciatus* group of *Bothrometopus* species (Kuschel & Chown 1995). The sister taxon relationship of *B. elongatus*, which is assigned to the *gracilipes* group, with *B. fasciatus* from the *fasciatus* group of *Bothrometopus* species (Kuschel & Chown 1995) in the COI gene tree also raises questions regarding the phylogenetic utility of these two major groups. Third, what was previously considered a single species on the Prince Edward Islands, *B. parvulus* Jeannel, is clearly two species that are certainly not sister taxa, but rather share relationships with different species from our sample taxa. Identification of this cryptic species increases the number of species within the *Ectemnorhinus* group of genera from 36 to 37.

Despite being a partial analysis of this group of weevils endemic to the South Indian Ocean Province Islands, the current study has important implications for interpretation of biogeographic and evolutionary dynamics in the region more generally. Perhaps the most significant point to emerge is that colonization of the Prince Edward Islands is likely to have taken place repeatedly from other islands in the South Indian Ocean Province. Thus, although *B. parvulus* and *B. randi* are sister species in the current tree (Fig. 1), the molecular clock based on a 2.3% nucleotide sequence divergence per million years estimate obtained from an arthropod mtDNA survey of Brower (1994), which has proven useful for studies of this group (see Grobler *et al.* 2006), indicates that divergence must have taken place approximately *c.* 3.3 m.y.a. (Fig. 2). This could not have happened on the Prince Edward Islands because the oldest date for the islands is *c.* 0.5 m.y., and there is no geological evidence to suggest that they are very much older than this (Boelhouwers *et al.* 2008). The date of the divergence between *B. huntleyi* and *B. brevis*, *c.* 2.0 m.y.a., also suggests that an early colonization of the Prince Edward Islands is unlikely. Instead, the dated phylogeny suggests that dispersal to the Prince Edward Islands must have occurred from elsewhere, sometime after the islands emerged, and on at least two separate occasions. Because we were unable to sample all taxa in the genus *Bothrometopus* (see Chown & Kuschel 1994, Kuschel & Chown 1995 for review) it seems likely that the colonization has been from species on the Crozet archipelago. *Bothrometopus randi* (the sister species of *B. parvulus*, based on this analysis) is known from Possession Island and other *Bothrometopus* species are widespread across Iles Crozet (Chown & Kuschel 1994). Such an hypothesis of colonization against the prevailing west wind drift is not new,

and was in fact proposed by Dreux and Voisin in a series of works on the group (e.g. Dreux & Voisin 1987, 1989). Thus, unlikely as their hypotheses may have seemed initially, they cannot, on present evidence, be rejected. Indeed, it also appears that *P. eatoni* colonized the Prince Edward Islands relatively recently (Figs 1 & 2) and that dispersal between Marion Island and Prince Edward Island has been quite common since their emergence.

Several independent lines of evidence support this proposal of repeated colonization across the region. Using a molecular phylogenetic approach, Stevens *et al.* (2006) demonstrated that repeated colonizations across the sub-Antarctic islands probably took place from the late Miocene (*c.* 7 m.y.a.) to approximately 0.3 m.y.a. Likewise, recent investigations of the ameronothroid mite genera *Halozetes* and *Alaskozetes* have shown colonization of the islands by species in these genera over the last ten million years (Mortimer *et al.* 2010). These dates also correspond closely with those for dispersals among populations of the springtail *Cryptopygus antarcticus* in the Scotia Arc and Antarctic Peninsula region (McGaughran *et al.* 2010b), and trans-Drake Passage dispersal of the nudibranch *Doris kerguelenensis* (Wilson *et al.* 2009). However, the divergence times differ substantially for those estimated for the bull kelp *Durvillaea antarctica*, which apparently recolonized the South Indian Ocean Province Islands after its removal during the last glacial maximum, *c.* 16 000 years ago (Fraser *et al.* 2009).

These dispersal dates indicate that for the terrestrial species much of the diversification considerably preceded the last glacial maximum and many events date to either the Pliocene–early Pleistocene, or as soon as a particular island group (such as the Prince Edward Islands) emerged. Thus, it appears likely that the groups survived several glacial cycles in refugia on the islands, and are certainly not post-glacial colonists. Such proposals have been made previously for various groups (see discussions in Chown 1990, Van der Putten *et al.* 2010). Indeed for the *Ectemnorhinus* group of genera, Chown (1989, 1994) suggested that the species typical of the epilithic biotope, (i.e. those in the genera *Bothrometopus*, *Palirhoeus* and *Disker*) probably radiated since the end of the Pliocene in the epilithic biotopes that must have come to predominate as a consequence of cooling (for revised climatic histories see Turner *et al.* 2009). The divergence times calculated on the basis of an arthropod mtDNA survey of Brower (1994) certainly support such a proposal. Whether the groups more typical of vegetated areas will show an equally deep history is not clear. However, the deep divergence time, approximately 6.46 m.y.a., found here between *Ectemnorhinus* (a genus in which species are typical of vegetated areas - Chown 1989, 1994) and *Bothrometopus* (restricted to epilithic biotopes) and the fairly substantial divergence dates among species within this genus (see also Grobler *et al.* 2006), suggests that they may well do so. That recent studies have supported the persistence of vascular plants on the South Indian Ocean Province Islands through

several glacial periods (e.g. Van der Putten *et al.* 2010) also suggests that survival during these periods is likely. In consequence, the proposal that the genus *Ectemnorhinus* diversified following the last glacial maximum (Chown 1994) must be rejected. Similar hypotheses of recolonization of terrestrial areas from refugia, such as marine refugia in the case of the ameronothroid mites have also been rejected on the grounds of new molecular evidence (Mortimer *et al.* 2010). However, within particular species it remains clear that volcanic and glacial cycles and refugia on particular islands have played important roles in population structuring. Such structure has thus far been identified for indigenous springtails, mites, and weevils (Grobler *et al.* 2006, Myburgh *et al.* 2007, Grobler *et al.* 2011), and seems also to apply to a vascular plant species and to other insects. Significantly, though, in a sub-Antarctic context such details are available only for the Prince Edward Islands, and to a lesser extent for Macquarie and Heard islands.

