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Wood-decaying Fungi and Beetles

A Multilateral Approach to Studying Fungus-insect Communities

Kohmei Kadowaki

A thesis submitted in partial fulfilment of the requirements for the degree of PhD in Biological Sciences, The University of Auckland, 2010.
Abstract

Fungus-insect interactions permeate through the intricate tapestry of terrestrial ecosystems, but remain a persisting mystery in ecology. The wood-decaying basidiomycete *Ganoderma* produces perennial sporocarps (fruiting bodies) that provide food (spores) and habitat (hymenial surface) for three endemic beetles; *Zearagytodes maculifer* (Leiodidae) and two *Holopsis* species (Corylophidae) that occur in sympatry in the Waitakere Ranges, Auckland, New Zealand. *Ganoderma* spore release from individual sporocarps undergoes density fluctuations of two orders of magnitude that are neither clearly associated with temperature and humidity nor spatially synchronous. Such resource dynamics did not exert a strong effect on spore-feeding beetles since they use a minute percentage of spores discharged from the hymenial surface and are therefore not resource-limited. Competition at sporocarp patches is pervasive in the beetle community, as beetles range over a restricted area on the hymenial surface to feed and are likely to be space-limited. The three spore-feeding beetles display different host specificity, spore consumption patterns, dispersal behaviour, and seasonality. Remarkably, the two *Holopsis* species differ in their ontogenetic niches. The long rostrum of *Holopsis* sp. 1 gives it competitive superiority. All these factors combined to create a complex competitive network among the three species, but competitive coexistence is likely to occur as *Z. maculifer* and *Holopsis* sp. 2 evade competition with *Holopsis* sp. 1 via different colonisation strategies across sporocarp patches. There was no evidence for a fungus-insect mutualism: *Z. maculifer* does not disperse *Ganoderma* spores as passage through the beetle gut destroyed the spores and virtually none germinated. Altogether, the unique lifestyles of spore-feeding beetles “living on the surface” in long-lasting, exposed habitats dictate much of this tiny but extraordinary ecosystem of the fungus-insect community, characterized by the structuring forces of weak fungus-insect interactions and strong insect competition.
Acknowledgements

It is a great pleasure to acknowledge my dissertation supervisors, Jacqueline Beggs and Richard Leschen who improved this thesis in innumerable ways. Jacqueline has always encouraged me in my confronting a series of frustrations, not least of which, I appreciated her generosity, enabling me to exercise freedom in my PhD research, and her patience ploughing through the entire manuscript with constructive comments. Rich has taught me how to write good English, shared his extensive knowledge of fungus beetles and always entertained me with his slightly unusual sense of humour and Mexican culture that he shares with his wife Elena. My great thanks to an unflappable technician Kelly Booth who helped dissect many tiny beetles, Manpreet Dhami who counted spores, Barbara Paulus and Paula Wilkie who offered superb mycological assistance and Peter Wilson who kindly helped chainsaw and transport heavy dead logs. I profited as well from invaluable comments by Christine Bezar, Peter Buchanan, Tadashi Fukami, Robin Gardner-Gee, Greg Gilbert, Ilkka Hanski, Stephen Hartley, Greg Holwell, Marcel Holyoak, Brian Inouye, Peter Johnston, Atte Komonen, John McLean, Mark McPeek, Masashi Murakami, Barbara Paulus, Anne Thistle, Anne Tomlinson, Nobuko Tuno, Yong Wang, Carola Warner, Darren Ward, Mike Winterbourn, and many anonymous reviewers. I thank Birgit Rhode for photography, Grace Hall and Stephen Thorpe for identifying arthropods, Priscilla Cameron for help with literature, Pierre Ancion, Disna Gunawardana and Olga Panassenko for translation, Russell Millar, Yong Wang and Thomas Yee for statistical advice, Adrian Turner and Catherine Hobbis for technical assistance, Max Suckling, Ashraf El-Sayed and Lloyd Stringer for allowing me to use wind tunnel, Auckland Regional Council for providing permits and access to the Waitakere Ranges. I also acknowledge the Long-term International Doctoral Scholarship funded by the Ministry of Education-Japan, the University of Auckland, Landcare Research, the Entomological Society of New Zealand, and a Marsden grant of the Royal Society of New Zealand (145/3608546) for funding. My final appreciation goes to the many people who I have come across during the time spent on the PhD in New Zealand. I will never forget a lady I met while tramping, who talked to such an obscure, foreign entomologist during my fieldwork “thank you for studying the nature of New Zealand”. I can only hope that the thesis is worthy of the great wisdom, curiosity and enthusiasm of New Zealanders.
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Chapter 1 Mushrooms, bracket fungi and insects

PLATE 1. From the Waitakere Regional Park, looking toward the urban Auckland city, New Zealand
(Photo: K. Kadowaki)
1. Introduction to fungus-insect ecology

Introduction

Fungi and insects mark the largest and most diverse groups of living organisms in terrestrial ecosystems (Boddy and Jones 2008). Fungi offer various above-ground structures (i.e. fruiting bodies) as habitat for insects (Hanski 1989), while insects host a range of saprotrophic fungi, often in specialised organs (Vega and Blackwell 2005; Grebennikov and Leschen 2010). Entomogenous fungi attack the egg, larval, or adult stages of insects for prey (Humber, 1984; Evans 1988; Vega et al. 2009), and insects may deleteriously affect fungal fitness by feeding on sporocarps (Guevara et al. 2000b). Insects are prey, predators, parasites, mutualists, and competitors for fungi, and the two groups interact in ecologically complex and evolutionarily dynamic ways. Over the past century, mycologists have documented microscopic features of fungi associated with particular insect taxa (Thaxter 1914, 1920; Cooke 1977; Humber 1984; Harrington et al. 2005; Vega et al. 2009), while entomologists have recorded local insect fauna associated with fungal sporocarps (i.e. fruiting bodies) (Donisthorpe 1935; Scheerpeltz and Höfler 1948; Benick 1952; Rehfous 1955). Despite two landmark compilations by Blackwell and Wheeler (1984) and Wilding et al. (1989), current knowledge about fungus-insect communities remains too fragmented to empirically evaluate their ecosystem roles. Vega and Blackwell (2005) recently updated the study of fungus-insect communities from a mycological perspective. The present review will complement that work from an entomological perspective.

To identify trends and knowledge gaps in fungus-insect studies, I sought to conduct an extensive bibliographic survey of literature published from 1800 to 2010, using Google Scholar (http://scholar.google.com/). I examined the following attributes: (a) insect taxa, (b) type of fungus studied (ephemeral mushroom, long-lasting bracket fungus, or others), and (c) the type of ecosystem role, including host fungus-consumer insect interactions, fungus-insect mutualism, fungus-insect competition, insect competition for fungal resources, and insect
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predator-prey/host-parasitoid interactions. For this purpose, I used the following key words and/or pairs (in alphabetical order): bracket fungus; competition; community; fruiting body; fungivorous; fungus; host-parasite; host selection; interaction; life history; mushroom; mutualism; mycophagous; mycophagy; population; predator-prey; saproxylic; sporocarp. The study of host insect-fungal pathogen interactions and fungus-fungus competition has been substantial and there is much relevant literature (see reviews by Ferron 1978; Hajek and Leger 1994; Vega and Blackwell 2005; Boddy et al. 2008). I excluded these areas of research that are peripheral to my focus on fungal habitats for insects.

**Fungus-insect communities**

**Fungi:** Molecular phylogenetics has estimated the existence of >1.5 million species of fungi, and the fungal groups associated with insects are largely limited to the Basidiomycota (Agaricomycotina, Ustilagomycotina, Urediniomycotina) and Ascomycota (Pezizomycotina, Saccharomycotina, Taphrinomycotina) (Blackwell 2010). Agaricomycotina produces a diverse array of fruiting bodies, including gilled mushrooms (agarics), chanterelles, stinkhorns, corticioid fungi, polypores, cyphelloid fungi, false truffles, coral fungi, bird’s nest fungi, puffballs, and other forms that defy easy description (e.g. *Sparassis*) (Hibbett 2007). Urediniomycotina includes rust fungi and parasites of scale insects, and Ustilagomycotina includes smut fungi (Blackwell 2010). Ascomycota includes various parasites, pathogens, and endosymbionts of insects (Blackwell 2010).

**Insects:** The estimated diversity of insects is about 30 to 80 million species worldwide (Gullan et al. 2005), but the number of species associated with fungal habitats is difficult to even roughly estimate. Mycophagy, the consumption of fungi for food, has evolved multiple times among insects occurring in such diverse lineages as Coleoptera, Diptera, Lepidoptera, Hemiptera, Collembola, Isoptera, and Hymenoptera (Blackwell and Wheeler 1984). Mycophagy occurs ubiquitously in Coleoptera, especially among the primitive
1. Introduction to fungus-insect ecology

Staphyliniformia, Eucinetiformia, and Bostrichiformia (Lawrence 1989). Recent phylogenetic work suggests that there is a large radiation of taxa feeding on macrofungi, and about half of the major Coleoptera lineages may have had mycophagous ancestors (Leschen and Buckley 2007).

**Ecosystem roles of fungus-insect communities**

The extensive literature survey included over 477 published articles and book chapters, and revealed diverse ecosystem roles within fungus-insect communities (Fig. 1-1a). Eighty-five percent of the fungus-insect studies focused on one of five ecosystem roles by describing or manipulating it. Fifty-two percent of studies concerned host fungus-consumer insect interactions, followed by studies of fungus-insect mutualisms (33%). Competition between insects and predator-prey interactions were less studied, and very few studies examined insect-fungus competition (Fig. 1-1a). Two types of fungal habitats, ephemeral mushroom and long-lasting bracket fungus systems, have attracted equivalent amounts of attention (Fig. 1-1b). The increase in the number of overall fungus-insect studies accelerated beginning in the 1980s when Wheeler and Blackwell (1984) and Wilding et al. (1989) were published (Fig. 1-2). Even though early studies until the 1980s were dominated by host fungus-consumer insect interactions, the various ecosystem roles have been equally investigated since the 1990s (Fig. 1-2a). The number of ephemeral mushroom studies decreased beginning in the 1990s, whereas studies of long-lasting bracket fungi markedly increased, possibly due to the enhanced appreciation of fungal habitats in conservation ecology (Fig. 1-2b). The vast majority of focal papers (> 90%) were purely empirical, especially among bracket fungus studies, whereas the study of ephemeral mushroom systems fostered constant feedback from theory. Such disparity between the two lines of study can be traced to the distinct foci of interest; ephemeral mushroom studies have focused on host selection, competition, and predator-prey interactions of insect communities, whereas bracket fungi studies have
1. Introduction to fungus-insect ecology

concentrated on insect diversity, conservation, host relationships, and trophic interactions. The vast majority of research has been conducted in northern hemisphere temperate habitats.
1. Introduction to fungus-insect ecology

**Figure 1-1.** The percentage of ecological and entomological studies focusing on (a) ecosystem roles and (b) types of fungal habitats. Ecosystem roles include a total of five types: host fungus-consumer insect interactions (52%), insect-fungus mutualism (23%), competition among insects for fungal resources (7.8%), competition between fungi and insects (0.5%), insect predator-prey, host parasitoid, and host-parasite interactions (6.9%). Relevant articles examined the following two types of systems: ephemeral mushrooms (33%), long-lasting bracket fungi (34%), or both (4%). Articles that were not relevant to the corresponding classifications were removed from the total of 477 publications.
1. Introduction to fungus-insect ecology

Figure 1-2. Number of studies on fungus-insect communities, classified in terms of (a) the focal ecosystem role and (b) type of fungus.
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**Trends and knowledge gaps in fungus-insect studies**

*Host fungus-consumer insect interactions*

Sporocarp production varies not only spatio-temporally (Mehus 1986; Straatsma et al. 2001) but is also transient in many cases (Straatsma et al. 2001). Field studies have demonstrated that sporocarp production depends on a variety of ecological factors that span spatial and temporal scales (Table 1-1). These studies were necessarily correlative, and therefore the cause-effect links were often unclear. Sporocarp lifespans vary across taxa (Table 1-2).

Ephemeral mushrooms and persistent bracket fungi are not distinct categories but instead mark the extremes along a crude continuum of the temporal stability of fungal habitats.

Sporocarp lifespans also depend on weather conditions and visitation by fungivores (Lacy 1984b). Few studies have compiled a frequency distribution of sporocarp persistence.

**Table 1-1.** Factors affecting sporocarp production of fungi. The effects of these factors may interact to influence sporocarp production (Moore 2003; Moore et al. 2008).

<table>
<thead>
<tr>
<th>Factor</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil moisture, temperature, acidity</td>
<td>Hering 1966; Tyler 1985, 1989; Straatsma et al. 2001; Salerni et al. 2002; see also Fogel 1976</td>
</tr>
<tr>
<td>Host specificity to substrates, host genotypes</td>
<td>Last et al. 1984; Trudell and Edmonds 2004</td>
</tr>
<tr>
<td>Ectomycorrhizal or saprotrophic habit</td>
<td>Trudell and Edmonds 2004; but see Straatsma et al. 2001</td>
</tr>
<tr>
<td>Defoliation</td>
<td>Last et al. 1979</td>
</tr>
<tr>
<td>Amount of coarse woody debris</td>
<td>Yamashita et al. 2009; see also Wästerlund and Ingelög 1981</td>
</tr>
<tr>
<td>Fire</td>
<td>Hinton et al. 1989</td>
</tr>
<tr>
<td>Anthropogenic factors (harvesting, pollution, habitat fragmentation, climate change)</td>
<td>Ohenoja 1988; Penttilä et al. 2006; Gange et al. 2007; Dickie et al. 2009; see also Arnold 1991</td>
</tr>
</tbody>
</table>
1. Introduction to fungus-insect ecology

Table 1-2. Sporocarp lifespans from ephemeral mushrooms to long-lasting bracket fungi.

<table>
<thead>
<tr>
<th>Lifespan of sporocarp</th>
<th>Fungal taxa</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–3 days</td>
<td><em>Mycena</em> and <em>Galerina</em></td>
<td>Richardson 1970; Lacy 1984b</td>
</tr>
<tr>
<td>4–7 days</td>
<td><em>Russula</em>, <em>Lactarius</em>, <em>Entoloma</em>, <em>Amanita</em></td>
<td>Richardson 1970; Lacy 1984b; but see Hora 1959</td>
</tr>
<tr>
<td>12–20 days</td>
<td><em>Boletus</em>, <em>Hygrophorus</em>, <em>Paxillus</em>, <em>Hygrophoropsis</em></td>
<td>Richardson 1970; Lacy 1984b</td>
</tr>
<tr>
<td>40 days</td>
<td><em>Lentinula edodes</em></td>
<td>Sato and Suzuki 2001</td>
</tr>
<tr>
<td>68 days</td>
<td><em>Polyporus</em></td>
<td>Lacy 1984b</td>
</tr>
<tr>
<td>3–4 months</td>
<td><em>Trametes</em>, <em>Cryptoporus</em></td>
<td>Setsuda 1995; Guevara et al. 2000a</td>
</tr>
<tr>
<td>6–7 months</td>
<td><em>Polyporus</em></td>
<td>Paviour-Smith 1964</td>
</tr>
<tr>
<td>5 years</td>
<td><em>Ganoderma</em></td>
<td>Ingold 1953</td>
</tr>
<tr>
<td>35 years</td>
<td><em>Fomes</em></td>
<td>Paviour-Smith 1968</td>
</tr>
</tbody>
</table>

Fungal tissue is a high-quality diet for insects. Fresh fungal sporocarps contain as much as 80–90% water, and the dry weight consists of 40–50% carbon, with highly variable nitrogen concentrations that depend on fungal taxa, developmental stage, and morphological part (i.e. stems, caps, or gleba) (Martin 1979; Ingham 1992; Claridge and Trappe 2005). Fungi concentrate biologically important nutrients from nutrient-poor substances such as wood or litter. For example, the nitrogen in 13.6 g of *Betula* sapwood is required to supply 1 g of *Ganoderma applanatum* tissue, 36.1 g of wood are needed to supply 1 g of spores (Merrill and Cowling 1966), and more than 14 kg of wood are necessary to produce 1 kg of sporocarp (Moore et al. 2008). However, mycophagy requires its own particular set of digestive and metabolic capabilities, due to the special nature of fungal cell wall polysaccharides (e.g. chitin, β-glucans) and fungal sterols (e.g. ergosterol), as well as urea, ammonia, and a diverse array of secondary metabolites (Martin 1979; Martin et al. 1981). Many fungus beetles can digest β-glucans, but not chitin, with the exception of some Ciidae and Tenebrionidae beetles (Martin et al. 1981). In xylophagous insects, fungal enzymes acquired during feeding may contribute to the digestion of chitin and non-cellulosic β-glucans or detoxification in the guts of mycophagous insects, thus extending their digestive capability (Martin 1979, 1987; Kukor et al. 1988). Fungal-decayed (softened) wood or sporocarps are
1. Introduction to fungus-insect ecology

physically more efficient for insects to process than undecayed (hard) wood (Fisher 1940, 1941).

Ephemeral mushrooms provide a pulsed or intermittent resource supply for insects, whereas perennial bracket fungi provide a continuous supply. In ephemeral mushroom systems, mycophagous insects may display traits such as rapid larval development (Sevenster and Alphen 1993; Leschen 1994; Toda and Kimura 1997), parental care of eggs (Ashe 1986; Setsuda 1994), greater dispersal ability (Kimura 1992), wider host ranges (Hanski 1989; Sevenster and van Alphen 1993; Toda and Kimura 1997; see also Leschen 1990), or small clutch size to enhance risk-spreading (Hackman 1979; Bruns 1984; Hanski 1989).

Meanwhile, bracket fungi offer long-lasting larval habitats and spatially and temporally predictable adult habitats (Pace 1967; Hanski 1989); insects may grow slowly on such fungi (Liles 1956), have larger clutch sizes (Hanski 1989), weaker dispersal ability, and evolve obligate host associations (Lawrence 1973; Hanski 1989). Ephemeral mushrooms and long-lasting bracket fungi provide the context in which insect life history evolves, yet no comprehensive meta-analyses have been conducted on insect life history traits to unify facts, provide explanatory power, or control for phylogenetic effects. In fact, the study of host fungus-consumer insect interactions has been restricted to specific insect (Drosophilidae and Tenebrionidae) and fungal taxa (Agaricus, Polyporus, Fomes), perhaps because many fungal and insect species are rare in nature or not tractable for manipulative studies.