These results clearly indicate the need for further comprehensive molecular phylogenetic analyses of the biogeography of the region including a range of taxa. Only in this way will clearer reconstructions of the history and evolutionary relationships of the endemic and frequently enigmatic taxa in the region be established, and the hypotheses concerning the origins of the group (e.g. Jeannel 1964) assessed on a sounder basis. Moreover, they suggest that hypotheses concerning the historical biogeography of the region based solely on distributional data are perhaps no longer as useful as they once were. The distributional data must be accompanied by modern phylogenetic analyses for two reasons. First, the phylogenetic approach can reveal divergence times and relationships more straightforwardly than other approaches (acknowledging that a match with earth history must still be sought), thus helping to resolve biogeographic interpretation. Second, molecular evidence has been instrumental in revealing the presence of cryptic species, the existence of which can change interpretation substantially (Stevens *et al.* 2006, Torricelli *et al.* 2010). Given enhanced scientific cooperation across the Antarctic within a variety of scientific programmes, the development of comprehensive molecular phylogenies is likely to be achieved readily, and will almost certainly change current perspectives on the biogeography and biodiversity of the region, as this initial study has demonstrated.

Acknowledgements

Supplemental material

A supplemental table will be found at www.journals.cambridge.org/jid_ANS.

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Pre-freeze mortality in three species of aphids from sub-Antarctic Marion Island

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ARTICLE INFO

ABSTRACT

Understanding the mechanisms by which aphids survive low temperature is fundamental in forecasting the risk of pest outbreaks. Aphids are chill susceptible and die at a temperature close to that at which a small exothermal event is produced. This event, which can be identified using differential scanning calorimetry (DSC), normally occurs at a higher temperature than the supercooling point (SCP) and has been termed a pre-freeze event (PFE). However, it is not known what causes the PFE or whether it signifies the death of the aphid. These questions are addressed here by using a sensitive DSC to quantify the PFE and SCP and to relate these thermal events to the lower lethal temperature (LT_{50}) of sub-Antarctic aphids acclimated to low temperatures. PFEs were observed in each of the 3 species of aphids examined. They occurred over a narrower temperature range and at a higher temperature range than the SCP (-8.2 to -13.8 and -5.6 to -29.8 °C, respectively). Increased acclimation temperature resulted in increased SCPs in *Myzus ascalonicus* but not in *Rhopalosiphum padi*. The LT_{50} reduced by approximately 1 °C from -9.3 to -10.5 °C with reduced acclimation temperature (10–0 °C). The LT_{50} was close to the temperature at which the PFE occurred but statistically significantly higher than either the PFE or the SCP. In the majority of cases the PFE exotherm occurred well before the main exotherm produced by the bulk of the insect's body water freezing (SCP). However, in a few cases it occurred at the same temperature or before the super-cooling point making the term, pre-freeze event (PFE), rather misleading. The possible origins of the PFE are discussed.

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1. Introduction

Research into the cold tolerance of insects originates from studies on the overwinter survival of crop pest species due to their economic importance (Salt, 1933; Manson, 1943). From these early beginnings insects were categorised as being either freeze-avoiding or freeze-tolerant based simply on whether they could survive freezing of their body fluids. Freeze-avoiding insects reduce the temperature at which they freeze to as low as -30 °C and survive in a supercooled state. This is achieved by the accumulation of cryoprotectants and the removal of ice nucleators from their bodies. In contrast, freeze-tolerant insects generally freeze at relatively high sub-zero temperatures, ice being seeded (nucleated) in extracellular fluids. By seeding the formation of ice in such areas insects have a degree of control over when, where and how ice propagates around the body (Zachariassen and Hammel, 1976). In this way they are able to avoid intracellular ice formation which is always lethal in insects. Over the past 20 years

the classification of insect cold tolerance has expanded to take into account more recent discoveries (Denlinger and Lee, 2010), including the recognition that some insects with low freezing points die before they freeze. Such insects have been classified as chill-susceptible or chill-tolerant (Bale, 1993). Chill-susceptible species can survive a few degrees below the threshold required for growth and reproduction but die after exposure to sub-zero temperatures, which can be well above their freezing or super-cooling point (SCP). Chill-tolerant species likewise may die if exposed to sub-zero temperatures above their SCP, but can withstand a greater exposure to sub-zero temperatures than chill-susceptible species. Some chill-susceptible species die at a specific temperature above the SCP while others are killed by the accumulative effect of longer periods at a low temperature. This phenomenon is known as pre-freeze mortality (see reviews in Lee and Denlinger (2010)).

Several species of aphids have been shown to be chill-susceptible (Knight and Bale, 1986). Adults of the grain aphid, *Sitobion avenae* (Fabricius) have a SCP of approximately -24 °C, yet when cooled at 1 °C min^{-1}, mortality starts to be seen at -5 °C, with a lethal temperature of 50% of the sample (LT_{50}) of -8.1 °C and no survival below -15 °C. More detailed studies of

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the thermal events occurring in this species during cooling using differential scanning calorimetry (DSC) revealed a small exotherm at a temperature close to the LT_{50} (Knight and Bale, 1986). This exotherm is very small by comparison with the main exotherm produced when the bulk of the animal's body water freezes and was originally termed a pre-freeze event (PFE) because it occurred at a higher temperature than the SCP. Similar results were obtained for the lupin aphid *Macrosiphum albifrons* (Essig) (Knight and Bale, 1986). A further study by Clough and Bale (1987) investigated the effect of rearing temperature (5, 10 and 15 °C) on pre-freeze mortality in the peach potato aphid *Myzus persicae* (Sulzer). This species also produced a small pre-freeze exotherm close to the LT_{50} but with two distinct peaks. Aphids reared at 0 °C showed a slight decrease in temperature (approximately 1–2 °C) in the key features of the pre-freeze exotherms (peak start and finish) compared to those reared at 5 and 10 °C. However, the authors of this study concluded that because some aphids were able to survive temperatures below the very clearly defined temperature at which the pre-freeze exotherm occurred, pre-freeze mortality was not necessarily due to this thermal event.

Since these studies in the 1980s there have been no other reports of pre-freeze exotherms in any other species even though investigations into insect cold tolerance, including measurements of SCPs are prevalent. This paper describes a detailed study using a sensitive DSC to investigate the effect of acclimation temperature on pre-freeze mortality in three species of aphid from sub-Antarctic Marion Island (46°54′S, 37°45′E). The aims of the study are to investigate the nature of pre-freeze mortality in aphids inhabiting a comparatively harsh environment and determine whether low temperature mortality can be attributed to any pre-freeze thermal event.

2. Materials and methods

The life cycle of aphids can be complex and varies between species. They can overwinter either as eggs, which undergo diapause and are extremely cold-tolerant (Strathdee et al., 1995), or as nymphs and adults, which are less tolerant to low temperature but have the advantage of being able to develop and reproduce more rapidly when conditions become favourable in early spring. Some species of aphids (e.g. *M. persicae*) employ a mixed strategy producing both holocyclic (sexual, egg producing) and anholocyclic clones in the autumn. This adaptation has advantages in regions where winters are generally mild but where unpredictable cold spells may occur. More severe climates select for sexual reproduction resulting in a decrease in obligate parthenogenesis towards extreme latitudes. (Vorburger, 2004).

Although four species of aphids were originally recorded on Marion Island by Dreux (1971), only three (*Rhopalosiphum padi* (Linnaeus, 1758), *Macrosiphum euphorbiae* (Thomas, 1878) and *Myzus ascalonicus* (Doncaster, 1946)) were recorded by Crafford et al. (1986). All have a semi-cosmopolitan distribution and are thought to have been anthropogenically introduced to the island with fresh produce. They are now widely distributed on Marion Island (Hänel et al., 1998; Lee et al., 2007) and considered to be naturalised aliens (Crafford et al., 1986). Aphids have also been recorded on other Southern Ocean islands including Gough, Macquarie and Kerguelen (Jones et al., 2003; Greenslade, 2006; Hullé et al., 2003).

The three species of aphids investigated here are all of economic importance causing damage to crops world-wide. The summer stages of *R. padi*, the bird cherry-oat aphid, are a severe pest of cereals in Scandinavia and northern Europe and can transmit barley yellow dwarf virus in the UK (Leather et al., 1993). In temperate regions adults of this species feed on grasses during the summer but enter a holocyclic life cycle in the autumn when winged morphs fly to their winter host plant, the bird cherry tree (*Prunus padus*) where they produce overwintering eggs (Strathdee et al., 1995). As there are no bird cherry trees on Marion Island, only anholocyclic clones are produced. Adults feed mainly on *Poa cookii* (Poaceae), but may also be found on *Poa annua*. Recently, the parasitoid *Aphidius matricariae* was inadvertently introduced to Marion Island where it utilizes this species as its sole host (Crafford et al., 1986; Lee et al., 2007). *M. euphorbiae*, the Potato Aphid, primarily feeds on members of the rose family (Blackman and Eastop, 1984) and is a holocyclic species which may also have anholocyclic forms. On Marion Island it is anholocyclic and both winged and unwinged forms are found, typically on *Acaena magellanica* (Rosaceae), but also on *Cotula plumosa* (Asteraceae) (Crafford et al., 1986; Abraham, 2006). *M. ascalonicus*, the shallot aphid, is an anholocyclic species. On Marion Island its major hosts are *A. magellanica* and *C. plumosa* (Crafford et al., 1986; Abraham, 2006).

2.1. Collection of aphids

Aphids were removed from cut stems of host plants using a fine paintbrush within 2–8 h of collection or after a specific period of temperature acclimation. Aphid species were specific to particular host plants which aided collecting and sorting. *R. padi* was collected from *P. cookii* and *C. plumosa* sampled from Trypot Beach (S46° 53.136, E37° 52.100). *M. ascalonicus* was collected from *A. magellanica* leaves on plants close to Junior's Kop (S46° 52.717, E37° 50.214). Although *M. euphorbiae* is comparatively rare on the island, it was found on both *A. magellanica* and the Kerguelen cabbage (*Pringlea antiscorbutica*) from a site close to Junior's Kop. All experiments were conducted during April 2008 on sub-Antarctic Marion Island (Fig. 1).

2.2. Acclimation

Cut stems of *P. cookii* and *A. magellanica* hosting *R. padi* and *M. ascalonicus*, respectively, were placed in beakers of water and loosely covered with transparent polythene bags to prevent leaves from drying and to retain the aphids on the plant material. This material was acclimated in temperature-controlled cabinets (Labcon, Johannesburg, South Africa), with 12 h lighting at 0, 5, 10 or 15 °C for 10 days. The cabinets typically maintain temperatures within 0.5 °C of the set-point, as verified using thermochron DS1922 i-buttons (Dallas Semiconductor Corporation, Texas, USA).

2.3. Thermal analysis

Thermal analysis was conducted using a Mettler DSC820 (Mettler, UK) differential scanning calorimeter (DSC) cooled by a mechanical intra-cooler operating at −60 °C (Thermo Haake, EK 90/MT, Germany). A standard protocol cooling from 25 to 5 °C at 10 °C min^{-1}, then to −30 °C at 0.5 °C min^{-1} was used for all determinations unless otherwise stated. Groups of 10 aphids (*R. padi* and *M. ascalonicus*) were placed in 100 μL aluminium pans to obtain mean SCPs. The relatively large SCP exotherms were spread over a wide temperature range (−5.6 to −29.8 °C) making it straightforward to determine the freeze onset of individual aphids even when 10 were enclosed in one pan. This was not the case for the PFE, which is an order of magnitude smaller and occurs over a much narrow temperature range (−8.2 to −13.8), making it necessary to analyse a few aphids of each species individually in order to be able to characterise the thermal event. These samples were cooled at 1 °C rather than 0.5 °C min^{-1}.
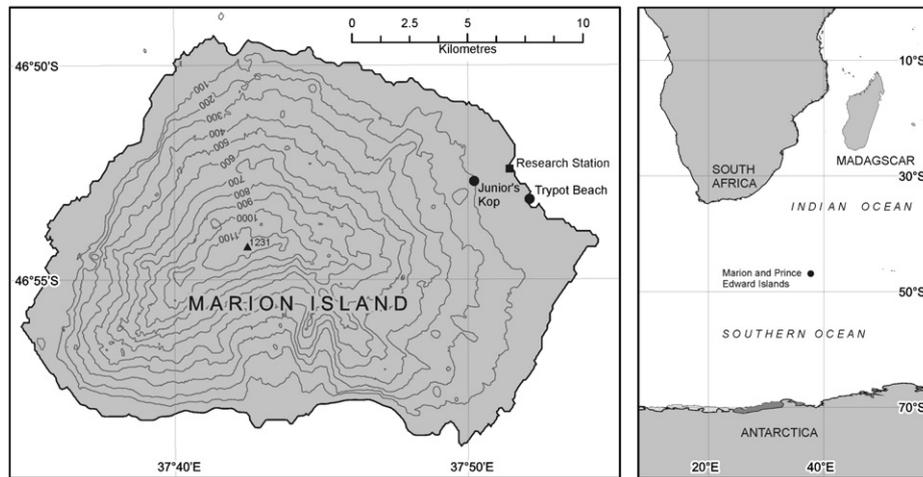
Fig. 1. Collection sites on Marion Island and location of Marion Island.