Fungal life histories have not been extensively studied in the context of counter strategies that avert insect mycophagy. Hackman and Meinander (1979) proposed five examples of such strategies: (a) delayed emergence of sporocarps from soil, (b) protection of unripe basidia by a volva or cortina, (c) secretion of milky sap (e.g. Lactarius), (d) toxic or repellent chemicals, and (e) intermittent or irregular occurrence of sporocarps (i.e. unpredictability). The foraging strategy of Basidiomycota shifts from a slow and dense
exploitative mycelial growth to a less dense explorative growth under high grazing pressure by collembolans (Boddy and Jones 2008). Fungal mycotoxins may cause insects to display growth inhibition, lower fecundity, or loss of fertility, mortality, or repellency (Wright et al. 1982; Wicklow 1988), although chemical defence by fungi may have evolved against mammals rather than insects (Camazine 1983; Camazine et al. 1983).

Host selection patterns of consumer insects are complex but appear to deviate significantly from random (Ashe 1984; Hanski 1989; Leschen 1990). Controversy has centred on two main explanations for these patterns: temporal availability and chemical defence of fungi. Kimura (1980) and Lacy (1984a) agree that resource availability (ephemerality of sporocarps) explains the degree of insect polyphagy, whereas other studies refute this conclusion or provide counter-examples (Hanski 1989; Leschen 1990). Other partial explanations for host selection patterns include chemical composition (Kimura 1980; Bruns 1984; Lacy 1984a; Yamashita and Hijii 2007), physical toughness of sporocarps (Lawrence 1973; see also Klopfenstein and Graves 1989), or apparency (Guevara and Dirzo 1999). The conflicts among authors may have arisen partially out of a discrepancy in the definitions of phylogenetic resolution to which the concept of host specialisation applies (i.e. species-, genus-, or family-level specialisation) (Leschen 1990). Phylogenetic signals behind host selection patterns have not yet been examined.

Genetics often reveal the causes and consequences of host selection. In Drosophila, genetic variation for food preference is additive (Jaenike 1985a; Courtney and Chen 1988), and exists not only among strains from different geographic areas, but also among strains from a single population (Jaenike and Grimaldi 1983). Quantitative genetic analysis has indicated substantial heritable variation in both the acceptance of a novel host Agaricus by ovipositing D. suboccidentalis and in the number of eggs deposited by accepting females (Courtney and Chen 1988). These two traits, host acceptance and egg investment, are
1. Introduction to fungus-insect ecology

Genetically correlated (Courtney and Chen 1988). A multigenerational genetic experiment in *Drosophila tripunctata* suggested no evidence for sympatric divergence of host races or for co-adapted complexes of genes related to host specificity (Jaenike 1989), which was in accordance with the genetic data for five species of *Drosophila* (Lacy 1983). Jaenike (1985b) demonstrated that the proximate determinant of monophagy in *Drosophila quinaria* is the failure of ovipositing females to recognise mushrooms as suitable breeding sites, rather than genetic trade-offs on different hosts (James et al. 1988).

Correlative studies have identified four major factors that help to determine the incidence and density of consumer insects: (1) sporocarp size, (2) developmental stage of a sporocarp, (3) micro- and macro-habitat, and (4) isolation effects. First, a larger sporocarp size indicates a greater carrying capacity (Thunes and Willassen 1997; Midtgaard et al. 1998; Rukke 2002), an increased likelihood of being detected by colonisers due to greater volatile emissions (Jonsell and Nordlander 1995; Fäldt et al. 1999), more resistance to fluctuating environments (Midtgaard et al. 1998; Rukke 2002), and longer persistence time to allow for more colonisers (Paviour-Smith 1968; Midtgaard et al. 1998; Rukke 2002; Komonen and Kouki 2005). These effects may be correlated with habitat surface area (Yamashita and Hijji 2003) or habitat complexity.

Second, the developmental stage of a sporocarp has a pronounced effect on insect density (Graves 1960; Nadvornaya and Nadvorny 1991; Okland and Hågvar 1994; Thunes 1994). The processes of insect colonisation of sporocarps can be categorised as follows (Schigel et al. 2006): the first to appear on the fungal substrate are spore-feeders (very weak destructors), followed by sporocarp-feeders (weak destructors), and then sporocarp-dwellers (effective destructors). Finally, at late stages of decomposition, anamorphic fungi grow over the rotting fruit body, and they host another wave of microphagous beetles. Typically, sporocarps undergo two alternative pathways of the decomposition process (Graves 1960);
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Some mature sporocarps become desiccated, often riddled with insect burrows or become entirely hollowed out, while others rapidly decay due to the proliferation of bacteria, moulds, and other fungi, perhaps aided by insects (Graves 1960).

Third, microhabitat effects include the water content of sporocarps (Thunes et al. 2000; Rukke 2002), height above ground (Elton and Miller 1954; Paviour-Smith 1964; Midtgaard et al. 1998; Rukke 2002; Jonsell et al. 2007), sun exposure (Kaila et al. 1997; Sverdrup-Thygeson and Ims 2002; Komonen and Kouki 2005; Komonen 2008) and the status of substrates for fungi (Thunes and Willassen 1997; Jonsell et al. 1999, 2004, 2005; Lindhe et al. 2005; Abrahamsson and Lindbladh 2006). Mycophagous insects are sensitive to many forest management practices that modify macrohabitat characteristics (Grove and Stork 1999; Grove 2002a, 2002b; Saint-Germain et al. 2006; see also Selonen et al. 2005). Two ciid beetles, Octotemnus glabriculus and Cis boleti, occur slightly more frequently in forests than in clearcuts, despite the lower resource availability of the host fungus Trametes in forests (Komonen and Kouki 2005). The spatial extent of surrounding old-growth forests may affect the abundance of mycetophilid flies (Økland 1996). A potentially powerful but infrequent flyer, Oplopephala haemorrhoidalis (Tenebrionidae), is restricted primarily to old-growth forests with a high density of suitable substrates that have been available for a long period of time, providing the prerequisite for population establishment (Jonsson 2003).

Fourth, small-bodied fungus specialists that are poor dispersers may be susceptible to isolation effects that may be alleviated by metapopulation structure (Whitlock 1992; Ingvarsson et al. 1997; Starzomski and Bondrup-Nielsen 2002). The immigration rates of mycophagous beetles decline with increasing distance between sporocarp patches (Heatwole and Heatwole, 1968; Jonsell et al. 1999). Density-dependent emigration may occur in Cis bilamellatus populations and may be caused by resource depletion, as eggs are continually produced and emerged adults tend to remain in the sporocarp (Paviour-Smith 1968).
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*Phalacrus substriatus* (Phalacridae) inhabiting smut fungi consists of many small extinction-prone populations, but this species can persist via metapopulation dynamics (Ingvarsson et al. 1997).

**Insect-fungus mutualism**

Insect-fungus mutualisms take on a wide range of forms. The simplest form of mutualism represents insect-mediated dispersal of fungal vegetative spores (Batra 1963; Kukor and Martin 1983; Wheeler and Blackwell 1984; Tuno 1998; Harrington 2005). Bark beetles may serve as vectors for the dispersal of spores and mycelia, thus enhancing colonisation of the cambium by the fungal partner (Six 2003; Harrington 2005; Sallé et al. 2005). Stinkhorn spores consumed by gleba-feeding flies retain the ability to germinate and may be dispersed by the flies into new terrain via faecal pellets (Fulton 1889; Tuno 1998).

Two extraordinary model systems exist that contrast with plant-animal pollination mutualism in different ways. First, *Phorbia phrenione* (Anthomyiidae) adult flies transport spermatia (gametes) between self-incompatible fungal stromata of the plant-pathogenic fungus *Epichloë*, and the subsequent fly larvae consume perithecia that produce fungal propagules (i.e. ascospores) (Parker and Bultman 1991; see also Rao and Baumann 2004; Rao et al. 2005). Second, the rust fungus *Puccinia monoica* inhibits flowering in its host plants (*Arabis* spp.) and radically transforms host morphology, creating elevated components of infected leaves that mimic true flowers of unrelated species in shape, size, colour, and nectar production (Roy 1993). These fungal pseudoflowers attract insects that fertilise the rust by carrying spermatia between different mating types (Roy 1993, 1994; Roy and Raguso 1997). The former system represents an obligate fungus-insect mutualism that parallels pollination mutualisms, whereas the latter system displays the abuse of opportunistic visitor flies by fungi via floral mimicry.
Social insects have complex mutualistic associations with fungi. Fungus-growing attine ants provide the symbiotic fungus (Lepiotaceae) with optimal conditions for growth; in exchange, the fungus serves as the main food resource for the ants (Mueller et al. 2001; Caldera et al. 2009). These ants also exhibit a mutualistic relationship with filamentous bacteria (actinomycetes) that produce antibiotics to suppress the growth of the virulent fungal pathogen *Escovopsis* in their fungal gardens (Currie et al. 1999; Currie 2001). Southern pine beetles *Dendroctonus frontalis* engage in a beneficial symbiosis with the fungus *Entomocorticium* and use actinomycete bacteria to protect their fungal food source from a competitor fungus, *Ophiostoma minus* (Scott et al. 2008). A termite, *Reticulitermes speratus*, tends the sclerotium-forming fungus *Fibularhizoctonia* sp., which mimic termite eggs in size, shape, and chemistry, whereby the presence of termite balls sometimes enhances egg survival, while the fungus gains competitor-free space in the termite nests (Matsuura et al. 2000, 2009; Matsuura 2006). The members of the termite subfamily Macrotermitinae tend their mutualistic fungus *Termitomyces*, which is a poor competitor, and passage through worker guts reduces germination of non-mutualistic symbiont spores (Aanen et al. 2002). A review of the associations of siricid woodwasps, fungus-growing termites, and fungus-growing ants suggested that the rate of speciation has been slower in the protected partner than in the host partner and that the protected partner might exhibit a low degree of host specificity (Martin 1992).

**Insect competition**

Exploitative larval competition is likely to be intense in some, if not all, sporocarps (Paviour-Smith 1968; Grimaldi and Jaenike 1984; Kadowaki 2010), in that larvae must complete development in the sporocarp where they are oviposited. Copious experimental evidence indicates that in ephemeral mushroom systems, competition involves high mortality induced by resource depletion and/or a decrease in body size of emergent adults and a resultant
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decrease in fecundity (e.g. Atkinson and Shorocks 1981; Grimaldi and Jaenike 1984; Gilpin et al. 1986; Ståhls et al. 1989; Courtney et al. 1990; Shorocks et al. 1990a; Worthen et al. 1993; Takahashi and Kimura 2005; see also Worthen et al. 1994), yet competition appears to have no effect on community composition (Worthen et al. 1993). In long-lasting bracket fungi, interspecific competition has been implicated by several studies (Paviour-Smith 1965; Lawrence 1973; Midtgaard et al. 1998), with additional support by observational studies (Thunes 1994; Kadowaki 2010; but see Paviour-Smith 1968).

Niche partitioning and spatial mechanisms are two commonly proposed explanations for insect species coexistence that counteract interspecific competition (Kadowaki 2010). The niche partitioning hypothesis posits that different insect species use different resources (resource partitioning), different developmental and/or life stages of a resource (successional niche partitioning; Guevara et al. 2000a; Jonsell and Nordlander 2004), or different organs and/or parts of a given resource (Mathewman and Pielou 1971; Hackman and Meinander 1979; Schigel et al. 2004). Guevara et al. (2000a) demonstrated niche partitioning in two ciid beetles, Octotemnus glabriculus and Cis boleti, among developmental stages of Trametes sporocarps via discrimination of age-dependent chemical composition of the sporocarps. The reproductive activities of O. glabriculus and C. boleti were independent of the presence or absence of the other species; therefore, such successional niche partitioning was not mediated by contemporary interspecific competition (Guevara et al. 2000a).

The spatial structure of fungal habitats can buffer interspecific competition in various ways. In ephemeral mushrooms, insect species are spatially aggregated in a number of sporocarp patches, by chance creating spatial refuges for inferior competitors (the so-called aggregation model of coexistence) (Atkinson and Shorocks 1981, 1984; Ives and May 1985; Shorrocks and Rosewell 1987; Ståhls et al. 1989; Rosewell et al. 1990; Shorrocks et al. 1990b; Hartley and Shorrocks 2002; but see Green 1986; Worthen and McGuire 1988).
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Spatial refuges may also result from the under-exploitation of large-sized sporocarps (Sevenster 1996; Toda et al. 1999). The movement of insects is restricted at the scale of ‘clumps of patches’, thereby increasing the number of patch-level spatial refuges for inferior competitors (Inouye 1999; Kadowaki 2010). Fitness trade-offs may facilitate coexistence in a spatial context. In ephemeral mushroom systems, Sevenster and van Alphen (1993) used simulations to propose that if a short developmental period increases larval competitive ability and a long adult life increases the chance of reaching new breeding sites in time and space, then a trade-off between these two life-history traits explains stable coexistence in drosophilid communities. Toda and Kimura (1997) argued against Sevenster and van Alphen (1993) by demonstrating a negative correlation between larval developmental time and adult life span in mycophagous drosophilids. Historical effects may modify spatial coexistence in fungus-insect communities. Two drosophilid competitors, *Drosophila phalerata* and *D. subobscura*, display priority effects for fitness parameters; the species that arrives at the resource later exhibits lower survival, smaller size, and longer developmental time than the species that arrives first; however, priority effects are unlikely to influence species coexistence in the field when larvae are spatially aggregated over host mushrooms (Shorrocks and Bingley 1994).

**Predator-prey interactions**

Potential predators in fungal habitats include ants, beetles (Graves and Graves 1966; Paviour-Smith 1968; Fäldt et al. 1999; Johansson et al. 2006), parasitoid wasps (Sevc 2001), parasitic nematodes (Jaenike 1995), mites (Freire et al. 2007), birds (Nadvornaya and Nadvorny 1991), and mammals (Conner et al. 1985). Experimental evidence has indicated that predatory ants reduce the survival of mycophagous flies (Lewis and Worthen 1992; Worthen et al. 1993, 1994, 1995), and predation on ovipositing adults by a staphylinid beetle also reduces the number and biomass of mycophagous flies (Worthen 1989). Attack rates by parasitoids on
mycophagous insects can be low (Grimaldi and Jaenike 1984) or relatively high (Paviour-Smith 1968). High parasitism rates by nematodes of mycophagous Drosophila result in reductions in female fertility (Montague and Jaenike 1985) and greater mortality rates (Jaenike 1995) but not reductions in larval survival (Jaenike and Anderson 1992). The rates of nematode parasite transmission increase with the ratio of adult flies to mushrooms at a small spatial scale (Jaenike and Anderson 1992) and also depend on patterns of precipitation at a larger spatial scale (Jaenike and Perlman 2002).

The consequences of host-parasite and host-parasitoid interactions on population dynamics are controversial. Parasitism may play a large role in the population dynamics of mycophagous drosophilids (Vet 1983; Janssen et al. 1988; Jansen 1989). A parasitoid wasp, Cephalonomia formiciformis (Bethylidae) has been shown to exert high mortality on ciid beetle populations but without population extinction (Paviour-Smith 1968). The probability of parasitism of emergent Drosophila by the nematode Howardula aoronymphium is generally independent of emergence numbers (i.e. density-independent parasitism) (Jaenike and James 1991). In a field experiment, Jaenike et al. (1995) demonstrated that parasite-induced mortality can regulate Drosophila population density by releasing Drosophila putrida and D. neotestacea that were parasitised by nematodes in the laboratory. Vertical transmission potentially contributes to the dynamics of natural populations of these parasites (Jaenike 2000) and can help to sustain populations of parasites through inevitable periods of low host population density (Jaenike and James 1991; Jaenike and Dombeck 1998). Jonsson and Nordlander (2006) documented suggestive evidence for a predator release effect in a colonisation experiment using Fomitopsis pinicola. Natural experiments via space-for-time substitution offer insight into the persistence of host-parasitoid interactions: Komonen et al. (2000) showed that forest fragmentation truncated the top species of parasitoid of fungivores
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in most isolated forests, suggesting non-random species extinctions in terms of food-web structure.

Predator-prey interactions may depend on other concurrent interactions. The deleterious effects of predatory ants on the survival of mycophagous flies depend on small-scale differences in soil moisture (Worthen et al. 1993, 1994). Due to conflicting evidence (Worthen et al. 1993, 1994), it is unclear whether the interactive effects were complex in reality or if the result itself was not repeatable. Predation by a beetle on ovipositing adults also relaxes interspecific competition among mycophagous flies (Worthen 1989; Ståhls et al. 1989). Such ‘keystone predator’ effects have been demonstrated in laboratory experiments with mycophagous flies (Worthen 1989) yet had no effect on community structure in field experiments (Worthen et al. 1993, 1994). The parasitic nematode *Howardula aoronymphium* may sufficiently reduce the competitive advantage of the superior competitor species to allow for the coexistence of *Drosophila quinaria* (Jaenike and Perlman 2002).

**Overview**

Interactions between host fungi and their consumers in part parallel plant-herbivore interactions (Cooke 1977; Shaw 1992; Guevara et al. 2000b). Shaw (1992) wrote: “The flies and beetles that graze fungal fruiting bodies are exceptions with no good analogue in other systems [i.e. plant-herbivore systems], since they are exploiting a rich but transient resource without affecting its fitness.” For such an “established” theory, notably few experimental tests have quantified the effects of insects on fungal fitness. As pointed out by Guevara et al. (2000b), little evidence exists for the ‘magnitude’ of antagonistic interactions in fungus-insect studies. Little quantitative research has addressed the effects of consumer insects on fungal physiology, metabolism (Boddy and Jones 2008), phenotypes, reproductive efforts, or life history trade-offs. Consequently, partial or incomplete descriptions of host fungus-insect
consumer systems in the literature may have inflated our awareness of certain types of the interactions relative to others.

Fungus-insect mutualism studies have not been extended to mushroom-forming fungi beyond several extraordinary model systems. Among studies of insect-mediated spore dispersal, most have attempted to narrow down insect candidates as dispersal agents but have not unequivocally shown increased or neutral effects on the survival of spores by ingestion, digestion, or sequestration, coupled with the acceptance of certain fungi as food by insects. Few removal experiments of mutualist partners have been conducted; therefore, the relative importance of alternative spore dispersal strategies for fungi is not understood. The consideration of only population-level averages may have promulgated the neglect of spatial variation in mutualistic interactions. It is useful to note that fungus-insect interactions may have components for which it is hard to tell where antagonistic interaction leaves off, and mutualistic interaction begins. These components may depend on the physiological traits of spores and digestive capability of spore consumers (Tuno et al. 2009).