Table 1
Sample medians with bootstrap standard errors.

Species	Variable	Treatment	n	Median (SE)
M. ascalonicus	SCP	FF	163	−16.00 (0.83)
		0	40	−18.75 (1.48)
		5	40	−14.90 (1.20)
		10	40	−13.10 (0.45)
	PFE	FF	9	−12.50 (0.67)
		0	7	−11.95 (0.62)
R. padi	SCP	FF	63	−26.80 (0.34)
		0	71	−27.30 (0.22)
		10	40	−27.55 (0.40)
		15	40	−27.15 (0.39)
	PFE	FF	9	−13.80 (0.48)
M. euphorbiae	SCP	FF	17	−19.00 (3.96)

As *M. euphorbiae* was comparatively rare, they were always analysed individually when they were found. The SCP was taken as the onset of the large exotherm produced by the latent heat of freezing of the animal's body fluids. Thermograms were analysed using Mettler Tolledo Star$_e$ software, which enabled the peak onset and integral to be calculated. SCPs and PFEs were measured for freshly collected specimens (Field Fresh=FF) and samples acclimated at specific temperatures for 10 days (see Table 1 for details). A few specimens of each species were analysed in the DSC a second time immediately after the first measurement to identify non-reversible thermal events. When an insect is repeatedly frozen, thawed and re-frozen, the animal's body water would be expected to freeze at about the same temperature each time (Worland, 2005) whereas some other thermal events are non-reversible and so are not seen in subsequent treatments (Knight and Bale, 1986).

2.4. Lower lethal temperature (LLT)

For each species, except *M. euphorbiae*, which was too rare to be investigated, six Eppendorf tubes each containing 10 aphids were placed in holes in a temperature controlled metal block in which they were cooled from 5 °C to the survival test temperature at 0.5 °C min^{-1} and held at this temperature for 1 h. They were then warmed to 5 °C at the same rate. The block was cooled by Peltier modules and the temperature controlled using a program-mable electronic system (Central Electronics Services, Stellenbosch University). Control aphids were treated in a similar way but held at a constant 5 °C for 1 h using the same apparatus. Aphids were assessed for survival immediately after treatment and after allowing a 24 h recovery period in an incubator at 5 °C. The lower lethal temperature was also measured for *M. ascalonicus* after acclimation at 0, 5 or 10 °C for 10 days. The limited period of time for the study on Marion Island did not permit the measurement of the lower lethal temperature of other species after acclimation.

2.5. Statistical analysis

One of the aims of this study was to investigate the likelihood of either the SCP or the PFE being responsible for the death of aphids at sub-zero temperatures. To meet this aim the statistical analysis compares the median temperature of death (LT_{50}) with the medians of the distributions of supercooling points (SCPs) and pre-freezing events (PFEs). To assess differences between LT_{50} and SCP/PFE, standard errors of the medians had to be calculated. This was achieved by bootstrapping, which involves re-sampling the data to generate a large number (5000) of the so-called bootstrap samples (Manly, 1997). For each bootstrap sample the median is calculated and a bootstrap standard error estimated from the standard deviation of the bootstrap values. The standard error of the observed median is the standard deviation of the bootstrap values, which is larger than the estimate for a Normal distribution (GenStat, 12th ed.).

Values of LT_{50} were estimated using probit analysis (Minitab release 15.1) and goodness-of-fit tested using two tests (Pearson and Deviance). Differences between the LT_{50} and the median values of SCPs and PFEs were then tested using a z-test. The z-value for a particular comparison was calculated as the "difference/SE [difference]" where

$$\text{SE [difference]} = \sqrt{(SE_1^2 + SE_2^2)}$$

Significance levels (two-tailed) for z are obtained from the Normal probability distribution.

As death could be due to either a high SCP or a PFE the more appropriate comparison is between the LT_{50} with the higher of SCP and PFE termed as T_{max}. However, the occurrence of SCPs at a higher temperature than the PFE was unusual (3 out of 25) and had only a very slight effect compared with taking the PFE as the highest event (see results for details).

3. Results

3.1. SCPs and PFEs

Pre-freeze events (PFEs) were observed for all three species. *M. ascalonicus* and *R. padi* exotherms each consisted of a single peak whereas the *M. euphorbiae* PFE consisted of three slightly over-lapping peaks (see figures 2, 3 and 4 for example thermograms). The majority of pre-freeze exotherms occurred at a higher temperature than the much larger exotherm produced by the bulk of the insect's body water freezing (SCP). However, there were a few examples where the SCP was produced at a higher or at the same temperature as the PFE (Fig. 3) making the term "pre-freeze" rather misleading. Examples of thermograms for each species are provided in Fig. 2 showing the range over which both the PFEs and SCPs occurred. In general, the PFEs occurred over a much narrower range than the SCPs (-8.2 to -13.8 and -5.6 to -29.8 °C, respectively, for field fresh *M. ascalonicus*). The range of SCPs for *M. ascalonicus* is much greater than that of *R. padi* and the SCPs of the former species show a

bimodal distribution (Fig. 5). When an aphid was re-frozen after thawing the PFE was only seen in the initial thermogram whereas the SCP occurred in subsequent refreezing of the same sample (Fig. 4). The SCP reoccurred at a similar temperature each time the sample was frozen. The integral of the melt endotherms was similar in value for both the first and second evaluations indicating that the PFE did not "relax" under these conditions.

Table 1 shows a summary of the sample SCP and PFE medians and bootstrap standard errors for field fresh (FF) and acclimated aphids. The sample size for SCPs is much larger than that for PFEs because it was possible to analyse ten aphids at a time whereas the PFEs could only be analysed for individuals. Increased acclimation temperature resulted in increased SCP values for *M. ascalonicus* with field fresh samples having a median SCP between those of samples acclimated at 0 and 5 °C, which is in the range of late summer field temperatures (see Fig. 6 for field temperatures). *R. padi* SCPs were lower than those of *M. ascalonicus* and showed little change with acclimation temperature. SCPs for field fresh *M. euphorbiae* were similar to those of *M. ascalonicus*.
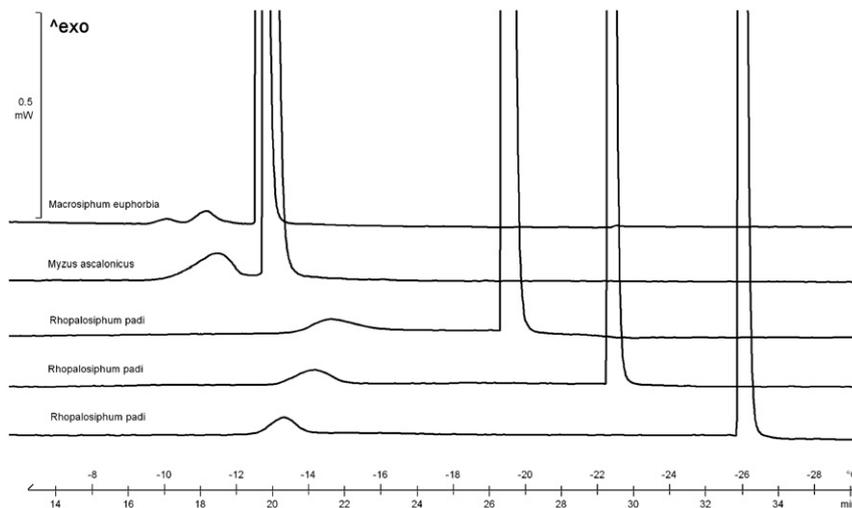

Fig. 2. Example thermograms for aphids cooled from 5 to -30 °C at 1 °C min^{-1}. PFEs are the small peaks followed by the much larger exotherm produced by freezing of the animal's body fluids. The PFE produced by *M. euphorbiae* is made up of 3 small overlapping peaks whereas *M. ascalonicus* and *R. padi* produce only one peak.
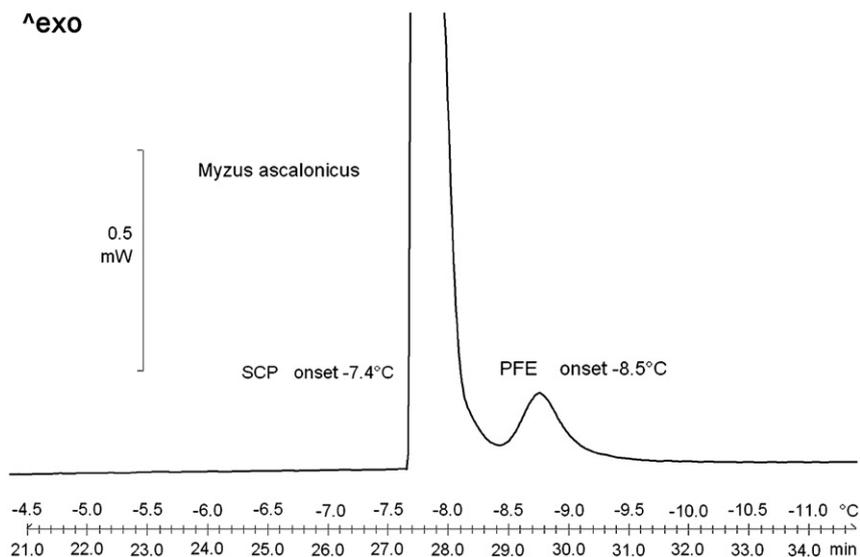