While mounting evidence from short-term experiments points to the importance of insect competition and predator-prey interactions in ephemeral mushrooms, such studies by themselves are not adequate to elucidate long-term impacts on insect populations and community dynamics. Indeed, one of two broad strategies to study these long-term impacts would be to superimpose repeated short-term experimental results under various conditions. This approach can, however, lead to the inevitable stumbling block that rearing fungus insects is often challenging; there has been no single report of the establishment of experimental populations of fungus specialists, whereas some work has been conducted on fungus generalists (e.g. Grimaldi and Jaenike 1984; Jaenike 1992). The alternative solution would be to directly examine these patterns from time-series data through model fitting.
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exercises. The application of this approach has also been limited by the lack of both high
good quality time series data and empirical efforts to aggressively combine theory and data.

The vast majority of studies has focused on only one ecosystem role within fungus-insect
communities and has therefore been unilateral in approach. This approach not only prevents a
complete understanding of a single fungus-insect system, but also overlooks the interactive
effects of multiple ecosystem roles on such systems. Extant fungus-insect communities may
be controlled by a mixture of host fungus-consumer insect interactions, fungus-insect
mutualisms, insect competition, and predator-prey interactions. Thus, a multilateral approach
would enrich the understanding of how various types of ecosystem roles combine to shape
fungus-insect communities. In addition, studies of ephemeral mushroom systems and those of
long-lasting bracket fungi have rarely referenced each other, thus hindering comparative
perspectives across a variety of fungus-insect systems.

These trends and knowledge gaps in fungus-insect ecology are summarised in Table 1-3.

### Table 1-3. Trends and knowledge gaps in fungus-insect ecology.

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Aims and layout of thesis

The preceding sections highlight two key missing approaches to the study of fungus-insect ecology: quantification of fungus-insect interactions, and a multilateral examination of concurrent ecosystem roles. These two approaches adopted in the thesis can create a synergy to establish recurring patterns and to understand the elementary processes that organise fungus-insect communities. Motivated by combining the multilateral and quantitative approaches, I use a variety of research techniques spanning from field observations, statistical modelling, and laboratory experiments to address three ecosystem roles: host fungus-consumer insects, insect competition, and insect-fungus mutualisms. The thesis aims to elucidate poorly understood factors: extrinsic factors that influence long-term resource dynamics and its spatial variation, the relative magnitude and symmetry of host fungus-consumer insect interactions, spatial dynamics of insect competition, and species coexistence mechanisms in space. Such a multilateral and quantitative examination of these factors can provide an integrated view of the causal links in any fungus-insect community (Fig. 1-3).

![Potential causal links in a hypothetical fungus-insect community](image)

**Figure 1-3.** Potential causal links in a hypothetical fungus-insect community: environmental variables influence fungi, and fungi and insects interact with each other, while insects compete for the fungal resource.
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**Study system**

This section provides background information on the taxonomy and biology of the study system.

**Ganoderma.**—*Ganoderma* Karst. (Ganodermataceae, Basidiomycota) produces perennial sporocarps on a variety of woody hosts, and three species are found in New Zealand (Buchanan and Wilkie 1995), two of which I encountered in my research: *Ganoderma* cf. *applanatum* (Pers.) Pat and *G. australe* (Fr.) Pat. These two species are distinguished based on stromal texture, hymenium, spore-tube morphology, spore size, and spore colour (Buchanan and Wilkie 1995). Sporocarps of *Ganoderma* grow perennially at decelerating rates (Fig. 1-4a). The hymenial surface discharges an astronomical number of spores following the trajectories shown in Fig. 1-4 b. Spores of *Ganoderma* species are uniquely double-walled, typically ovoid, echinulate, and enlarged or truncated at the apex; those of *G. applanatum* measure 9.0–11.0 × 6.0–7.5 μm, whereas spores of *G. australe* are 11.5–14.5 × 6.5–9.0 μm (Buchanan and Wilkie 1995).
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Figure 1-4. Macroscopic and microscopic structure of a perennial *Ganoderma* sporocarp. (a) Vertical section of *Ganoderma applanatum* sporocarp collected from a beech trunk in July 1949. The sporocarp consists of sterile pileus tissue and a myriad of longitudinal pore tubes; sterile pileus tissue is shown in black. Six annual layers of tubes are visible. The tubes are continuous from one layer to the next but are shown interrupted at the end of each year’s growth to distinguish the annual layers. Interestingly, not only sporocarps, but also the hymenial surfaces themselves, are perennial (Ingold 1953). (b) Part of a vertical section of sporocarp (Ingold and Hudson 1993). Spores are discharged along the trajectories indicated by the arrows. (c) Horizontal section through the pore region of a sporocarp (Ingold and Hudson 1993). (d) Details of a single pore (Ingold and Hudson 1993).

**Leiodidae.**—Leiodid beetles are moderately diverse and found worldwide. The reasonably diverse fauna of Leiodidae in New Zealand resulted from the “Gondwanan” distribution
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patterns rather than recent overseas dispersal (Newton 2005). The genus *Zearagytodes* Jeannel contains three described species (and four or more new species) and is endemic to New Zealand (Newton 1984, 1989; Seago 2005). *Zearagytodes maculifer* (Broun) (Leiodidae: Camiarinae: Agyrtodini) is found throughout the North Island, New Zealand (Kuschel 1990). The natural history of Leiodidae is too varied to concisely summarize, but the family includes many fungus-feeders (Newton 1984; Seago 2005). *Zearagytodes maculifer* is a very agile spore grazer that browses on the hymenial surface of mainly *Ganoderma* species (Kuschel 1990); larvae are slender, aposematic, and have elongate urogomphi.

**Corylophidae.**—Corylophids commonly have a prominent hood-like pronotum in adults, but larval characters tend to be highly derived with strongly modified mouthparts, antenna, and defensive glands (Ślipiński et al. 2009). Most Corylophidae feed as both larvae and adults on the spores or hyphae of moulds and other microfungi, and there are two types of associations with Basidiomycetes: 1) feeding on conidia of secondary fungi (mainly ascomycetes) covering the surfaces of old decaying polypore or mushroom fruiting bodies, and 2) feeding on basidiospores (Ślipiński et al. 2010). The genus *Holopsis* (Corylophidae: Corylophinae: Peltinodini) is contained in its own tribe Peltinodini (Ślipiński et al. 2009) and is worldwide in distribution, but absent from the Oriental and Afrotropical regions (Bowestead 1999). The genus contains five described species in New Zealand (Hudson 1923), but there are many undescribed species, including four or more in the Auckland area (Kuschel 1990).

In the Waitakere Ranges (Auckland, New Zealand), there are two new species, *Holopsis* sp. 1 and 2, which were confirmed in my research by dissection of adults (female genitalia are distinct in each species); these are referred to as *Holopsis* sp. 1 and *Holopsis* sp. 2, respectively. The two species of *Holopsis* spp. (Corylophidae: Corylophinae: Peltinodini) are smaller-bodied spore-feeders with extremely similar external morphology as
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adults, but larvae differ markedly in colour pattern and especially the head structures: *Holopsis* sp. 1 has a long rostrum while *Holopsis* sp. 2 lacks a rostrum. Lawrence (1989) conjectured that the larva of *Holopsis* sp. 1 was a ‘pore tube specialist’ that would have access to immature spores in the depth of pore tubes.

**Thesis layout**

This thesis is presented as a series of loosely connected, self-contained chapters. The chapters are grouped under the four main issues of extrinsic factors that affect fungi (Chapter 2), host fungus-consumer insect interactions (Chapters 3, 4), insect competition (Chapter 5), and fungus-insect mutualisms (Chapter 6).

Chapter 2 describes spore release dynamics of *Ganoderma*, focusing on spatial synchrony and environmental effects. This chapter presents the first field study that attempted to examine the forces controlling spore release at the sporocarp level, rather than measuring airborne spores, which has been the approach of previous studies. Chapter 3 examines the life history and several aspects of spore consumption in the obligate spore-feeding beetle, *Zearagytodes maculifer*. I make use of field census, laboratory experiments and field trials to investigate larval development time, generation patterns, host use patterns, and larval spore-breaking capability. Chapter 4 shows the ontogenetic allometries of two congeneric *Holopsis* beetle species and discusses links with niche theory. I decompose larval morphological character states into a hierarchy of allometric components by statistical analysis to find divergence of ontogenetic trajectories between the two species. Chapter 5 reveals spatial coexistence mechanisms of three competing spore-feeding beetles in the context of metacommunity ecology. I take advantage of high quality population dynamics datasets to investigate competitive rank orders and competitive interaction webs of the three spore-feeding beetles. In Chapter 6, I move away from insect competition to experimental analysis
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of a spore dispersal mutualism between *Ganoderma* and *Z. maculifer*, addressing the effects of beetle feeding on *Ganoderma* spore germination rates. In conclusion, Chapter 7 (General Discussion) revisits the knowledge gaps identified in Chapter 1, and provides a synthesis of the first six chapters to show how my study has changed our way of understanding fungus-insect communities. I then discuss limitation of my research, painful lessons from measuring insect competition, and ideas for future challenges.
Chapter 2 Periodicity of spore release from individual *Ganoderma* fruiting bodies in a natural forest

PLATE 2. A kauri tree *Agathis* which is one of the common hosts for *Ganoderma* spp. fruiting bodies (sporocarps) in the Waitakere Ranges, Auckland, New Zealand (*Photo: K. Kadowaki*).
2. *Ganoderma* spore release

**Introduction**

Fungal spores play the primary role in both survival of a fungal species under unfavourable conditions and dispersal into new habitats (Kramer 1982), but our knowledge about how environmental conditions affect spore release dynamics in Basidiomycetes is surprisingly limited. Haard and Kramer (1970) provided a synthesized framework that explains spore release patterns through a complex of factors: (1) circadian (Haard and Kramer 1970; McCracken 1972; Gilbert and Reynolds 2005), (2) seasonal (White 1919; Hasnain et al. 1984), (3) lifespan (i.e. sporocarp-size-dependent) (Haard and Kramer 1970; Nuss 1982), and (4) revival patterns after rainfall (Rockett and Kramer 1974a, b; Gilliam 1975; Li 2005).

Empirical studies have shown that spore release depends on several environmental factors, including temperature (Bohaychuk and Whitney 1973; McCracken 1970, 1987; Craig and Levetin 2000), humidity (Haard and Kramer 1970; McCracken 1972; McKnight 1990; Li and Kendrick 1995; Li 2005; also see White 1919), light intensity (Kramer and Pady 1970; Okhuoya 1989; also see Kramer and Long 1970), landscape features (Edman et al. 2004), and seasonality (e.g. White 1919; Haard and Kramer 1970; McCracken 1972; Hasnain et al. 1984; Craig and Levetin 2000).

Experimental *in vitro* studies have manipulated environmental factors to observe changes in spore release, using one or a few sporocarp(s) in laboratory or greenhouse conditions (White 1919; McCracken 1972; Rockett and Kramer 1974a). Field studies, on the other hand, have measured airborne spores by a variety of techniques (Sreeramulu 1963; Rockett and Kramer 1974c; Harrington 1980; Hasnain et al. 1984; Arnold and Herre 2003; Gilbert and Reynolds 2005), which measure spore numbers from many sporocarps in unspecified locations. No studies have quantified long-term spore release at the level of a sporocarp where fungi possibly perceive their environments and effect their fitness.
The present study measured monthly spore release dynamics of two species of the wood-decaying basidiomycete *Ganoderma* Karst. (Ganodermataceae) in the Waitakere Ranges, North Island, New Zealand. Spore release of the genus *Ganoderma* has been investigated by several authors (White 1919; Haard and Kramer 1970; McCracken 1987; Craig and Levetin 2000), but the effect of environmental variables on spore release and the presence of seasonal periodicity remain unknown at the sporocarp level. Here I first look at how environmental variables shape the dynamics of spore release across a number of different sporocarps in the landscape. I then explored two important features of spore release dynamics: seasonal periodicity and spatial synchrony. I examined several working models to identify the patterns of seasonality and spatial synchrony of spore release by an information-theoretic approach.

**Material and Methods**

**Fungi**

The world-wide genus *Ganoderma* produces conspicuous perennial sporocarps on a variety of woody hosts and there are three species widely recognized in New Zealand (Pennycook and Galloway 2004). In the Waitakere Ranges there are two common species, *Ganoderma* cf. *aplanatum* (Pers.) Pat and *G. australe* (Fr.) Pat., which are distinguished based on context texture, pore size, spore size and spore print colour (Buchanan and Wilkie 1995). Notably, *G. cf. aplanatum* has spores that are much smaller than those of *G. australe*. *Ganoderma* cf. *aplanatum* (= *G. mastoporum* (Lev) Pat, sensu auct. NZ) is the more common species, and has two different pore surface morphotypes that were once considered as separate species (Cunningham 1965): one type has an even smooth pore surface; the other has a distinctly pitted and rugose pore surface (Buchanan and Wilkie 1995). *Ganoderma* has a unique double-walled spore, typically ovoid, echinulate, and enlarged or truncated at the apex (Moncalvo et al. 1995).
2. *Ganoderma* spore release

**Study site**

The Waitakere Ranges are a series of hills covering 30 000 ha on the coast 25 km west of Auckland City, rising up to a height of 460 m a.s.l. and comprise dense regenerating native forest (Esler and Astridge 1974). The study area consists of two large-area sites, the Cascade Kauri (36˚53'S, 174˚31'E) and the Upper Huia Dam (36˚56–57'S, 174˚30–32'E), separated by 8 km (Fig. 2-1a). The vegetation differs between sites: the Cascade Kauri site is *Agathis*-dominated forest, whereas the Upper Huia Dam site is mixed broadleaf–podocarp forest.
2. *Ganoderma* spore release

![Map of study areas](image)

**Figure 2-1.** (a) Study area and study sites; (b) Cascade Kauri area; (c) Upper Huia Dam area; and (d) sporocarps observed (identifications indicated bottom left). In (d), bar length indicates sporocarp duration (during the period from November 2007 to October 2008). Months are shown with a one-letter code starting from N (November 2007) and finishing in O (October 2008). The grey zone in the sporocarp 1-5B at Cascade Kauri indicates where the sporocarp was perturbed by mould but rejuvenated in a month.
2. *Ganoderma* spore release

**Spore survey**

Dead logs with *Ganoderma* sporocarps were sampled at each site, nine at Cascade Kauri and seven at Upper Huia Dam sites (Fig. 2-1b, c). The mean distance between dead logs was 0.6 km in Cascade Kauri, and 1.8 km in Upper Huia Dam. Each dead log chosen had one or more sporocarps of *Ganoderma* and I chose up to three sporocarps per dead log (Fig. 2-1d).

Reflecting the natural proportion of sporocarp occurrence in the areas, there were more *G. cf. applanatum* sporocarps (25) than *G. australe* (5). If a sporocarp decayed or was destroyed by disturbance, it was omitted from the next measurement, whereas newly emergent sporocarps (width < 5.0 cm) were recorded as new sporocarps and inspected in later surveys. Sporocarp number was 25 at the start of the study and 21 at the end, with a total of 30 studied overall (n = 15 per site) (Fig. 2-1d).

The spore release survey was carried out at monthly intervals from November 2007 to October 2008. A spore print was collected over 24 h from each sporocarp by attaching a sheet of plastic plate (3.7 cm × 2.3 cm) on the centre of the exposed pore surface. Spore-feeding beetles are common on *Ganoderma* sporocarps in these forests (Kadowaki et al. 2011, Chapter 3), and were excluded by the small gap (<1.0 mm) between the plate and the pore surface. I chose two consecutive non-rainy days (occasionally with showers) for sampling in order (1) to avoid rain washing spores out of the plastic plates and (2) to reduce a possible confounding effect between seasonality and weather conditions. In the laboratory, each spore print was suspended into 10–20 ml of tap water, and the spore density was estimated using a haemocytometer under a microscope (×100 magnification). Each spore sample was counted three times and averaged.

Several environmental variables were measured for each sporocarp: temperature and relative humidity were measured monthly, and light intensity and pore surface area every other month. Temperature and relative humidity were recorded with a hand-held meter (4200
2. *Ganoderma* spore release

Pocket weather meter, Kestrel Weather Australia) at the starting and finishing times of spore collection. Light intensity was quantified by percentage of canopy cover (%), measured by a spherical densiometer (Concave, Ben Meadows Company). The outer edge of the pore surface was traced with a permanent marker on standardized-weight tracing paper (1.12 × 10^{-2} g cm^{-2}) attached to the pore surface, and the trimmed pieces of papers were dried and weighed to estimate pore surface area (cm^2). I also measured height above ground (cm) where a sporocarp was situated on a log. The daily spore release by each sporocarp was obtained by multiplying spore density per cm^2 by total pore surface area. Pileus thickness was measured as the distance between the top of the pileus to the centre of the pore surface where spore collection was made.

**Statistical analysis**

I first conducted separate linear regression analyses of spore release per plate with respect to each environmental variable. A linear model with identity link and a Gaussian error term was used. These analyses consider neither the interdependence of repeated measurements from the same sporocarps, nor spatial dependence between sporocarps.

To understand the dynamics of spore release per plate, I constructed one mechanistic model firmly anchored in mycology (e.g. Haard and Kramer 1970), and several phenomenological models. These five types of models reflect different underlying mechanisms in spore release dynamics: environmental conditions, seasonal constancy, time trends, seasonal periodicity and spatially asynchronous fluctuations (Table 2-1). The linear mixed-effects models were employed to accommodate repeated measurements (12 times in total) but did not consider nested site-effects for simplicity.
2. Ganoderma spore release

### Table 2-1. Mixed-effects models of spore release per plate dynamics of Ganoderma cf. applanatum.

<table>
<thead>
<tr>
<th>Models</th>
<th>Mixed-effects models’ formulae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental condition</td>
<td>( y_{ij} = (\beta_0 + b_{0j}) + \beta_1 \text{Temp} + \beta_2 \text{RH} + \beta_3 \text{CnpCov} + \beta_4 \text{CarpSize} + \beta_5 \text{Height} + e_{ij} )</td>
</tr>
<tr>
<td>Seasonal constancy</td>
<td>( y_{ij} = (\beta_0 + b_{0j}) )</td>
</tr>
<tr>
<td>Time-trend I</td>
<td>( y_{ij} = (\beta_0 + b_{0j}) + \beta_1 \text{Month} + e_{ij} )</td>
</tr>
<tr>
<td>Time-trend II</td>
<td>( y_{ij} = (\beta_0 + b_{0j}) + (\beta_1 + b_{1j}) \text{Month} + e_{ij} )</td>
</tr>
<tr>
<td>Seasonal periodicity I</td>
<td>( y_{ij} = (\beta_0 + b_{0j}) + \beta_1 \sin \left( \frac{(2i - 1)\pi}{12} \right) + \beta_2 \cos \left( \frac{(2i - 1)\pi}{12} \right) + e_{ij} )</td>
</tr>
<tr>
<td>Seasonal periodicity II</td>
<td>( y_{ij} = (\beta_0 + b_{0j}) + \beta_1 \sin \left( \frac{(2i - 1)\pi}{12} \right) + \beta_2 \cos \left( \frac{(2i - 1)\pi}{12} \right) + \beta_3 \sin \left( \frac{(2i - 1)\pi}{6} \right) + \beta_4 \cos \left( \frac{(2i - 1)\pi}{6} \right) + e_{ij} )</td>
</tr>
<tr>
<td>Spatial asynchrony</td>
<td>( y_{ij} = (\beta_0 + b_{0j}) + (\beta_1 + b_{1j}) \sin \left( \frac{(2i - 1)\pi}{12} \right) + (\beta_2 + b_{2j}) \cos \left( \frac{(2i - 1)\pi}{12} \right) + e_{ij} )</td>
</tr>
</tbody>
</table>

The estimated spore density in the \( i \) th month for the \( j \) th sporocarp. The regressors include Temp = temperature, RH = relative humidity, CnpCov = Canopy cover (light intensity), CarpSize = Sporocarp size, Height = height above ground, Month = month (\( i = 1, 2, \ldots, 12 \)) and \( \beta_0, \beta_1, \ldots, \beta_5 \) denote the fixed effects, while random effects are \( b_j \sim N(0, \sigma_b^2) \). The error term is \( e_{ij} \sim N(0, \sigma^2) \).

The environmental condition model depends on the linear relationship between spore release and temperature, relative humidity, canopy cover, sporocarp size and height from ground (Table 2-1). Environmental variables were centered to zero to reduce correlation between intercepts and estimates before analysis. The seasonal constancy model predicts that Ganoderma spore release patterns are constant over time at different background levels among sporocarps, thus providing a null expectation against the other phenomenological models of time trends and seasonal periodicity. The time trend model I depends on the shared slope of increasing or decreasing trends among different sporocarps, whereas the time trend model II accommodates differential increasing or decreasing trends across sporocarps. The seasonal periodicity models I and II accommodate synchronous cyclic fluctuations expressed by harmonics of trigonometrical functions (Lindsey 1995), fitting twelve months and six months cycles respectively. The spatial asynchrony model is a generalized version of
seasonal periodicity I model (Table 2-1), and it uses to express asynchronous periodicity in spore release across different sporocarps. Although spatial asynchrony model does not explicitly use any spatial coordinates, it can depict differential dynamics of spore release across sporocarps in spatially implicit manner.

I selected the most parsimonious model in terms of Akaike’s Information Criterion (AIC) (Vaida and Blanchard 2005) as well as the Bayesian Information Criterion (BIC), and the significance of deviance reduction was then tested among the models. Data with missing values (destroyed sporocarps and new sporocarps) were excluded from the mixed-effects models. Because of such missing values for *G. australe* sporocarps, there was only one longitudinal series left so *G. australe* data were excluded from analysis. Analyses were conducted with R 2.12.0 (R Development Core Team 2010).

**Results**

**Overview**

*Ganoderma* sporocarps produced an astronomical number of spores consistently over the year. For each *Ganoderma* sporocarp, mean spore release (day$^{-1}$ cm$^{-2}$ pore surface area) varied from $1.22 \times 10^7$ at minimum to $4.31 \times 10^7$ at maximum. The monthly total spore release amounted to $1.32 \times 10^{10}$ spores. The average spore density was 3.5 times higher in summer than in winter, although January saw a low density around (the range of means = $8.60 \times 10^9$ at minimum in March 2008 to $3.04 \times 10^{10}$ at maximum in December 2007 for an intermediate-sized sporocarp (pore surface area 195.7 cm$^2$). Sporocarp destruction and emergence occurred eight times and four times respectively in a year (Fig. 2-1d). Two sporocarps were destroyed by disturbance, another two were removed (presumably by illegal collectors), and the remaining four were killed by mould or unknown mortality factors. The mean sporocarp size (pore surface area) was 195.7 cm$^2$ (range = 13.7–664.4 cm$^2$). Some sporocarps did not increase in size over the year, while others showed a dramatic increase in
2. *Ganoderma* spore release

Size. The mean rate of monthly sporocarp growth of the sporocarps that survived 12 months was 40.75 cm$^2$ (range = 0 – 114 cm$^2$ month$^{-1}$). Pileus thickness was correlated with pore surface area ($r = 0.483$, $p = 0.0167$), so pore surface area is hereafter used as an index of sporocarp size.

![Figure 2-2](image_url)

**Figure 2-2.** The effects of environmental variables on spore release per plate (3.7 cm × 2.3 cm); a) temperature (°C); b) relative humidity (%); c) canopy cover (%); d) height above ground (cm); e) *Ganoderma* species (*G. cf. applanatum* or *G. australe*); f) sporocarp size or pore surface area (cm$^2$). Spore density cm$^2$ was $ln$-transformed. None of the environmental variables significantly affected spore density. Repeated measurements were not considered for significance testing.
2. *Ganoderma* spore release

**Spore release dynamics**

None of the environmental variables significantly influenced spore release at the sporocarp level in separate general linear model analyses (Fig. 2-2). The effects of temperature and relative humidity were positive but not significant ($t = 1.138$, df = 1, $p = 0.257$, and $t = 1.214$, df =1, $p = 0.231$, t-test, respectively; Fig. 2-2a, b). The effect of canopy cover or height above ground were negligible ($t = –0.156$, df =1, $p = 0.876$, and $t = –0.185$, df =1, $p = 0.853$, t-test respectively; Fig. 2-2 d). The two *Ganoderma* species only marginally differed in spore density ($t = 1.879$, df =1, $p = 0.083$; Fig. 2-2e); *G. australe* produced larger but not significantly more spores than *G. cf. applanatum*. No effect of sporocarp size was found ($t = 0.923$, df =1, $p = 0.358$; Fig. 2-2f).

The most parsimonious model among seven working models was the spatial asynchrony model. Figure 2-3 presents the best-fitting curves derived from the spatial asynchrony model. The environmental condition model gave an unsatisfactory fit (Table 2-2). The time trend model II with random slopes could not improve the fit of time trend model I ($\chi^2 = 3.4982$, df = 2, $p = 0.1739$, ANOVA). Seasonal periodicity models were comparable to time trend models. The spatial asynchrony model and seasonal constancy model lowered the deviance, and outperformed the other models. Although I tried to fit a more complex spatial asynchrony model with a second set of harmonics, it did not reduce much of the deviance (not shown in Table 2-2; AIC = 385, $\chi^2 = 5.3043$, df = 5, $p = 0.3799$, ANOVA).
2. *Ganoderma* spore release

**Figure 2-3.** Fitted curves of spatial asynchrony models of spore release per plate (3.7 cm × 2.3 cm) of *Ganoderma cf. applanatum*. Each curve/symbol represents a different sporocarp (n = 11): A = 1-4A, B = 1-4B, C = 1-5A, D = 1-5C, E = 1-7B, F = 1-8A, G = 1-8B, H = 1-9, I = 2-1A, J = 2-4A, K = 2-4B (see Fig. 2-1 for locations and duration of sporocarps).
2. *Ganoderma* spore release

**Table 2-2.** Fitted mixed-effects models of spore release per plate dynamics.

<table>
<thead>
<tr>
<th>Model</th>
<th>AIC</th>
<th>ΔAIC</th>
<th>BIC</th>
<th>ΔBIC</th>
<th>Log-likelihood</th>
<th>REML Deviance</th>
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<tr>
<td>Random effects</td>
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<tr>
<td>Intercept</td>
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<td>0.285</td>
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<tr>
<td>Residual</td>
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<td>0.858</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Seasonal constancy         | 388   | 13    | 396.6 | 1.4   | −191.0         | 382          |
| Time trend I               | 393.3 | 18.3  | 404.9 | 9.7   | −192.7         | 378.9        |
| Time trend II              | 393.1 | 18.1  | 410.4 | 15.2  | −190.5         | 375.5        |
| Seasonal periodicity I     | 394.6 | 19.6  | 409   | 13.8  | −192.3         | 378.8        |
| Seasonal periodicity II    | 402.1 | 27.5  | 422.3 | 27.1  | −194.1         | 377.3        |
| Spatial asynchrony         | 375   | 0     | 395.2 | 0     | −180.5         | 357.1        |
| Intercept                  | 16.8  | 0.285| 59    |       |                |              |
| Residual                   | 0.764 | 0.874|       |       |                |              |

<table>
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<th>Variable</th>
<th>Estimate</th>
<th>SE</th>
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<th>Variable</th>
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<td>Intercept</td>
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2. *Ganoderma* spore release

For detailed model formulae, see Table 2-1. Abbreviations of variables denote: Temp = temperature, RH = relative humidity, CnpCov = canopy cover, CarpSize = sporocarp size, Height = height above ground, \(\operatorname{trg1} = \sin \frac{2i-1}{12} \pi\), \(\operatorname{trg2} = \cos \frac{2i-1}{12} \pi\), \(\operatorname{trg3} = \sin \frac{2i-1}{6} \pi\), \(\operatorname{trg4} = \cos \frac{2i-1}{6} \pi\). Parameters were obtained by restricted maximum likelihood estimation (REML).
Discussion

Spore release by *Ganoderma* spp. was highly variable across sporocarps. Daily spore release in my study was approximately 10-fold greater than that estimated by White (1919) for the same genus *Ganoderma* in Canada (i.e. $3.0 \times 10^7$ spores a day in White [1919]). I found myself unable to relate spore release patterns of *Ganoderma* to temperature and relative humidity. This accords with White (1919), but contrasts with Haard and Kramer (1970) who found that increasing temperature and/or relative humidity increased spore release in *Ganoderma*. This contrast might be explained by the temporal scales of the studies: Haard and Kramer (1970) studied circadian patterns, while White (1919) and my study focused on longer term patterns. I also found that spore release per plate did not depend on sporocarp size, which might partly reflect constancy in the number of pores per pore surface area cm$^2$ over the entire sporocarp. I did not detect a difference in spore release between the two *Ganoderma* species, but this is difficult to interpret due to the unbalanced number of samples.

I confirmed a 3.5-fold difference in spore release between summer and winter, similar to the 2–4-fold difference found in an airborne spore study of *Ganoderma* in Auckland, New Zealand (Hasnain et al. 1984). An exceptionally low level of spore release in January might be caused by drought; a loss of moisture in the wood substrate may cause a decline of spore release. However, such seasonality explained only a small proportion of the variability of spore release at the level of a sporocarp. This might highlight a fundamental difference between Hasnain et al. (1984) and my survey, both in the spatial scale of the observational unit and the methodology of spore sampling. While spore release was measured at the smaller scale of a sporocarp in my study, Hasnain et al. (1984) measured airborne spores by Burkard volumetric trapping, which might have masked differential fluctuations of spore release between sporocarps.
2. *Ganoderma* spore release

Contrary to my expectations, the phenomenological model of spatial asynchrony outperformed the environmental condition model (Table 2-2). Evidence for spatial synchrony was not confirmed since the spatial asynchrony model was superior to the seasonal periodicity model I. The rationale of the spatial asynchrony model is that sporocarps show seasonal cycles in spore release, but their cycles may not necessarily be spatially synchronous if sporocarps perceive their environments and effect fitness differently. The relatively well-fitted model of seasonal constancy also supported the notion that temporal fluctuations in spore release might have resulted simply from randomness at different background levels among sporocarps (Table 2-2). These models are necessarily phenomenological, and do not explain the underlying mechanisms behind what appears to be spatial asynchrony or seasonal constancy. I also acknowledge that there is considerable room to improve the fit of the statistical models. The large-sized residual deviance in the best-fit model suggests two possibilities. Firstly, there might be some kind of periodicity occurring at longer time intervals than the circadian intervals, or simply daily random fluctuations may dominate spore release in *Ganoderma* (e.g. Haard and Kramer 1970). Secondly, there might be missing variables or interactive effects of environmental variables I have not considered here. For example, physiochemical processes such as water budget (Haard and Kramer 1970) or ecological factors like fungal competition inside dead logs may cause spatial asynchronous effects on spore release. The interactive effects of environmental variables could be examined in experimental studies. Furthermore, one limitation of the present study is that I measured temperature and relative humidity at the start and end of the 24-h spore collection period, rather than using a data logger. This could have impeded my ability to detect significant effects of temperature and relative humidity on spore release. I generally surveyed sporocarps from 1000 to 1400 hours in a fixed sequence, so daily change of microclimates might be
2. *Ganoderma* spore release

confounded with rising temperature and relative humidity data. Given this caveat, I could not rule out the possibility that environmental heterogeneity influence spore release dynamics.

It is an empirical challenge to identify the controlling forces in highly fluctuating spore release (e.g. Haard and Kramer 1970; Hasnain et al. 1984; Craig and Levetin 2000). With the current limited knowledge, it is difficult to argue whether or not such large fluctuations are a universal property of spore release in *Ganoderma*. Nevertheless, the present study offers three insights. Firstly, one way to resolve such difficulties is that spore release can be measured more often in a month to minimize the overriding effects of high-amplitude daily fluctuation. Secondly, one could measure the environmental variables within substrates (i.e. dead logs), rather than outside or around a sporocarp. Specifically, larger sporocarps may be more resistant to fluctuations of environmental variables. Hence, larger sporocarps may have delayed response to these factors compared with smaller sporocarps. Thirdly, it is also interesting to ask whether weather interacts with seasonality; seasonality might be more pronounced on rainy days than non-rainy days where the background level of spore release prevails. Such measurements may perhaps improve the predictive power of my models and my ability to unravel the underlying mechanisms if spore release at the sporocarp level is not spatially synchronous. Although I present very simple statistical models that are widely applicable in other spore release studies, more advanced statistical modelling is required for analysing highly complex, fluctuating data (e.g. autoregressive models without a priori choosing the intervals of periodicity, generalized least square models that correct for spatially explicit trends, partitioning variance componenets in mixed-effects models, hierarchical Bayesian models).

In conclusion, the present study reveals a highly fluctuating pattern and weak seasonality in spore release of the wood-decaying basidiomycete *Ganoderma* at the level of the sporocarp. Data also argued against spatial synchrony; the monthly spore release pattern
2. *Ganoderma* spore release

cannot necessarily be explained by simple seasonal cycles across sporocarps. Such fluctuating patterns at the individual sporocarps might have been masked in previous studies. Future studies are required to determine factors at the sporocarp level that can contribute to what appear to be spatial asynchrony of spore release across different sporocarps.
Chapter 3 Spore consumption and life history of Zearagytodes maculifer (Broun) (Coleoptera: Leiodidae) on Ganoderma, its fungal host

Plate 3. A sporocarp (fruiting body) of Ganoderma cf. applanatum (top left) and G. australe (top right), and congregating adults of Zearagytodes maculifer on the hymenial surface of G. cf. applanatum (bottom left) and the larvae (bottom right) (Photos: K. Kadowaki)
3. Life history of *Zearagytodes maculifer*

**Introduction**

Fungus–insect relationships have been studied for decades (Scheerpeltz and Höfler 1948; Benick 1952; Blackwell and Wheeler 1984; Komonen 2003), but their roles in shaping life history traits and host selection of mycophagous insects remain unclear. For ephemeral mushrooms, mycophagous insects may possess rapid larval development (Sevenster and van Alphen 1993; Leschen 1994; Toda and Kimura 1997), parental care of eggs (Setsuda 1994), and small clutch size to enhance risk-spreading (Hackman 1979; Bruns 1984; Hanski 1989).

In such spatially and temporally unpredictable habitats, adult mycophagous insects develop a wider host range (i.e. they are polyphagous); enabling ovipositing females to maximise reproductive success (Hanski 1989; Sevenster and van Alphen 1993; Toda and Kimura 1997; see also Leschen 1990). In contrast, bracket fungi offer long-lasting larval habitats and spatially and temporally predictable adult habitats (Pace 1967; Hanski 1989); insects may grow slowly on them (Liles 1956), have larger clutch sizes (Hanski 1989) and evolve obligate host associations (Lawrence 1973; Hanski 1989). Two comparative analyses (Ashe 1984; Hanski 1989) showed that chemical defence by fungi plays a minor role in governing fungus–insect interactions. However, most empirical studies on fungus–insect interactions have been limited to inferential evidence of host records (Jonsell and Nordlander 2004; Yamashita and Hijii 2007), have produced negative or enigmatic results (Hanski 1989; Leschen 1994), and have not considered phylogenetic effects. Furthermore, life history and host selection studies have rarely been experimental.

Spore-feeding insects and their host fungi are excellent models for examining life history traits and host selection, especially as the insects are external feeders that can be observed easily and manipulated, experimentally. The New Zealand species *Zearagytodes maculifer* (Broun) (Leiodidae: Camiarinae: Agyrtodini) is one of the most common spore-feeders on long-lived bracket fungi of the genus *Ganoderma* (Newton 1984). Both *G.*
3. Life history of *Zearagytodes maculifer*

cf. *applanatum* (referred to hereafter as *G. applanatum*) and *G. austral* act as hosts for both adult and larval beetles, which feed on spores on the hymenial surface of sporocarps (the fruiting bodies of fungi) (Fig. 3-1 a, b).

![Figure 3-1. The spore-feeding beetle Zearagytodes maculifer: (a) dorsal view of a larva, (b) adults and larvae, (c) experimental arenas for rearing, (d) schematic representation of the structure of an experimental arena, (e) egg, (f) physical egg protection with faecal pellets (spore mass).](image)

An ideal test of host selection requires the direct measurement of fitness-related parameters (e.g. survival, larval development time, or pupal size), but the rearing of specialist mycophagous insects is difficult (Hanski 1989). I therefore focused on two short-term parameters that could be readily measured: larval spore consumption rate and spore-breaking ability. Since larvae do not move between sporocarps, their ability to break spores to obtain
nutrient may have consequences for fitness. Hence, one might expect greater ability of larvae to break spores of more preferred fungal species.