Fig. 3. An example thermogram showing the SCP occurring before the PFE in *M. ascalonicus*.
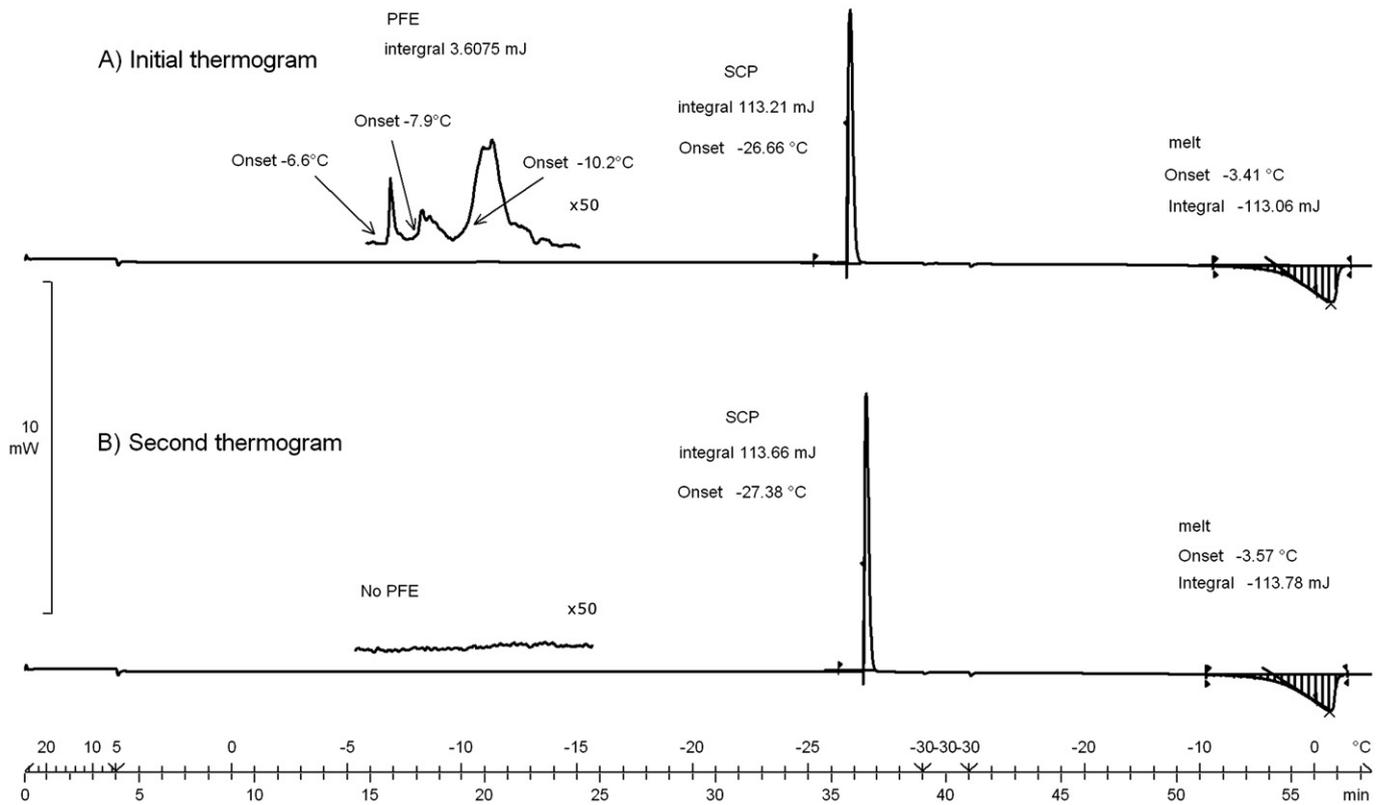
Fig. 4. Thermograms showing the effect of refreezing the same sample (*M. euphorbiae*). The upper trace shows the first time the sample was frozen with the PFE amplified 50 times compared with the SCP. The lower trace is the same sample refrozen under the same conditions. The SCP occurred at a similar temperature but there was no PFE.
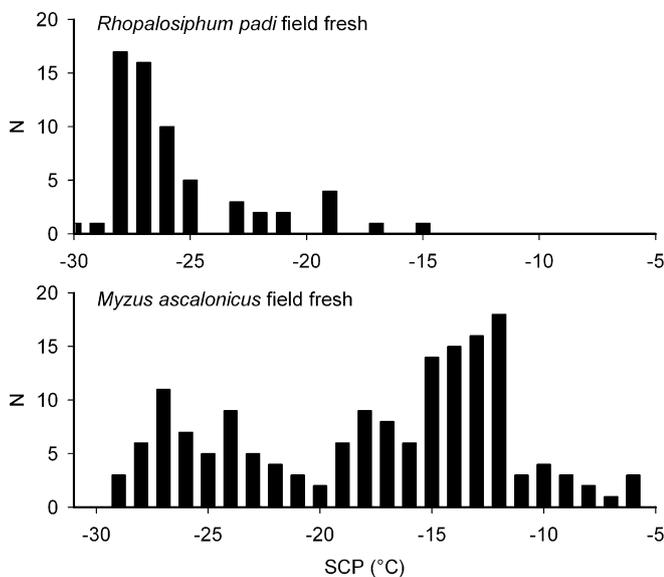
Fig. 5. Supercooling point distributions for field fresh *M. ascalonicus* and *R. padi* collected on Marion Island.

Fig. 6. Monthly mean and minimum temperatures recorded during 2008 at 100 metres above sea level on Marion Island using i-button data loggers (Thermochron, DS1922L, Dallas Semi-conductors, USA, 0.5 °C resolution).

3.2. Lower lethal temperature

Probit analysis was used to calculate the LT_{50} values for freshly collected aphids and, in the case of *M. ascalonicus,* after acclimation at different temperatures (Fig. 7). Table 2 shows details of two goodness-of-fit tests for the survival data. Fitted

curves for field fresh *M. ascalonicus* and *R. padi* are shown in Fig. 8a and b. There is no statistical significant lack-of-fit for either Pearson ($P=0.29$) or Deviance ($P=0.18$) for field fresh *M. ascalonicus*. For *R. padi* there is a significant lack-of-fit with both Pearson ($P=0.026$) and Deviance ($P=0.011$), although visual examination of Fig. 8b shows that the probit curve provides a reasonable fit with no obvious systematic lack-of-fit.
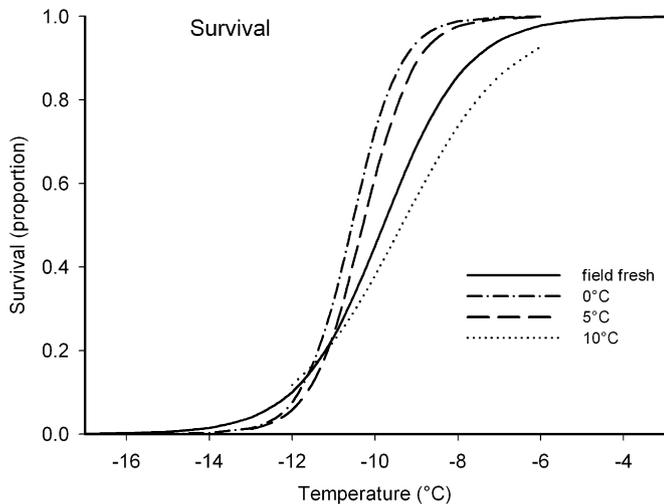
Fig. 7. Survival curves (probit analysis) for *M. ascalonicus* after acclimation at different temperatures.

Table 2
Probit analysis relating survival to temperature.

Species	Treatment	LT_{50} (SE)	Pearson (P)[a]	Deviance (P)[a]	df
M. ascalonicus	FF (control)	−9.81 (0.13)	15.22 (0.29)	17.56 (0. 18)	13
	0	−10.53 (0.07)	16.26 (0.09)	13.98 (0.17)	10
	5	−10.28 (0.07)	3.98 (0.68)	4.97 (0.55)	6
	10	−9.37 (0.11)	18.84 (0.002)	20.50 (0.001)	5
R. padi	FF	−9.69 (0.21)	24.62 (0.026)	27.27 (0.011)	13

[a] Two goodness-of-fit tests (Pearson and Deviance) are given with corresponding values of chi-square statistic, and the degrees of freedom (df) and associated *P*-value in brackets.