The host fungus *Ganoderma applanatum* has two morphotypes that differ in the structure of their hymenial surfaces and once were considered to be separate species (Cunningham 1965). One type has an even, smooth pore surface, whereas the other has a distinctly pitted and rugose surface (Buchanan and Wilkie 1995). In small patches of bush in suburban Auckland where *Z. maculifer* is absent, the pore surface always appears to be smooth, but in the Waitakere Ranges almost all sporocarps have a rugose surface (K. K., pers. obs.). Newton (1984) noted that adult *Z. maculifer* also fed on hyphae, and it is possible that its feeding on hymenial tissue induces a pitted, rugose surface.

The aim of this paper is to describe aspects of the life history and feeding behaviour of *Z. maculifer*. Seasonal abundance and host relationships of *Z. maculifer* with *Ganoderma* species are reported, and I determined the duration and number of larval instars using a combination of field experiments and laboratory observations. Spore consumption rates and host selection patterns were tested using the two *Ganoderma* species and related to field observations. Finally, I considered the effects of larval spore-feeding on fungal host fitness, and whether adult grazing induces a change in host morphotype of *G. cf. applanatum*.

**Material and methods**

**Study area**

The Waitakere Ranges (30 000 ha) are 25 km west of central Auckland City, New Zealand and rise from sea level to 460 m a.s.l. Two large plots designated Cascade Kauri (36°53' S, 174°31' E) and Upper Huia Dam (36°56'–57' S, 174°30'–32' E), were chosen as field survey and experimental sites (Fig. 3-2). The vegetation is dense regenerating native forest but
3. Life history of *Zearagytodes maculifer*

differs between sites: Cascade Kauri is *Agathis*-dominated forest, whereas Upper Huia Dam has mixed broadleaf–conifer forest.

**Study system**

The genus *Zearagytodes* contains three described species (and four or more undescribed species) and is endemic to New Zealand (Newton 1984, 1989; Seago 2005). *Zearagytodes maculifer* is found throughout the North Island, and occurs in kauri-dominated forests and small suburban reserves within the Auckland region (Kuschel 1990).

Species of the bracket fungus *Ganoderma* (Ganodermataceae, Basidiomycota) produce conspicuous perennial sporocarps on a variety of woody hosts (Buchanan and Wilkie 1995); three species have been recognised in New Zealand. Two of them, *G. applanatum* and *G. australe* are common (Buchanan and Wilkie 1995). However, because of variation in some morphological features, distinguishing between these species can be confusing. Buchanan and Wilkie (1995) showed that *G. australe* has a smooth pore surface, softer context than *G. applanatum*, indistinctly stratose to non-stratose tubes and larger, reddish-brown (rusty-brown) spores. In contrast, both rugose and smooth pore surfaces are found in *G. applanatum* whose spores are yellowish-brown. Spores of *Ganoderma* species are uniquely double-walled, typically ovoid, echinulate, and enlarged or truncated at the apex (Moncalvo et al. 1995). Those of *G. applanatum* measure 9.0–11.0 × 6.0–7.5 μm, whereas spores of *G. australe* are 11.5–14.5 × 6.5–9.0 μm (Buchanan and Wilkie 1995).

**Field and laboratory experiments**

*Seasonality and host relationship*

I examined the number of individuals of *Z. maculifer* (both larvae and adults) and daily spore release patterns over a total of 25 *Ganoderma* sporocarps at monthly intervals from November 2007 to October 2008. I selected nine logs with *Ganoderma* sporocarps at a site in
3. Life history of *Zearagytodes maculifer*

Cascade Kauri (Fig. 3-2 b, d) and seven logs at the Upper Huia Dam (Fig. 3-2 c, d). The mean distance between logs was 0.6 km at Cascade Kauri and 1.8 km at the Upper Huia Dam. Due to sporocarp deaths or emergence, sporocarp number was 25 at the start of the study and 21 at the end, with a total of 30 studied overall (n = 15 per site) (Fig. 3-2d), including 25 sporocarps of *G. applanatum* and five of *G. australe*. The initial mean area of hymenial surface was approximately 192 cm\(^2\) ± 31.2 (mean ± SE, n = 25). Fortunately, larvae and adults of *Z. maculifer* are always visible on hymenial surfaces, and therefore I obtained an absolute count of *Z. maculifer* populations. I measured daily spore release by attaching a plastic plate beneath the hymenial surface for 24 hours, and then counting the number of spores collected on per cm\(^2\) in the laboratory. The detailed methods and results were reported elsewhere (Kadowaki et al. 2010, Chapter 2).

Figure 3-2. (a) Location of the two study areas, Cascade Kauri and Upper Huia Dam in the Waitakere Ranges; (b, c) location of patches where fungi were observed in the two study areas; (d) months in which sporocarps were found in each patch between November 2007 and October 2008. The grey zone in the sporocarp 1-5B at Cascade Kauri indicates where the sporocarp was perturbed by mould but rejuvenated in a month. This figure is the same as Fig. 2-1 (Chapter 2).
3. Life history of *Zearagytodes maculifer*

*Eggs*

I measured egg size and hatching time. Eight eggs were collected from the three sporocarps (located at sporocarps 1-5, Fig. 3-2) at Cascade Kauri on 7 March 2009 and preserved in 70% ethanol for size measurements. I further collected 18 eggs on 10 March 2009 from the same sporocarps to rear individually on wet filter paper in a Petri dish at ambient conditions, which were checked daily for hatchlings. The exact age of the latter eggs at the time of the collection (10 March 2009) was not known, but as they were not seen on 7 March, I assumed they were less than three days old.

*Larvae*

Larval morphometrics were quantified by examining 60 ethanol-preserved specimens that were collected haphazardly from Cascade Kauri (located at sporocarps 1-4, 1-5, 1-9; Fig. 3-2) from November 2008 to February 2009. Three measurements (head capsule width, body length and urogomphal length) were made on each larva to the nearest 0.01 mm using a linear micrometer inserted in the eyepiece of a Leica MZ16 microscope. Four final-instar larvae were excluded from analysis since urogomphi had been detached and could not be measured. I visually assessed the peaks in frequency distributions of the three measured body parts.

*Larval development*

I conducted field trials to determine the rate of larval development in *Z. maculifer*. Sixteen experimental arenas were set-up on nine *G. applanatum* sporocarps: five arenas on five sporocarps at Cascade Kauri, and 11 arenas on four sporocarps at Upper Huia Dam (two or three arenas per sporocarp). An arena was built by attaching a plastic ring (diameter = 4 cm, height = 1.5 cm) to the rough hymenial surface using 10.5 g of brown plasticine (Fig. 3-1 c, d). At least 3 days were required for the plasticine to become tightly attached to the surface.
3. Life history of *Zearagytodes maculifer*

The top of each arena was covered by a square sheet (7.5 × 7.5 cm) of fine white mesh fabric (0.2 × 0.2 mm in mesh size) secured by a rubber band to prevent larvae moving into and out of the arena. The hymenium was brushed lightly before affixing an arena and left for two weeks allowing any newly hatched beetle larvae or other insects to be removed. Two first instar larvae of *Z. maculifer* obtained from other sporocarps were then introduced to each arena with a soft brush on 10 and 11 March 2009 at Cascade Kauri and Upper Huia Dam respectively. Instar development and survival were recorded every three or four days based on numbers of exuviae present and body size. Mesh coverings were replaced after each observation. The trial was terminated either after 4 weeks or when all larvae had pupated or died. Mean daytime temperature was 21.4°C, and humidity 67%, during the observation periods (1100–1300 h).

*Spore consumption and spore-breaking capability*

Spore consumption and spore-breaking ability were measured in a two-way factorial experiment using four instars of *Z. maculifer* and two *Ganoderma* species, replicated four times in an array of 32 Petri dishes. Spores were collected on 5–8 August 2008 from 12 *G. applanatum* sporocarps and one *G. australe* sporocarp at Cascade Kauri. Only fresh, unbroken spores were used. Larvae were collected at the same site and kept together in a large plastic container with a wet paper towel flooring on 14 September 2008. Immediately prior to the experiment larvae were starved for 4 h and gently sprayed with water for 30 s to remove spores adhering to their bodies. A single larva was placed in each Petri dish (3 cm diameter, 1 cm high) that contained 10 mg of spores on damp filter paper and was kept at ambient temperature (c. 15°C). After 24 hours, the total number of faecal pellets per individual was examined, and larvae were then preserved in 70% ethanol to measure head capsule width. The spore breaking capability of each larva was calculated by counting the number of broken spores out of 150 randomly selected spores in each of five faecal pellets.
3. Life history of *Zearagytodes maculifer*

For assessing broken spores, faecal pellets were mounted individually on glass slides and viewed at 200 × magnification. Spores were regarded as broken if they lacked full contents (Nakamori and Suzuki 2005). An approximate estimate of the average number of spores consumed by a single larva over its entire life was estimated by multiplying larval development time (field trial) by daily spore consumption rate (laboratory experiment) and the mean number of spores per faecal pellet. The latter factor was estimated using data from fourth instar larvae (averaged from five faecal pellets), since it was too labour-intensive to estimate these for all the four instars. I then obtained an estimate of the maximum number of spores consumed by *Z. maculifer* larval populations in relation to the total number of spores discharged by a sporocarp.

Spore consumption data were analysed using generalised linear models. Two explanatory variables were used: larval instar (three levels, 1, 2, and 3-4 instars combined, ordinal), and fungal species (two *Ganoderma* species, categorical). Third and fourth instars were pooled because limited numbers of fourth instar larvae were available at the time of collection. Because the variance of residuals increased with the mean level of the response, the assumption of constant variance was problematic. Hence, a quasi-Poisson error term with a log-link was used for analysis (Ver Hoef and Boveng 2007). The baseline constraint of the fungal species variable was set to *G. applanatum*.

Logistic regression was used to model the cumulative proportion of broken spores (Lindsey 1995). Although I conducted multiple counts from faecal pellets produced by each larva, I was primarily interested in the variability among faecal pellets from different individuals. Therefore, a mixed-effect approach was used by incorporating ‘individual larva’ as random intercepts (i.e. repeated measurements). The same two explanatory variables (larval instar and fungal species) were used as above.
3. Life history of Zearagytodes maculifer

**Grazing effect on host morphotypes**

To determine whether adults of *Z. maculifer* induced a rugose hymenial surface in *G. applanatum*, I established 12 new arenas on six sporocarps, which were located at sporocarps 1-5 and 2-4 (Fig. 3-2). Hymenial surfaces were initially smooth. Three adult *Z. maculifer* were introduced into six arenas and left to feed for five weeks (7 May to 13 June 2009), while the other six arenas were controls lacking beetles. Survival of beetles was checked weekly when mesh covers were replaced. Morphology of the hymenial surface was assessed as either smooth, slightly rugose, or highly rugose by moulding plasticine over it to record microstructure at the beginning and at the end of the trial.

All statistical analyses were implemented in R.2.7.1. (R Development Core Team 2008) with the lme4 package (Pinheiro and Bates 2000).

**Results**

*Seasonality and host relationship*

A total of 11 731 larvae and 8 906 adults of *Z. maculifer* were recorded. Seasonal abundance of *Z. maculifer* showed no clear pattern for adults, but larval abundance tended to be higher in winter (Fig. 3-3). The average number of months in which larval populations were found was 8.15 across 16 sporocarps that persisted for 12 months. Overall, 99.6% of larvae were found on *G. applanatum* and only 0.4% on *G. australe*. Adults were also very rare on *G. australe* (0.06 – 0.07%).
3. Life history of *Zearagytodes maculifer*

**Figure 3.3.** (a) Mean (SE) spore density per pore surface cm$^2$ each month for all sporocarps examined in the two study areas; (b) mean (SE) monthly abundance per sporocarp of larvae and adults of *Zearagytodes maculifer*.

**Eggs**

Eggs were ovoid (length 0.52 ± SD 0.044 mm, maximum width 0.39 ± SD 0.040 mm, n = 8), lacked micro-sculpture and were white to grey at maturity (Fig. 3-1e). Egg surfaces were partly covered by masses of fungal spores. Eggs were deposited singly in gaps on the fungal hymenium, and were concealed by faecal pellets of females (Fig. 3-1f). Eggs were also deposited on plasticine in the field trial. In the laboratory, duration of the egg stage was 5–7 days (n = 16). One egg failed to hatch and another died after 7 days. Survival from egg to first instar was 88.9%.

**Larval instars and development time**

The frequency distribution of head capsule width measurements showed four discrete, non-overlapping peaks corresponding to “observed” instars 1-4 (Fig. 3-4c). Although the first
3. Life history of *Zearagytodes maculifer*

Instar was well defined by urogomphus length, later instars were not clearly separated (Fig. 3-4b). The overlapping distribution of body length was not concordant with the distribution of head capsule width (Fig. 3-4c). The field trial observed four larval intervals (Table 3-1). Total development time for the combined egg, larval and pupal stages was approximately 25 days (Table 3-1). However, only four individuals completed pupation, which averaged 4–8 days (Table 3-1). Most larvae died on the mesh cover while searching for an appropriate place to pupate. Larvae that successfully pupated were found on the hymenial surface, or in gaps between the hymenium and the plasticine. Survival from first to fourth instar in the field trial was 60% (n = 30).

*Figure 3-4.* Frequency distribution of (a) head capsule width, (b) urogomphus length and (c) body length measurements for *Zearagytodes maculifer.*

**Spore consumption and spore-breaking capability**

A generalized linear model showed that neither larval instar nor *Ganoderma* species influenced larval spore consumption rates over a 24 h period (t = −0.517, df = 1, p = 0.348 and t = −2.596, df = 1, p = 0.117, respectively; Fig. 3-5a, b). The mean proportion of spores broken by *Z. maculifer* was less than 10%, and the spores of *G. australe* were significantly more difficult to break than those of *G. applanatum* (z = −2.912, df=1, p < 0.0001; Fig. 3-5d). Instar number did not affect larval spore-breaking capability (z = −0.019, df = 1, p= 0.985; Fig. 3-5c). The number of spores found per faecal pellet produced by fourth instar larvae was
3. Life history of *Zearagytodes maculifer*

$318 \pm SD 129$.

**Table 3-1.** Mean (SD) larval body length, head width, urogomphus length and instar duration (larvae and pupae) for *Zearagytodes maculifer*. $n^1 =$ larvae measured; $n^2 =$ larvae reared in the field.

<table>
<thead>
<tr>
<th>Instar</th>
<th>Body length (mm)</th>
<th>Head width (mm)</th>
<th>Urogomphus length (mm)</th>
<th>$n^1$</th>
<th>Instar duration (days)</th>
<th>$n^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.2 (0.18)</td>
<td>0.30 (0.01)</td>
<td>2.0 (0.20)</td>
<td>17</td>
<td>4.9 (2.0)</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>2.0 (0.42)</td>
<td>0.40 (0.01)</td>
<td>3.2 (0.31)</td>
<td>15</td>
<td>5.2 (2.2)</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>2.2 (0.30)</td>
<td>0.46 (0.01)</td>
<td>4.2 (0.38)</td>
<td>7</td>
<td>4.4 (2.4)</td>
<td>24</td>
</tr>
<tr>
<td>4</td>
<td>2.3 (0.32)</td>
<td>0.51 (0.01)</td>
<td>4.3 (0.20)</td>
<td>17</td>
<td>6.2 (2.9)</td>
<td>18</td>
</tr>
<tr>
<td>Pupa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.6 (1.3)</td>
<td>4</td>
</tr>
</tbody>
</table>

**Figure 3-5.** Effects (mean + SE) of instar and *Ganoderma* species on (a, b) faecal pellet egestion rate (number of pellets per larva per 24 h), and (c, d) spore-breaking capability of *Zearagytodes maculifer* larvae.
Effect of sporophagy on host morphotype

The hymenial surfaces of both control and treatment sporocarps were visually similar at the end of the experiment and all were scored as smooth.

Discussion

Life history

The number of larval instars in Leiodidae ranges from two in cave-dwelling Leptodirini to five in Leptinus species parasitic on mammals (Newton 2005). Zearagytodes maculifer had four larval instars, and therefore fell within the known range. First to third instars had distinct, non-overlapping peaks in head capsule width distribution. Fourth instars were only slightly larger in body length than third instars, although a field rearing trial showed four instar intervals. I could not convincingly exclude the possibility that the beetle has three instars, since the apparent four “numeric” instars might be due to heterogeneous larval growth patterns between sexes or related to nutrient uptake.

Few data are available about the size of leiodid eggs (Newton 2005), but those of Z. maculifer were larger in size to the eggs of Phanerota fasciata (Staphylinidae) (Ashe 1981), Scaphisoma impunctatus (Leschen 1988), and were similar to those of Bolitophagus reticulates (Jonsson 2003). Deposition of eggs singly as found in Z. maculifer, has also been reported in several other fungal-feeding beetles, e.g. Bolitotherus cornutus (Tenebrionidae) (Pace 1967), Pleurotobia tristigmata (Staphylinidae) (Ashe 1990), and Gyrophaena (Staphylinidae) (White 1977). Physical egg protection by faecal matter is also known from Scaphisoma (Staphylinidae), Endomychus (Endomychidae), Bolitotherus (Tenebrionidae) (Leschen 1994). Time taken for development of Z. maculifer from egg to adult in the field (25 days) was similar to that of Endomychus biguttatus (Endomychidae) on the fungus Schizophyllum commune (Leschen and Carlton 1988), but was much shorter than reported for
3. Life history of *Zearagytodes maculifer*

*B. cornutus* on *G. applanatum* (88 days, Liles 1956) and *Cis bilamellatus* (Ciidae) (56 days, Paviour-Smith 1968). Thus, the egg-to-adult period was equivalent to, or shorter than, that of other fungivorous beetles living on bracket fungi.

The life cycle of *Z. maculifer* showed little indication of seasonality and both adults and larvae were present throughout the year, though larval abundance peaked during winter. This weak seasonality is unusual for Leiodidae. Chandler and Peck (1992) showed that leiodid beetles living on slime moulds and fungi in a New Hampshire (USA) forest had a narrow, unimodal peak in adult emergence, whereas a univoltine life cycle with summer diapause was found in *Catops nigricans*, a saprophagous species of European leiodid (Topp 1990). The persistence of bracket fungi, and the mild environment of forests in northern New Zealand may facilitate continuous breeding in *Z. maculifer*.

**Host selection**

Spore consumption rates did not differ significantly among larval instars, or on the two *Ganoderma* species, though rates showed considerable variation. In fourth instar larvae this variation may have been partly due to cessation of feeding prior to pupation.

Spore-breaking ability of *Z. maculifer* larvae was low (<10%) compared with the 50–90% recorded by Nakamori and Suzuki (2005) for a collembolan *Hypogastrura* fed spores of *Hypsizygus marmoreus*, and suggests that *Ganoderma* spores may be better defended than those of *H. marmoreus*. However, the relative efficiency of the spore-crushing mechanisms of the respective collembolan and coleopteran species is not known. As *Z. maculifer* larvae are spore-feeding specialist (Lawrence 1989), it would be particularly interesting to know whether a low percentage of spore breaking can limit nutrient uptake and whether spore-breaking ability provides a robust measure of nutrient uptake from its fungal host.
3. Life history of *Zearagytopes maculifer*

The impact of spore feeding by *Z. maculifer* on fitness of its fungal hosts is likely to be neutral. The average number of spores consumed by a larva in its life is estimated to be $7.87 \times 10^4 - 2.98 \times 10^5$ (range), and when multiplied by 118 (the maximum number of larvae per sporocarp) represents a very small percentage (0.1–0.4 %) of the spores discharged by an intermediate-sized sporocarp (ca. 192 cm$^2$) of *G. applanatum*. My results contrast with those of Guevara et al. (2000b), who showed that feeding by ciid beetles had a negative impact on the fitness of the fungus *Trametes versicolor* by reducing its short-term reproductive potential. However, my results are similar to those of Økland and Hågvar (1994) who argued that the sporulating sporocarp of *Fomitopsis pinicola* was able to tolerate high grazing pressure by a spore-feeding staphylinid beetle *Gyrophaena boleti*. In contrast to *Z. maculifer* and *G. boleti*, ciid beetles feed almost entirely on the stroma, rather than the spores and hymenium, respectively. Finally, grazing by adults of *Z. maculifer* did not induce a change in the morphology of *G. applanatum* from smooth to rugose. Its polymorphism therefore remains unexplained.
Chapter 4 Ontogenetic allometries in *Holopsis* spp. larvae (Coleoptera: Cucujoidea: Corylophidae)

PLATE 4. The foraging larvae of *Holopsis* sp. 2 with a short rostrum and *Holopsis* sp. 1 with an elongate rostrum (Corylophidae) on *Ganoderma* hymenial surface (top), and the foraging activity of *Holopsis* sp. 1 (bottom left and right: tip of the rostrum, before and after consuming spores from white rejuvenating basidia of *Ganoderma* cf. *applanatum*)(Photos: K. Kadowaki).
4. Ontogenetic allometries in *Holopsis* spp.

**Introduction**

Ontogenetic variation in body size – appendage ratios between species is the result of the prior evolutionary divergence via natural selection (Klingenberg and Spence 1993; Frankino et al. 2005), while ontogenetic uniformity in the scaling relationship indicates the presence of developmental constraints or stabilizing selection (Maynard Smith et al. 1985; Arnold 1992). Identifying factors that govern such scaling relationships between body size and appendages is not straightforward, however, since selection can act both on body size and appendages (Fairbairn 1992). Changes in body size either during growth or over evolutionary time generally entail changes in shape (Huxley 1932; Gould 1966; Maynard Smith et al. 1985), and such departure from geometric similarity can arise from size-mediated changes in shape or shape-mediated changes in size (Fairbairn 1992; Frankino et al. 2005). Allometric theory offers a sound analytical tool to decompose morphological character states into a hierarchy of static, ontogenetic and evolutionary allometries in a set of closely related species, by which I call allometric decomposition theory (Cock 1966; Gould 1966; Klingenberg and Zimmermann 1992). Static allometry (or size allometry) results from variation among individuals that belong to specific age classes, while ontogenetic allometry (or growth allometry) involves covariation among characters during growth; and evolutionary allometry refers to covariation among changes in different traits between closely related species in a phylogenetic framework (Cock 1966; Klingenberg 1996b).

Intraspecific and interspecific comparative studies have demonstrated ontogenetic and evolutionary allometries in flowers (Kampny et al. 1993; Wang and Wang 2005), corals (Kullmann 1972), hemimetabolous insects (Matsuda 1961; Brown and Davies 1972; Fairbairn 1992; Klingenberg 1996b; Rodrigues et al. 2005), fish (Frédérich et al. 2008; Frédérich and Sheets 2009) and birds (Grant 1981; Boag 1983; Price and Grant 1985; Burns 1993). In these studies, there were three ways of data collection: longitudinal data sets based
4. Ontogenetic allometries in *Holopsis* spp.

on measurements of the same individuals at several developmental stages (e.g. Klingenberg 1996a), cross-sectional data with different specimens in several known stages (e.g. Klingenberg and Zimmermann 1992; Rodrigues et al. 2005), or mixed cross-sectional data collected without information on ontogenetic stages (e.g. Fairbairn 1992; Burns 1993).

Several statistical techniques have been proposed to analyse data collected as above: dimension reduction by common principal components analysis (Klingenberg 1996b; Klingenberg et al. 1996; Mitteroecker et al. 2004), size correction by regression residuals (Burnaby 1966; McCoy et al. 2006), path analysis that use size as a latent variable (Bookstein 1991) and Procrustes-based geometric morphometrics (Bookstein 1991; Frédéric et al. 2008; Frédéric and Sheets 2009). However, some long-standing and practical problems have not been addressed adequately with current available methodology. One such example is to apply allometric decomposition theory to organisms that cannot be reared or where the ages or instars of individuals are unknown *a priori*.

In arthropods, the strongly sclerotized exoskeleton of the cuticle prevents continuous growth, and these rigid structures leads to stepwise growth by moulting, i.e. the replacement of the old cuticles by a new one which was formed during the intermoult cycle prior to ecdysis (Gullan and Cranston 2005). Such stepwise growth of immature insects has been mathematically formulated in various ways (Dyar 1890; Brown and Davies 1972; Klingenberg and Zimmermann 1992; Nijhout and Wheeler 1996). For example, Dyer’s rule predicts the geometric progression, or \( \log y = \log a + (\log b)\times x \), where \( y \) is size, \( x \) is the instar number, and \( a \) and \( b \) are coefficients (Dyar 1890). However, there are a large number of arthropod species whose ages or instars cannot be determined due to the difficulty of rearing, and therefore a much more flexible framework to apply allometric theory is needed. In such situations, one needs to estimate not only the ages or instars from each specimen collected from the field, but also the allometric components derived from classification based on the
4. Ontogenetic allometries in *Holopsis* spp.

estimated instars. In what follows, I present a case study that applies allometric
decomposition to spore-feeding beetles that feed on the spores of wood-decaying bracket
fungi, and demonstrate that normal mixture models offer a useful statistical tool for
allometric studies.

In New Zealand, two sympatric fungus beetles in the genus *Holopsis* Broun (Insecta: Coleoptera: Cucujoidea: Corylophidae), coexist on the bracket fungus *Ganoderma* cf. *applanatum* (Kadowaki et al. unpublished, Chapter 5). These two species are very similar as adults, but larvae differ markedly in colour pattern and especially the head structures: *Holopsis* sp. 1 has a long rostrum while *Holopsis* sp. 2 lacks a rostrum. Lawrence (1989) conjectured that the larva of *Holopsis* sp. 1 was a ‘pore tube specialist’ that would have access to immature spores in the depth of pore tubes; however, the link between rostrum length and foraging behaviour remains speculative. I firstly describe statistical methods to simultaneously estimate the number of components (instars), mixing proportions of components, and variance–covariance in morphological characters to relate the results to allometric theory. Secondly, I examine the ontogenetic allometries in the two *Holopsis* species, and discuss the underlying mechanisms of ontogenetic variations by comparing the two species. Thirdly, I focus on rostrum length, which is likely to be related to the foraging ecology of these beetles. In particular, I test the following two specific hypotheses: (1) that the two *Holopsis* species differ in spore consumption patterns, especially the likelihood of consuming immature spores that can perhaps be accessed only by *Holopsis* sp. 1 with a longer rostrum, and (2) that increasing rostrum length in later instars of *Holopsis* sp. 1 entails more efficient consumption of immature spores.
4. Ontogenetic allometries in *Holopsis* spp.

**Material and methods**

**The beetles**
Corylophids commonly have a prominent hood-like pronotum in adults, but larval characters tend to be highly derived with strongly modified mouthparts, antenna, and defensive glands (Ślipiński et al. 2009). The genus *Holopsis* (Corylophidae: Corylophinae: Peltinodini) is contained in its own tribe Peltinodini (Ślipiński et al. 2009) and is worldwide in distribution, but absent from the Oriental and Afrotropical regions (Bowestead 1999). The genus contains five described species in New Zealand (Hudson 1923), but there are many undescribed species, including four or more in the Auckland area (Kuschel 1990). In the Waitakere Ranges (Auckland, New Zealand), I have confirmed two new species, *Holopsis* sp. 1 and 2. The perennial wood-decaying bracket fungus *Ganoderma cf. applanatum* (Ganodermatacea, Basidiomycota) is the host of both species in the Waitakere Ranges (Kadowaki et al. 2010, Chapter 2). *Ganoderma cf. applanatum* has a unique double-walled spore (9.0–11.0 × 6.0–7.5 μm), typically ovoid, echinulate and enlarged or truncated at the apex (Buchanan and Wilkie 1995).

**Data collection**

**Morphometrics**
Larval morphometrics were analysed on the basis of examination of 311 ethanol-preserved specimens: *Holopsis* sp. 1 (178 individuals) and *Holopsis* sp. 2 (133), which were collected from 29 May to 23 June 2009 from populations in Cascade Kauri in the Waitakere Ranges (36°53’ S, 174°31’ E), Auckland, New Zealand. Four measurements (body length [BL], body width [BW], head size [HS] and rostrum length [RL]; Fig. 4-1) were recorded to the nearest 0.01 mm under a binocular microscope (Leica MZ16, Singapore).
4. Ontogenetic allometries in *Holopsis* spp.

![Figure 4-1. Measured body size and appendages.](image)

Body length (BL) measurements were from the tip of the pronotum to the end of the elytra, as the head is concealed in corylophids. Body width (BW) was measured at the widest points from the dorsal view. Head size (HS) was quantified by the shortest distance between two stemmata, while RL was measured at the shortest points from stemmata to the tip of the rostrum. These measurements were straight-line distances between two clear reference points.

Dissection

I determined the proportion of mature vs. immature spores consumed by two *Holopsis* species. It was not possible to collect immature spores for choice experiments because the spores were deep within the pore tubes of sporocarps. Therefore, I counted the number of immature spores present in the gut of field-collected larvae by dissection. A total of 77 *Holopsis* sp. 1 and 25 *Holopsis* sp. 2 individuals (specimens collected on 23 June 2009 and 27 Feb 2010) were dissected under the microscope (× 20). The whole digestive tract was taken from each individual and smashed with a cover slip on a glass slide. I quantified the proportions of mature to immature spores by assessing 150 randomly selected spores under the microscope (×400). Immature spores are transparent in colour and smaller than mature spores, which were brown with full armature. Note *Ganoderma* sporocarps harbour both
4. Ontogenetic allometries in *Holopsis* spp.

mature and immature spores, irrespective of sporocarp size at any season, so the availability of immature spores is not limited spatially and temporally.

**Statistical analysis**

My analysis consists of three stepwise processes: (1) estimation of instar identity and number by mixture models, (2) model assessment of mixture models, and (3) computation of allometries componenets using variance–covariance matrices.

**Mixture models**

For the first step, one can fit a univariate or multivariate normal distribution (if log-transformed, a lognormal distribution) to morphological character(s). However, in case where one has no *a priori* knowledge of instar identity and number of instars, one should estimate the probability that a given set of measured character(s) from an individual belongs to each of the estimated number of instars. In other words, one needs to treat the factor of an ‘instar’ as missing information (or a latent variable), and simultaneously estimate instar identity and number from unlabeled datasets. In such cases, normal mixture models offer a useful statistical tool (McLachlan and Peel 2000) that can disentangle a number of overlapping distributions of morphological characters into biologically meaningful components (i.e. instars) without *a priori* knowledge. I applied normal mixture models to *Holopsis* spp. data. Prior to analysis, data were *ln*-transformed to ensure homoscedasticity.

In mixture models, parameter estimation can be done by Expectation-Maximisation algorithm (Dempster et al. 1977) that alternates in the two iterative steps, one which finds conditional probabilities that a set of measured character(s) belongs to each of the instars, and the other which computes the estimated mean and variance of morphological characters of all the instars by maximum likelihood theory by updating the mixing proportion of the estimated
number of instars in the entire sample. For the formal mathematical introduction of mixture models to allometric decomposition theory, see Appendix 4.1.

**Model specification**

In the second step, one should investigate how robust the results of instar number and identity are, using the same dataset. For doing this, one needs to assess how frequently the estimated labelling of instars makes misclassification errors in simulated datasets, and how the use of number and combinations of morphological character variables changes the estimated instar number and identity. There are two corresponding criteria I employed for mode specification: classification error rates in relation to dimensions (number of morphological variables), and model selection for the number of components (instars). Firstly, I explored 1-D (one-dimensional or univariate), 2-D (bivariate) and 4-D mixture models to test whether my ability to classify specimens into components (instars) could improve with more variables (1-D < 2-D < 4-D). Entomologists have used head capsule width as a measure of size and instars, hence I used head size (HS) for 1-D, HS and RL for 2-D, and all the traits for 4-D mixture models. Parametric bootstrap was performed 1000 times for each species to obtain classification error rates.

Secondly, I examined statistical evidence for the number of components (instars) by model selection criteria (Akaike Information Criterion and Bayesian Information Criterion). The AIC and BIC differ in theoretical basis, objectives, and performance, and BIC penalizes the -2*log-likelihood more severely than AIC to each additional parameter. Theoretically, BIC is consistent at choosing the correct number of components (instars) when the sample size approaches infinity. Here it appears that BIC is more sound than AIC, since the sample size is reasonably large and the true number of instars is likely an integer. I explored a
4. Ontogenetic allometries in *Holopsis* spp.

biologically realistic range of two to six components (or instars) in both species across 1-D, 2-D and 4-D mixture models.

*Ontogenetic allometries*

Based on the best-fit mixture models, I obtained variance–covariance matrices for the two *Holopsis* species to determine ontogenetic allometries. The variance-covariance matrix of morphological characters illustrates pairwise covariation among the morphological characters at all the instars. Here I applied only homoscedastic normal mixture models that rely on equal variances in components, focusing on ontogenetic allometry rather than static allometry. Hence, the variance–covariance matrices were identical across instars. I graphically assessed the growth trajectories to illustrate evolutionary allometry in the two *Holopsis* species. I also computed discriminant functions between instars and confidence contours of each of the estimated instars (Appendix 4-1).

*Spore-feeding strategy*

I tested by $\chi^2$ statistics whether two *Holopsis* species differ in the proportion of individuals that consumed immature spores. I examined the correlation between the proportion of immature spores in larval digestive tracts and rostrum length in *Holopsis* sp. 1. Analysis was performed in R (R Core Development Group 2010) with the original codes written by Yong Wang for mixture models.

*Results*

*Model specification*

The analysis showed that both *Holopsis* species have four instars. Model selection for the number of components across 1-D to 4-D models indicated the presence of four components in *Holopsis* sp. 2 and five components in *Holopsis* sp. 1 in terms of BIC (Table 4-1).

Remarkably, *Holopsis* sp. 1 had rostrum length polymorphism in the fourth instar, even
4. Ontogenetic allometries in *Holopsis* spp.

though these morphotypes could not be separated in the third instar (Fig. 4-2); some individuals possessed a disproportionately longer rostrum and narrower head width in the fourth instars, whereas the others had a relatively shorter rostrum and wider head. Therefore, both *Holopsis* species possess four instars during their larval period. Furthermore, the examination of 1-D (HS), 2-D (HS and RL) and 4-D (BL, BW, HS and RL) by classification error rates demonstrated that the usage of all the four morphological characters available gave the most accurate results (Table 4-1). In *Holopsis* sp.1, the potential polymorphism in the fourth instar was not distinguished for classification; I considered a classification correct when a bootstrapped sample fell into either morphotype of the fourth instar.

**Table 4-1.** Results of model assessment: (a) model selection for the number of instars (components) in terms of AIC; and (b) model selection results for BIC (see also Appendix 4-1, for detailed results); (c) classification error rates in 1-D, 2-D and 4-D mixture models by parametric bootstrap (B = 1000). Better models entail lower classification error rates.

<table>
<thead>
<tr>
<th></th>
<th>1D</th>
<th>2D</th>
<th>4D</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) AIC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sp. 1</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>sp. 2</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>(b) BIC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sp. 1</td>
<td>2</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>sp. 2</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>(c) classification error rates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sp. 1</td>
<td>0.1368</td>
<td>0.0456</td>
<td>0.0160</td>
</tr>
<tr>
<td>sp. 2</td>
<td>0.0329</td>
<td>0.0163</td>
<td>0.0132</td>
</tr>
</tbody>
</table>
4. Ontogenetic allometries in *Holopsis* spp.

**Table 4-2.** Mean morphological traits (mm) and mixing proportions estimated by 4-D mixture models. *Holopsis* sp. 1 possesses five components (two separate components in the fourth instar refer to 4A and 4B), and *Holopsis* sp. 2 possesses four components. Notations: BL, body length; BW, body width; HS, head size; RL, rostrum length; and π, mixing proportions in specimens.

<table>
<thead>
<tr>
<th>Species</th>
<th>Instar (component)</th>
<th>BL</th>
<th>BW</th>
<th>HS</th>
<th>RL</th>
<th>π</th>
</tr>
</thead>
<tbody>
<tr>
<td>sp. 1</td>
<td>1</td>
<td>0.543</td>
<td>0.297</td>
<td>0.074</td>
<td>0.099</td>
<td>0.123</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.693</td>
<td>0.358</td>
<td>0.081</td>
<td>0.183</td>
<td>0.220</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.942</td>
<td>0.481</td>
<td>0.097</td>
<td>0.317</td>
<td>0.229</td>
</tr>
<tr>
<td></td>
<td>4A</td>
<td>1.331</td>
<td>0.679</td>
<td>0.108</td>
<td>0.587</td>
<td>0.316</td>
</tr>
<tr>
<td></td>
<td>4B</td>
<td>1.410</td>
<td>0.732</td>
<td>0.133</td>
<td>0.429</td>
<td>0.111</td>
</tr>
<tr>
<td>sp. 2</td>
<td>1</td>
<td>0.563</td>
<td>0.339</td>
<td>0.102</td>
<td>0.062</td>
<td>0.249</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.746</td>
<td>0.446</td>
<td>0.127</td>
<td>0.082</td>
<td>0.159</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.953</td>
<td>0.579</td>
<td>0.167</td>
<td>0.107</td>
<td>0.366</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.263</td>
<td>0.792</td>
<td>0.222</td>
<td>0.135</td>
<td>0.226</td>
</tr>
</tbody>
</table>

**Ontogenetic allometry**

Table 4-2 summarises mean character states and the mixing proportions estimated by the specified mixture models (see Figs. 4-2, 4-3 for each species). The two species were similarly-sized in body length, but *Holopsis* sp. 2 had a wider body width and larger head size.