Table 3 shows the statistical significance for the differences between both the SCP and PFE with the LT_{50} data shown in Tables 1 and 2. For both *M. ascalonicus* and *R. padi* there is strong evidence that the LT_{50} is higher than the SCP and although the difference for the PFE is smaller, it is still significantly different from zero. An effective illustration of these differences is to plot the sample cumulative distribution of SCP/PFE, and the survival–temperature relationship on the same graph (Fig. 9), which shows how different the SCP curve is to the PFE and survival curves.

In order to take account of the very few cases where the SCP occurred at a higher temperature than the PFE, the LT_{50} can be compared with T_{max} which is the highest of the SCP and the PFE for any thermogram. For *M. ascalonicus* this gives a new median value of −12.28 °C, which is only slightly higher than the median value of the PFE for field fresh samples in the earlier analysis, i.e. −12.50 °C. Application of the bootstrap method to estimate the standard error of the median of T_{max} using data pooled over treatments gives a bootstrap standard error equal to 0.267. Comparing LT_{50} with median T_{max} gives a z-value of 8.05 ($P = < 0.001$). In other words the LT_{50} is significantly higher than the median of the maximum SCP and PFE.

4. Discussion

The survival and proliferation of aphids is of particular interest because of the devastating effect they can have on crops. Forecasting the abundance of aphids is based on the relationship between the severity of winter conditions and their ability to survive sub-zero temperatures (Bale, 2002). A full understanding
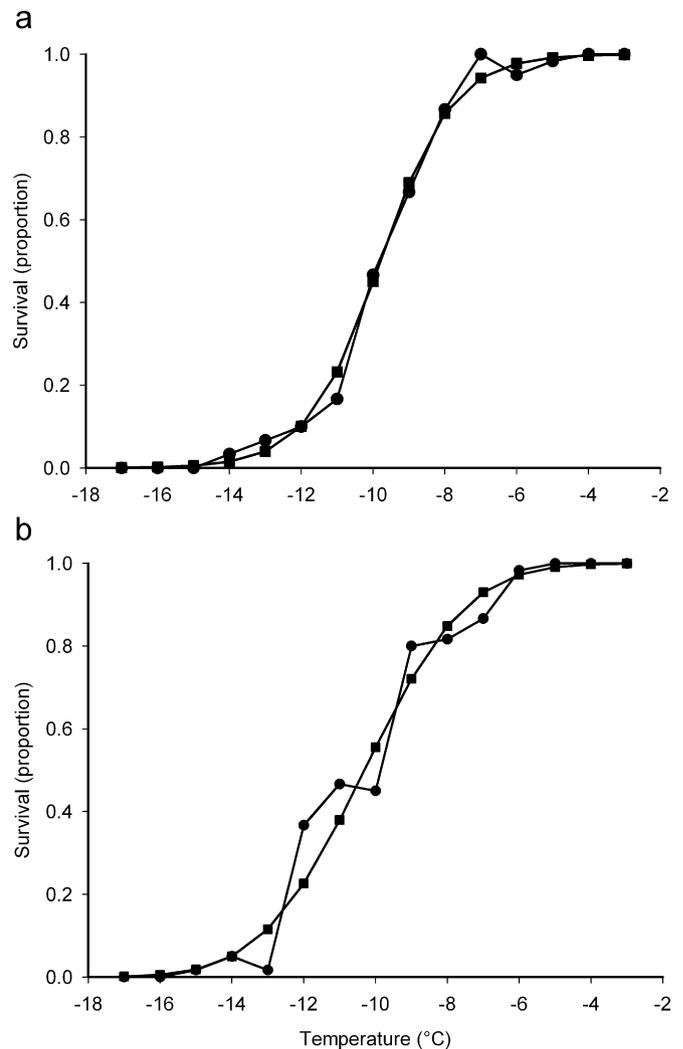

Fig. 8. Survival curves showing goodness-of-fit for field fresh aphids: (a) *M. ascalonicus* and (b) *R. padi*. Experimental data points (●); fitted curve (■).

Table 3
Relationship between SCP, PFE and $LT_{50.}$

Species	Treatment	Comparison of LT_{50} with:	z	P
M. ascalonicus	FF	SCP	7.33	0.001
	0	SCP	5.45	0.001
	5	SCP	4.51	0.001
	10	SCP	8.05	0.001
M. ascalonicus	FF	PFE	3.93	0.001
	0	PFE	2.28	0.05
R. padi	FF	SCP	43.21	0.001
		PFE	6.27	0.001

of the lower lethal temperature of insects is fundamental for such predictions and knowing what actually causes them to die will help provide better management and control. Knowing the upper and lower temperature thresholds also helps to anticipate the geographical range over which a particular species may spread and become established (Hazell et al., 2010a, b). In an era of climate change the survival, establishment and consequential effect of invasive species on local flora and fauna are of particular interest in sub-Antarctic regions that are particularly affected by global warming (Frenot et al., 2005).
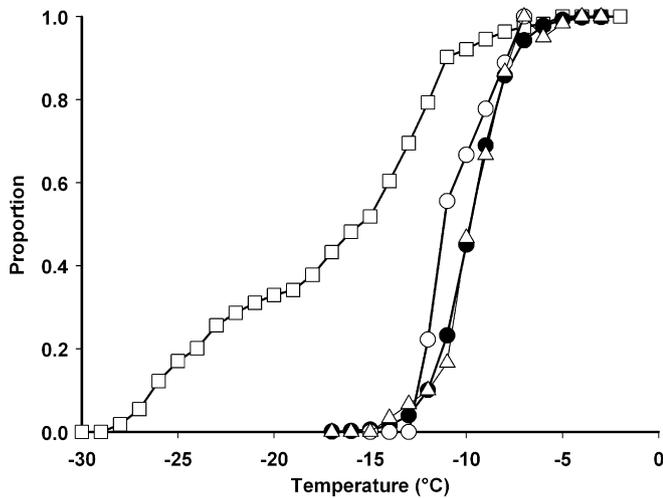
Fig. 9. Cumulative distribution of SCPs (□), PFEs (o), survival (Δ), and the calculated survival curve (●) (probit analysis) for field fresh *M. ascalonicus* in relation to temperature.