*Holopsis* sp. 1 undergoes a markedly accelerating growth in rostrum length compared with *Holopsis* sp. 2.

The variance–covariance matrices presented in Table 4-3 highlight three key results: (1) variations in characters were uniformly larger in *Holopsis* sp. 1 than in *Holopsis* sp. 2 despite their similar body size; (2) changes in head/rostrum shape associated with body length or width differed between the species; and (3) the covariations associated with head size was larger in *Holopsis* sp. 2, while the covariations associated with rostrum length was larger in *Holopsis* sp. 1. Fig. 4-4 illustrates the marked differences in ontogenetic allometries of the two *Holopsis* species in a bivariate allometric plot (HS and RL).
4. Ontogenetic allometries in *Holopsis* spp.

**Table 4-3.** Variance–covariance matrix estimated by 4-D mixture models, showing ontogenetic allometries in two *Holopsis* species. Notations: BL, Body length; BW, body width; HS, head size; RL, rostrum length. Data were ln-transformed.

<table>
<thead>
<tr>
<th>Species</th>
<th>Traits</th>
<th>BL</th>
<th>BW</th>
<th>HS</th>
<th>RL</th>
</tr>
</thead>
<tbody>
<tr>
<td>sp. 1</td>
<td>BL</td>
<td>0.008604</td>
<td>0.006143</td>
<td>0.001247</td>
<td>0.003265</td>
</tr>
<tr>
<td></td>
<td>BW</td>
<td>0.008437</td>
<td>0.000961</td>
<td>0.003547</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>0.007003</td>
<td>−0.00128</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RL</td>
<td>0.020935</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sp. 2</td>
<td>BL</td>
<td>0.008482</td>
<td>0.004957</td>
<td>0.003109</td>
<td>0.002195</td>
</tr>
<tr>
<td></td>
<td>BW</td>
<td>0.005286</td>
<td>0.002722</td>
<td>0.001596</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HS</td>
<td>0.005298</td>
<td>0.000251</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RL</td>
<td>0.012194</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Spore-feeding strategy**

Dissection of larval digestive tracts revealed that 29.9% of *Holopsis* sp. 1 larvae consumed immature spores, while no *Holopsis* sp. 2 larvae did. The two species significantly differ in the proportion of individuals that consumed immature spores ($\chi^2 = 8.0071$, df = 1, $p = 0.0047$). The mean proportion of immature spores compared to mature spores was very low in *Holopsis* sp. 1, $1.41 \pm 0.367\%$ (mean ± SE), suggesting that mature spores are the principal resource for both species. There was no significant correlation of rostrum length and the proportion of immature spores consumed in *Holopsis* sp. 1 (Pearson’s $r = −0.128$, $t = −0.593$, df = 21, $p = 0.56$), and therefore I found no evidence for foraging polymorphism associated with the rostrum length polymorphism in *Holopsis* sp. 1.
4. Ontogenetic allometries in *Holopsis* spp.

**Figure 4-2.** *Holopsis* sp. 1; (A) scatterplots for the original data set, (B) scatterplots after log-transformed data set; (C) histogram and fitted 1-D mixture for log(HS); (D) scatterplots between log(HS) and log(RL) with 2-D mixture discriminant functions (dotted lines) and the contours with Mahalanobis distance $d = 1, 2$ to the centroids (red dots). See Appendix 4-1 for discriminant function equations.
4. Ontogenetic allometries in *Holopsis* spp.

**Figure 4-3.** *Holopsis* sp. 2; (A) scatterplots for the original data set, (B) scatterplots after log-transformed data set; (C) histogram and fitted 1-D mixture for log(HS); (D) scatterplots between log(HS) and log(RL) with 2-D mixture discriminant functions (dotted lines) and the contours with Mahalanobis distance $d = 1, 2$ to the centroids (red dots). See Appendix 4-1 for discriminant function equations.
4. Ontogenetic allometries in *Holopsis* spp.

**Figure 4-4.** Interspecific ontogenetic allometries of the two *Holopsis* species in a bivariate allometric plot with centroids (red dots) and Mahalanobis distance contours ($d = 1, 2$).
4. Ontogenetic allometries in *Holopsis* spp.

**Discussion**

I have described a new statistical method to applying allometric decomposition theory to insects whose instars are unknown, and demonstrated that ontogenetic allometries markedly differed between the two *Holopsis* species. The analysis by multivariate normal mixtures indicated four instars in both *Holopsis* species, while *Holopsis* sp. 1 possesses a developmental polymorphism. The rostrum length was not correlated with ability to obtain immature spores in the pore tubes in *Holopsis* sp. 1. I found that both species predominantly depend on mature spores for food.

**Normal mixture models and allometric theory**

Previous studies have used head capsule width (i.e. 1-D) as a measure of size and estimated the number of instars by the simple frequency method or Janetschenk method (Janetschek, 1967), which has been criticized (Bliss and Beard 1954; Fink 1982). In many cases where such simple methods failed to detect clear peaks of instars, the differences might have been subtle or masked by overlapping distributions (e.g. Kondratieff and Voshell 1980), and only powerful multivariate analysis can identify such differences. The present study shows that, despite the unclear peak patterns in head capsule width distributions (1-D mixtures), the simultaneous use of four morphological variables (4-D mixture) allowed me to distinguish instars whose appendage distributions appeared highly overlapping in the 1-D models (Figs 4-2, 3). I found it possible to clarify the number of instars (or components) by exploring a number of components (instars) across 1-D, 2-D and 4-D models. These two methods deal with different aspects of the robustness of mixture models, and should be combined in model specifications.

Multivariate normal mixture models are more widely applicable to allometric studies than dimension reduction methods (e.g. PCA). Firstly, describing morphological characters in
multivariate space appears more sound and interpretable in theory than dimension reduction methods. This is true especially when the relative importance of ontogenetic and evolutionary allometries is of major interest. Dimension reduction methods summarise variation into the two main axes whose biological interpretation is nontrivial, while multivariate mixture models decipher pairwise covariation among morphological parts in an interpretative way. Even though the present study employed homoscedastic mixtures that assume equal variances in components to focus on ontogenetic allometries, heteroscedastic mixtures can disentangle the relative contributions of static and ontogenetic allometries. Secondly, mixture models can identify potential developmental polymorphisms without any external knowledge, as demonstrated here. Intraspecific polymorphism occurs in many natural populations but cannot always be detected easily (Ehlinger and Wilson 1988, Robinson et al. 1993; Skúlason and Smith 1995; Smith and Skúlason 1996), particularly when the differences between polymorphism are confounded with ages or instars. No statistical methods are currently available to explicitly fit growth trajectory curves in multivariate mixture models (see Nijhout and Wheeler 1996). Thirdly, mixture models can be used to study insects whose larval growth is heterogeneous. For example, mayflies show high individual variation in larval growth and morphological patterns, leading to conflicting conclusions about their instar numbers (Fink 1980, 1982). The highly overlapping growth patterns of coryphoid larvae have also perplexed entomologists in identifying the instar numbers; Hinton (1941) observed three instars in *Sericoderus lateralis* (Gyllenhal) (Coryphidae), while Polilov and Beutel (2010) found three instars or more and allometric growth in internal organs in the same species. Mixture models can also potentially accommodate such heterogeneous growth patterns between generations or ecomorphs, and thus provide a significantly increased capacity in allometry studies.
4. Ontogenetic allometries in *Holopsis* spp.

**Ontogenetic allometries**

Maynard Smith et al. (1985), in comparing two different taxa whose members have been exposed to a similar range of ecological conditions, predicted that when one taxon shows variants of a kind not shown by the other, the latter taxon has been subject to some degree of developmental constraint. My results in *Holopsis* sp. 1 exemplified this prediction: the two *Holopsis* species were similar in having a comparatively short rostrum at least from the first to second instars, while at the fourth instars, *Holopsis* sp. 1 develop a longer rostrum that was not possessed by *Holopsis* sp. 2. This suggest that population polymorphism of a shorter rostrum in *Holopsis* sp. 1 parallel interspecific diversity in characters in *Holopsis* spp., and developmental constraints may influence short rostrum length of *Holopsis* sp. 2. For a robust theory about the evolution of rostral polymorphism, one also needs to better understand the character distributions for *Holopsis*, a worldwide group containing many species, but whose natural history is poorly known and with larvae rarely collected. Apart from the species studied in this chapter, most species of *Holopsis* do not have a rostrum (one species from Honduras, two species from Australia, and four species in New Zealand were examined) and having a short rostrum is present in one additional New Zealand species collected from the South Island, and based on the limited sample, the short rostrum appears to be allometric.

From a developmental perspective, I also found interspecific heterochrony (i.e. a developmental change in the rate of a particular morphological change) between the two *Holopsis* species in terms of rostrum lengths and body widths, as shown by different growth trajectories (Fig. 4-4). Klingenberg (1998) applied heterochronic terms to bivariate allometry plots with the strict understanding they may not reflect underlying ‘true’ heterochronies. The interspecific heterochronies might occur at later stages of growth when the tissue or organs have differentiated via alteration in mitosis, rather than changes in local sensitivity to hormonal mitogens. Rostrum polymorphism in *Holopsis* sp. 1 may also suggest intraspecific
4. Ontogenetic allometries in *Holopsis* spp.

heterochrony. The intraspecific heterochrony in head and rostrum structures might also occur via a similar mechanism of alteration in mitosis, given the timing of divergence. Collectively, the intraspecific and interspecific heterochronies in *Holopsis* spp. indicate that increasing rostrum length and head size are compensatory changes not only at population level but also at species level.

**Interspecific difference in spore consumption patterns**

Spore consumption patterns in *Holopsis* species accords with such interspecific ontogenetic differences: *Holopsis* sp. 1 can access immature spores even though they predominantly feed on mature spores, while *Holopsis* sp. 2 consumes only mature spores on the hymenial surface. Therefore, Lawrence’s (1989) assertion that *Holopsis* sp. 1 is a ‘pore tube specialist’ on immature spores requires further scrutiny. Even though *Holopsis* sp. 1 larvae do not consume immature spores in the depths of pore tubes very often, they may access a larger area of hymenium that includes more entrances of pore tubes bearing discharging spores, leading to a more efficient spore-feeding strategy compared with *Holopsis* sp. 2. Whether or not such spore-feeding patterns translate into population dynamics among or between species in the *Ganoderma* system is an open question.

**Rostrum length polymorphism**

The maintenance of rostrum length polymorphism in *Holopsis* sp. 1 in theory may result from selection and its counteracting forces acting on rostrum length (and head size). A longer rostrum might not only accrue a benefit but also incur a cost to larval fitness in *Holopsis* sp. 1. A longer rostrum may involve the greater ability to consume immature spores with little external armature, which might be likely to be more digestible. This was not supported by the gut dissection analysis, which found that rostrum polymorphism in *Holopsis* sp. 1 did not correspond to immature/mature spore use. Another possible explanation relating to feeding is that a longer rostrum entails the ability to access a larger hymenium area to obtain more
4. Ontogenetic allometries in *Holopsis* spp.

abundant spores. I have observed that larvae cross their rostra like ‘swords’ in combat, and these interactions are surprisingly audible: polymorphism in rostrum lengths may relate directly to larval fitness. Although these explanations rely on the assumption that rostrum length is heritable, current knowledge about its genetic basis is too scarce to address other possibilities.

In conclusion, I report intraspecific and interspecific ontogenetic allometries for two sympatric fungus beetles in the genus *Holopsis*. Using a new statistical method of mixture models, I identified ontogenetic allometries in *Holopsis* spp. whose instars were unknown *a priori*, and discovered that population polymorphism of a shorter rostrum in *Holopsis* sp. 1 parallels interspecific diversity in characters in *Holopsis* spp. I examined the link between morphological characters (rostrum length) and trophic function (spore-feeding ability), and showed that the two species significantly differ in their ability to consume immature spores. Despite ontogenetic differences in *Holopsis* sp. 1, a longer rostrum did not correlate to higher probability of feeding on immature spores that are situated in the depth of pore tubes that are otherwise not physically accessible.
Appendix 4-1. A formal introduction of mixture models to allometric decomposition theory

Mixture models offer a useful statistical tool when a set of measurements comes from specimens of one of the instar groups but one does not know which one (McLachlan and Peel 2000). Since ‘instar’ is virtually a missing value (i.e. incomplete data), I refer to \( x = (y, z) \) as a data set that consists of a set of measurement data \( y \) and unobserved instar data \( z \).

I postulate a mixture density \( f(x | \Phi) \) as follows:

\[
f(x | \Phi) = \sum_{i=1}^{R} \pi_i \phi(x | \mu_i, \Sigma) ,
\]

where the sum of instar mixing proportions is equal to unity, \( \sum_{i=1}^{R} \pi_i = 1 \); and \( \phi(x | \mu_i, \Sigma) \) is the normal density with mean \( \mu_i \) and variance \( \Sigma \) (McLachlan and Peel 2000).

Suppose that \( y \) is represented as \( n \) observations \( y = (y_1, y_2, ..., y_n) \), that there exists a finite set of \( R \) states (the unknown number of instars or components), and that each \( y_i \) is associated with an unobserved instar \( z = (z_1, z_2, ..., z_n) \) where \( z_i \) is the indicator vector of the length \( R \) whose components are all zero except for one equal to unity indicating the unobserved state of \( y_i \) (Dempster et al. 1977, McLachlan and Peel 2000). I assume (1) that \( z_1, z_2, ..., z_n \) are independently and identically drawn from a density \( v(\cdot | \Phi) \) with parameters \( \Phi \), and (2) that there is a set of \( R \) densities \( u(\cdot | r, \Phi) \) for \( r = (1, 0, ..., 0), (0, 1, 0,..., 0), ..., (0, ..., 0, 1) \) such that \( y_i \) given \( z_i \) are conditionally independent with densities \( u(\cdot | z_i, \Phi) \). Dempster et al. (1977) then denotes

\[
U(y_i | \Phi) = (\log u(y_i|(1, 0, ..., 0), \Phi), \log u(y_i|(0, 1, ..., 0), \Phi), ... \log u(y_i|(0, 0, ..., 1), \Phi)) \quad (4-2)
\]

and

\[
V(\Phi) = (\log v((1, 0, 0, ..., 0)| \Phi), \log v((0, 1, 0, ..., 0)| \Phi), ... \log v((0, 0, 0, ..., 1)| \Phi)) . \quad (4-3)
\]
4. Ontogenetic allometries in *Holopsis* spp.

The log-likelihood can be defined as

$$\log f(x | \Phi) = \sum_{i=1}^{d} z_i \mathbf{T} \mathbf{U}(y_i | \Phi) + \sum_{i=1}^{d} z_i \mathbf{T} \mathbf{V}(\Phi)$$

(4-4)

where \( x = (y, z) \), the complete data with instar labels (Dempster et al. 1977; McLachlan and Peel 2000).

I used the EM (Expectation−Maximization) algorithm to estimate the unobserved instar (or component) vector, the unknown number of components \( R \), and the mean and variance of morphological characters at all the components (instars). The EM algorithm consists of an iteration of the following steps: the E-step requires us to find the elements of \( z_i \), which are the conditional probabilities that \( y_i \) belongs to each of the \( R \) states, while in the M-step, the log-likelihood (eqn 4-4) weighted by the \( R \) estimated components of \( z_i \) is maximized separately, updating the mixing probabilities (Dempster et al. 1977; McLachlan and Peel 2000).

For best-fit models, I computed discriminant functions between instars, where \( \mathbf{B}' \mathbf{X} = \mathbf{A} \), where \( \mathbf{B} \) is an \( i \times 4 \) matrix, \( \mathbf{A} \) an \( i \times 4 \) matrix, \( \mathbf{X} = (\mathbf{BL}, \mathbf{BW}, \mathbf{HS}, \mathbf{RL}) \), and \( i \) is the number of instars (or components). The confidence contours were obtained by Mahalanobis distance, \( d = 1, 2 \) to the centre (its coverage probability 0.393 and 0.865, respectively), \( d = ((\mathbf{x} - \mu)^T \Sigma^{-1} (\mathbf{x} - \mu))^{1/2} \), where \( \mathbf{x} = (x_1, x_2, x_3, x_4)^T \) from a component mean \( \mu = (\mu_1, \mu_2, \mu_3, \mu_4)^T \) and the covariance matrix \( \Sigma \).
4. Ontogenetic allometries in *Holopsis* spp.

**Table A4-1.** Model selection for the number of *Holopsis* spp. instars (components) in 4-D normal mixture models.

<table>
<thead>
<tr>
<th>Component</th>
<th>log-likelihood</th>
<th>AIC</th>
<th>ΔAIC</th>
<th>BIC</th>
<th>ΔBIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) sp. 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>351.2</td>
<td>−664.4</td>
<td>149.2</td>
<td>−604.0</td>
<td>94.1</td>
</tr>
<tr>
<td>3</td>
<td>370.2</td>
<td>−692.4</td>
<td>121.2</td>
<td>−616.0</td>
<td>82.1</td>
</tr>
<tr>
<td>4</td>
<td>390.5</td>
<td>−723.1</td>
<td>90.5</td>
<td>−630.8</td>
<td>67.3</td>
</tr>
<tr>
<td>5</td>
<td>437.1</td>
<td>−806.3</td>
<td>7.3</td>
<td>−698.1</td>
<td>0.0</td>
</tr>
<tr>
<td>6</td>
<td>445.8</td>
<td>−813.6</td>
<td>0.0</td>
<td>−689.5</td>
<td>8.6</td>
</tr>
<tr>
<td>(b) sp. 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>420.7</td>
<td>−803.4</td>
<td>51.5</td>
<td>−748.5</td>
<td>22.6</td>
</tr>
<tr>
<td>3</td>
<td>432.5</td>
<td>−817.0</td>
<td>37.9</td>
<td>−747.6</td>
<td>23.5</td>
</tr>
<tr>
<td>4</td>
<td>456.4</td>
<td>−854.9</td>
<td>0.0</td>
<td>−771.1</td>
<td>0.0</td>
</tr>
<tr>
<td>5</td>
<td>461.2</td>
<td>−854.3</td>
<td>0.6</td>
<td>−756.0</td>
<td>15.1</td>
</tr>
<tr>
<td>6</td>
<td>462.4</td>
<td>−846.8</td>
<td>8.1</td>
<td>−734.0</td>
<td>37.1</td>
</tr>
</tbody>
</table>

**Table A4-2.** Discriminant functions between *Holopsis* spp. instars in 4-D mixture models, obtained by $\mathbf{B}' \mathbf{X} = \mathbf{A}$, where $\mathbf{B}$ is an $i \times 4$ matrix ($b_{BL}$, $b_{BW}$, $b_{HS}$, $b_{RL}$), $\mathbf{A}$ an $i \times 1$ matrix, $\mathbf{X} = (BL, BW, HS, RL)$, and $i$ is the number of adjacent pairs of components.