Aphids are generally cyclical pathenogens with several parthenogenetic generations and one sexual generation. More severe climates tend to select for sexual reproduction resulting in a decrease in obligate parthenogenesis towards extreme latitudes (Vorburger, 2004). However, on Marion Island, where there are no primary host plants (trees), all three species reproduce by obligate parthenogenesis (Crafford et al., 1986). On Gough Island, *R. padi* is restricted to overwintering on herbaceous material in frost-free coastal and lowland areas (Jones et al., 2003). Similar observations have been made on the French sub-Antarctic islands where aphid colonies are mostly found at a short distance from the coast and generally within the first 100 m a.s.l, with no aphids above 250 m (Hullé et al., 2003).

Chill-susceptible insects are killed by physiological mechanisms induced by low temperature but with effect above the SCP. In some species death may occur at a specific sub-zero temperature while others are killed by the accumulative effect of long periods at a low temperature (Koštál et al., 2004). In either case cold hardening in response to reduced environmental temperature can increase the insects' tolerance to low temperature (Powell and Bale, 2004, 2005). Laboratory studies have shown that aphids reared for three generations at 10 °C have a reduced LT_{50} (adults, -13.5 °C) compared with those reared at 20 °C (-9.5 °C) (Powell and Bale, 2005). Some species of aphids (e.g. *S. avenae*) are also capable of rapid cold hardening resulting in extended survival at critical low temperatures (-12 °C) (Clough et al., 1990). It might therefore be expected that aphids established on a sub-Antarctic island would also have increased cold-tolerance compared to their temperate counterparts. However this does not appear to be the case as the LT_{50} for field fresh specimens of *M. ascalonicus* and *R. padi* were -9.81 and -9.69 °C, respectively. The SCP of *S. avenae* in the UK has been shown to be about -24 °C (Knight and Bale, 1986), which falls between *M. ascalonicus* and *M. euphorbiae* at -16 and -19 °C and *R. padi* at -26 °C (field fresh adults). In a study by Powell and Bale (2004), *S. avenae* died after exposure to temperatures below -9 °C, which is similar to the LT_{50} for *M. ascalonicus* and *R. padi* in this study. These data suggest that aphids established on sub-Antarctic Marion Island are no more cold-hardy than those inhabiting temperate regions. The wide range of SCPs recorded for *M. ascalonicus* is unusual for an aphid species and the occurrence of aphid SCPs as high as -5.6 °C is extremely unusual (J.S. Bale, pers. com.). Aphids feed on phloem sap, a nucleator-free diet, and

so it is difficult to speculate why individuals should freeze at such a high temperature. The bimodal distribution of SCPs suggests that a proportion of the population is affected by active ice nucleators which could be internal or on the surface of the aphid. This unusual observation is worthy of further investigation. As the lower lethal temperature of *M. ascalonicus* and *R. padi* is significantly higher than the SCP, death must be due to something other than freezing of body fluids. Comparing the median SCP and PFE with the LT_{50} for both *M. ascalonicus* and *R. padi* in this study provides evidence that the PFE is more likely to be responsible for death than the SCP in both species although statistically some aphids die even before the PFE.

4.1. Possible causes of the PFE

Unlike the SCP, the PFE is non-reversible. Therefore it is not a freezing event but more likely a non-reversible change in state or structure such as damage to a protein. Thermotropic membrane phase transitions at low temperatures can cause cells to become "leaky" by widening membrane pores (Byrne and Chapman, 1964). This results in loss of endogenous constituents such as ATP, amino acids and small molecular weight compounds (Strange and Dark, 1962). Pullin and Bale (1988) investigated the effect of cooling below the PFE on leakage of ions from cells and ATP levels. Observation of aphids after cooling confirmed that mortality is not immediate and some degree of recovery can occur after several hours but those which are not capable of coordinated walking after 4 h are unlikely to recover. ATP levels were maintained and temporarily increased for up to 48 h after cooling. This suggested that catabolic pathways were intact but that the insects may not have been capable of utilising the energy produced. Ion leakage only increased 72 h after treatment, and was probably due to cell death rather than the cause of it. The period of uncoordinated movement, which is sometimes seen after cooling, to below the PFE may be indicative of neurophysiological damage or disruption of the metabolic pathways.

Cold injury to the intercellular prokaryotic symbiont found in specialized cells in aphids is another possible source of the PFE. Aphids are highly dependent on their symbionts which aid nutrition as the phloem sap on which they feed is nutritionally poor. Parish and Bale (1991) found these symbionts to be increasingly damaged as temperature reduced. Such an event provides an alternative explanation as to why aphids do not always die immediately after being cooled below the PFE but sometimes show movement for up to 24 h later.

Phase transitions of lipids can also produce similar peaks to the PFE exotherm seen in aphids. As the transition temperature is dependent on the degree of unsaturation, multiple peaks can be seen where different compartmentalised lipids occur. Insects living at low temperatures are thought to have a mechanism for rapidly increasing the degree of unsaturation in fatty acid chains enabling them to maintain homeoviscosity (Overgaard et al., 2005; Koštál, 2010; Lee and Denlinger, 2010; Van Dooremalen and Ellers, 2010). However, such transitions are normally reversible. As the PFE in aphids only occurs during the first freeze and no specific melt event is seen, it is unlikely to be due to a lipid phase transition unless perhaps the lipid has become dispersed or modified during the thermal event.

In conclusion, the narrow temperature range over which the PFE occurs suggests that it may be due to a physical or molecular change, e.g. a protein deactivation. The exotherm is seen in all stages of aphids, therefore it cannot be attributed to eggs or unborn young enclosed within the animal's body. As the LT_{50} is statistically higher than the PFE it is not possible to say conclusively that this thermal event is directly responsible for

death. It is more likely that it signifies a change which disrupts normal cell processes, which can in some cases be repaired.

It is perhaps surprising that similar thermal events have not been found in other insects. This may be because the relatively small pre-freeze thermal exotherm can only be detected using sensitive thermal analysis instrumentation. However, despite using this technique to study the cold-tolerance mechanisms of many different arthropods, we are not aware of other examples. This suggests that the exotherm is specific to aphids. Finally, as the exotherm is now known to occur occasionally at the same temperature or even lower than the SCP, the term "pre-freeze thermal event" could be misleading.

Acknowledgements

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