<table>
<thead>
<tr>
<th>Instar intervals</th>
<th>$b_{BL}$</th>
<th>$b_{BW}$</th>
<th>$b_{HS}$</th>
<th>$b_{RL}$</th>
<th>a</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) sp. 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–2</td>
<td>−19.38</td>
<td>5.748</td>
<td>−16.72</td>
<td>−28.04</td>
<td>−22.43</td>
</tr>
<tr>
<td>2–3</td>
<td>−14.91</td>
<td>−11.51</td>
<td>−25.09</td>
<td>−23.59</td>
<td>−46.93</td>
</tr>
<tr>
<td>3–4A</td>
<td>−16.98</td>
<td>−16.28</td>
<td>−14.43</td>
<td>−24.86</td>
<td>−82.69</td>
</tr>
<tr>
<td>3–4B</td>
<td>−16.84</td>
<td>−29.06</td>
<td>−39.55</td>
<td>−9.298</td>
<td>−78.97</td>
</tr>
<tr>
<td>(b) sp. 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–2</td>
<td>−0.05</td>
<td>−33.66</td>
<td>−23.97</td>
<td>−18.05</td>
<td>−19.91</td>
</tr>
<tr>
<td>2–3</td>
<td>9.26</td>
<td>−31.93</td>
<td>−40.37</td>
<td>−18.14</td>
<td>−29.69</td>
</tr>
<tr>
<td>3–4</td>
<td>11.01</td>
<td>−47.48</td>
<td>−34.86</td>
<td>−14.06</td>
<td>−65.14</td>
</tr>
</tbody>
</table>
Chapter 5 Competition–colonisation dynamics of spore-feeding beetles on the long-lived bracket fungi *Ganoderma* in New Zealand native forest

Plate 5. Transient competitive coexistence of *Zearagytodes maculifer* (Broun) (Leiodidae) larvae and *Holopsis* sp. 1 (Coryphidae) larvae on the hymenial surface of *Ganoderma* cf. *applanatum* in the Waitakere Ranges (Photo: K. Kadowaki)
5. Spatial dynamics of spore-feeding beetles

Introduction

Identifying the mechanisms behind species coexistence in patchy environments is a persisting challenge in community ecology. Since the 1990s the proliferation of theoretical explanations for coexistence has overwhelmed empiricists by the sheer number of possible mechanisms to be simultaneously tested for and has made empirical testing dauntingly difficult because of the complexity of the proposed mechanisms involving space (Chesson 2000, Amarasekare 2003, Leibold et al. 2004). Theoretical works predict that competition and colonization determine the fate of competitors in a variety of ways, and the assumptions that the theories make are necessarily oversimplified and stylized in terms of spatial and temporal scales where competition and colonization occur. Natural systems are, however, often idiosyncratic in terms of distributional extent of a local population and rate of population changes, leading to the mismatch between theory and study system (Harrison et al. 1995, Amarasekare 2000). Furthermore, mounting evidence suggests that extant communities are likely to be controlled by a mixture of coexistence mechanisms (Levine and Rees 2002, Yu et al. 2004) rather than a single one. The current challenges for empiricists are therefore (1) to choose the appropriate spatial organizational framework in study designs (i.e., patches, gradients, hierarchies, and their complexes; Tally 2007) and (2) to investigate parameters controlling not only local population dynamics and local competitive relationships but also extinction-colonization dynamics at the metacommunity scale.

Competition-colonization dynamics theory predicts that coexistence among locally exclusive competitors can occur if (1) patch dynamics are driven by local disturbance and thus offer colonization opportunities for an inferior competitor and (2) a superior (and/or dominant) competitor is dispersal-limited and an inferior competitor with superior colonization ability therefore can exploit patches until the arrival of a superior competitor (Levins and Culver 1971, Nee and May 1992). Such dynamics driven by a trade-off between
5. Spatial dynamics of spore-feeding beetles

competitive and colonization ability of species have been motivated by mechanistic studies that have examined correlations between life-history traits associated with competitive/dispersal abilities (Sevenster and van Alphen 1993, Toda and Kimura 1997, Clark et al. 2004) and by direct testing in a microcosm experiment (Cadotte et al. 2006). The importance of competition-colonization (hereafter, CC) trade-offs in driving spatial dynamics has been supported by community patterns (Levine and Rees 2002, Rodríguez et al. 2007) and by extinction trajectories caused by habitat destruction (Tilman et al. 1994, Lei and Hanski 1998), but many other studies have failed to detect significant effects of CC trade-offs on coexistence (Harrison et al. 1995, Turnbull et al. 1999, Amarasekare 2000, Levine and Rees 2002, Jakobsson and Eriksson 2003, Mouquet et al. 2004), and some authors (e.g., Yu and Wilson 2001) have expressed doubt about the importance of CC trade-offs.

One of the two broad strategies for determining whether CC trade-offs permit species coexistence in natural systems would be to remove experimentally the effects of dispersal limitation for a superior competitor in replicated sets of patchy environments (Pacala and Rees 1998). Unfortunately, such experiments would require many large-scale replicated units and would be unfeasible in most natural systems. The alternative strategy is direct examination of evidence for the multiple hallmarks of CC trade-offs through model-fitting exercises with observational time-series data. Analysing local dynamics would allow estimation of the competitive rank order of species, which is necessary for determining whether or not CC trade-offs can explain the observed transitions in local community states. Few studies have unambiguously demonstrated the underlying dynamics of local displacement competition as well as patch transition patterns at the metacommunity scale. Population growth rates of competitors and resource dynamics have rarely been measured simultaneously in nature, and when a pattern consistent with CC trade-offs was detected, the
5. Spatial dynamics of spore-feeding beetles

mechanisms behind local dynamics were often unclear (e.g. Tilman et al. 1994, Levine and Rees 2002).

I studied communities of mycophagous insects living in long-lived bracket fungi. These are an excellent model system; each sporocarp (i.e., patch) is an arena for competitive interactions among larvae, and the mobile adult stages disperse among sporocarps (Whitlock 1992, Jonsell et al. 1999, Starzomski and Bondrup-Nielsen 2002). Such communities are thus spatially structured, and repeated assessment of local communities is feasible without damage to sporocarps. I could therefore collect not only cross-sectional data on local resource–multiconsumer communities but also data on the extinction-colonization dynamics of the metacommunity.

In the Waitakere Ranges, New Zealand, three obligate spore-feeding beetle species coexist on the wood-decaying bracket fungi Ganoderma cf. applanatum and G. australe (Fig. 1). Two species graze on mature spores on the pore surface of the sporocarp, whereas the other species has a specialized long rostrum that allows access to immature spores not available to the other two competitors. I therefore hypothesized that the species with the long rostrum is superior to the other two species in exploitative competition.

I investigated species coexistence mechanisms of the three spore-feeding beetles, focusing on CC dynamics. I explored local beetle dynamics to establish the competitive rank order and competitive relationships of the three beetle species. I then examined, for the patterns that demonstrate CC dynamics, (1) extinction, colonization, and population persistence time; (2) effects of patch productivity (daily spore release per square centimetre) and patch size (pore surface area); and (3) spatial synchrony/asynchrony of population dynamics. I also conducted a wind-tunnel experiment to compare the dispersal behaviours and associated physiological traits of the three species. I made four predictions: first, that the
5. Spatial dynamics of spore-feeding beetles

hypothesized inferior competitors would have (a) greater probability of local population extinction and lesser probability of colonization and/or (b) shorter persistence time in the presence of the hypothesized superior competitor than in its absence; second, that the hypothesized inferior competitors would be able to dominate only in less productive and/or smaller patches and that the superior competitor would predominate in more productive and/or larger patches; third, that the inferior competitors would have larger spatial extents of population synchrony than the superior competitor because of their greater dispersal ability or mobility; and fourth, that inferior competitors would be more willing to leave patches and colonize new terrain, be more likely to develop flight muscles, and have larger hindwings than superior competitors.

Figure 5-1. The wood-decaying bracket fungi *Ganoderma* spp. and three spore-feeding beetles. Sporocarp of (a) *G. cf. applanatum*, and (b) *G. australe*, (c) spore of *G. cf. applanatum*, size: 9.0–11.0 μm × 6.0–7.5 μm, (d) spore of *G. australe*, size: 11.5–14.5 μm × 6.5–9.0 μm, larvae of (e) Zearagytodes maculifer (Leioididae) (f) Holopsis sp. 1 (Corylophidae) with a long rostrum, and (g) Holopsis sp. 2 (Corylophidae) with a short rostrum. Scale bar = 1.0 mm.
Materials and methods

The study area

The Waitakere Ranges are a series of hills covering 30 000 ha on the coast 25 km west of Auckland City, rising up to a height of 460 m a.s.l. and covered in dense regenerating native forest (Esler and Astridge 1974). The study area consists of two large-area plots, the Cascade Kauri (36˚53'S, 174˚31'E) and the Upper Huia Dam (36˚56–57'S, 174˚30–32'E), separated by 8 km. The vegetation differs between plots: the Cascade Kauri is Agathis-dominated forest, whereas the Upper Huia Dam is mixed broadleaf–podocarp forest.

The study organisms

Ganoderma Karst. (Ganodermataceae, Basidiomycota) produces perennial sporocarps on a variety of woody hosts and there are three species present in New Zealand (Buchanan and Wilkie 1995), two of which I encountered in my study: Ganoderma cf. applanatum (Pers.) Pat and G. australis (Fr.) Pat (Fig. 5-1a, b). Ganoderma cf. applanatum (hereafter G. applanatum) and G. australis are distinguished based on stromal texture, hymenium, spore-tube morphology, spore size and spore print (Buchanan and Wilkie 1995). Notably, G. applanatum has spores that are much smaller than those of G. australis (Fig. 5-1c, d).

The three beetle species examined in my study are all endemic to New Zealand. Zearagytodes maculifer (Broun) (Leiodidae: Camiarinae: Agyrtodini) is a larger-bodied spore-feeding beetle (Kuschel 1990; Fig. 5-1e) while the other two species, Holopsis spp. (Corylophidae: Corylophinae: Peltinodini) are smaller-bodied (Fig. 5-1f, g). Larvae and adults of the three species graze mainly on Ganoderma spores. Zearagytodes maculifer is widespread in suburban reserves distant from the Waitakere Ranges (Kuschel 1990), and this species might be a superior disperser. Larvae of Z. maculifer are slender with aposematic colouring and elongate urogomphi. Two Holopsis species are confirmed in my study by
5. Spatial dynamics of spore-feeding beetles

dissection of adults (female genitalia are distinct in each species) and these are referred to as *Holopsis* sp. 1 and *Holopsis* sp. 2. Intriguingly, *Holopsis* sp. 1 possesses an elongate rostrum during its larval stage (Fig. 5-1f) while *Holopsis* sp. 2 does not (Fig. 5-1g). Lawrence (1989) termed *Holopsis* sp. 1 a “pore tube specialist” and conjectured that the species has access to even immature spores in the depth of pore tubes. The gut content of *Holopsis* sp. 1 larvae contains immature spores, although they often consume mature spores (Kadowaki et al. unpublished manuscript, Chapter 4).

The generation time of spore-feeding beetles is nearly four weeks (Kadowaki et al. 2011), and one would therefore expect relatively rapid population dynamics. Heavy infestations by beetle larvae never destroyed sporocarps, and newly emergent adults tend to stay and breed in the same sporocarps for several generations in a year. Little is known about their dispersal behaviour. Spore-feeding beetles do not seem to compete with other fungivorous competitors; spore-feeders colonize only while sporocarps are alive, whereas most fungivorous insects (e.g. internal feeders) colonize dead sporocarps (e.g. Jonsell et al. 1999; Komonen and Kouki 2005). I observed predatory insects and the other spore-feeding insects infrequently. I did not observe any parasitoids of the spore-feeding beetles.
5. Spatial dynamics of spore-feeding beetles

**Figure 5-2.** The study area (a) included two plots, Cascade Kauri (b) and Upper Huia Dam (c). Circles are sites with *Ganoderma* cf. *applanatum*, while triangles are *G. australe* sites. Panel (d) shows duration of observation of patches. The gray zone of patch 1-5B indicates that the patch was perturbed by mold but rejuvenated the next month. This figure is the same as Fig. 2-1 (Chapter 2), and Fig. 3-2 (Chapter 3).

**Field study**

I employed a spatially hierarchical sampling design, ranging over three spatial scales (i.e., patch, site and plot; Fig. 5-2). The two large-area plots of Cascade Kauri and Upper Huia Dam comprised nine and seven sites respectively, each containing dead logs as host substrate for *Ganoderma*. The mean distance between sites was 0.6 km in Cascade Kauri, and 1.8 km in Upper Huia Dam. A patch was defined as a single sporocarp or suites of aggregated sporocarps that occurred closer than 30 cm from each other. I initially chose no more than three patches in each site. The total number of patches that I surveyed changed in a year because of patch destruction and de novo patch creation. A decayed or destroyed sporocarp (by disturbance) was omitted from the next survey, while newly emergent, mature sporocarps (sporocarp width >5.0 cm) were recorded and inspected in the later surveys. Thus, sporocarp
5. Spatial dynamics of spore-feeding beetles

number was 25 at the start of the study and 21 at the end, with a total of 30 studied overall (Fig. 5-2). Reflecting the natural proportion of sporocarp occurrence, there were more *G. appplanatum* patches (25 sporocarps) than *G. australe* (5 sporocarps).

Each patch was surveyed monthly for beetle and spore measurements as well as environmental variables from November 2007 to October 2008. The number of individuals of larvae and adults for each beetle species was recorded by digital photographic documentation, and later counted with ImageJ program (freely available from http://rsb.info.nih.gov/ij/) and its plugin Cell Counter. Larvae of the three species are always visible and distinguishable in both the field and in photographs (Fig. 5-1e–g). Thus, this census gave absolute counts of larval communities. However, the external morphology of adults of the two *Holopsis* species are very similar, so results for adult *Holopsis* spp. were pooled. I measured spore release (patch productivity) for each patch by attaching a sheet of plastic plate (37 mm × 23 mm) beneath the pore surface. Because I was interested in resource dynamics itself (spore release), beetle larvae were excluded by the small gap (<1.0 mm) between the plate and the pore surface. I estimated spore density per plastic plate (the unit area) in the laboratory by the method described elsewhere (Kadowaki et al. 2010, Chapter 1). The contour of the pore surface was traced with a permanent marker on standardized-weight tracing paper (1.12 × 10⁻² g cm⁻²), and the trimmed pieces of papers were dried and weighed to be converted into area (cm²) (i.e. patch size). The daily spore release by each patch was obtained by multiplying per area spore density (patch productivity) by total pore surface area (patch size). Temperature and humidity were recorded monthly with a hand-held meter beside a patch; percentage of canopy cover (%) was quantified every other month by a spherical densitometer; and height above ground (cm) of a patch was measured.

**Wind tunnel experiment**

I investigated dispersal behaviours and the associated physiological traits of the three beetle
5. Spatial dynamics of spore-feeding beetles

species: flight willingness (time required to take-off), direction of take-offs of adults at two wind speed levels, flight muscle, weight and hindwing length. Over three days, I used an artificial wind tunnel (200 cm × 100 cm × 100 cm) to experiment a total of 53 individuals (21 Z. maculifer, 13 Holopsis sp. 1 and 19 Holopsis sp. 2) sourced from 17 sporocarps from the study sites on 18 April 2010. These adults varied in ages or sexes, as it was not possible to sex the beetles or obtain tenerals or similar aged beetles because all the three species have no sexual dimorphism and pupate in soil or crevices of dead logs. Individual adults were kept separately in a Petri dish (radius = 3 cm, height =1.5 cm) with moistened pieces of Ganoderma sporocarps on damp filter paper, and were starved for 24 h only before the trial. Conspecific adults for simultaneous flight trials were randomly assigned to five (or four) groups, each of which includes no more than four individuals that were not distinguished within a group. A pair of groups was experimented twice a day at different wind speeds (very slight breeze 3–5 cm s\(^{-1}\), and moderate breeze 60–70 cm s\(^{-1}\), generated by a voltage-regulated fan) in a reversed order for each species. I placed conspecific adults on the centres of white square papers (14 cm × 14cm) for platform on four metal stands (Appendix 5-1). I set a lure (0.50 g fragments of Ganoderma hymenium) in a 5 cm glass tube (radius = 1.5cm) on a tripod 40 cm forward the front of a platform (Appendix 5-1). I acclimatized the beetles for 3 min by covering them with Petri dishes, and then gently removed the dishes. I measured the time until take-off, flight direction (upwind, downwind or others) for 20 min for each trial. Individual adults were weighed to the nearest 1 mg on electrobalance to the nearest 1 mg, and were examined for physiological traits, including hindwing length, presence of flight muscle, sex, and presence of eggs if mature females. The measurement of hindwing length was made on each individual to the nearest 0.01 mm using a linear micrometer inserted in the eyepiece of a
5. Spatial dynamics of spore-feeding beetles

Leica MZ16 microscope.

**Statistical analyses**

*Local dynamics.* I parameterized local spore-feeder dynamics to estimate intrinsic rate of population increase, per capita competitive effects and responses among beetle species. I explored six working models that depict a wide array of possible population dynamics (Table 5-1), by using monthly fluctuations in larval abundance as an indicator of the population density of competitors. The justification for using larval abundance is described in Appendix 5-2.

The Lotka-Volterra model of interspecific competition has the following general function form (Ayala et al. 1973):

\[
\frac{dN_i}{dt} = D_i[N_i(t), N_j(t); r_i, K_i, \alpha_{ij}, \beta_{ij}, \ldots]
\]  

(5-1)

where \(D_i\) is an unspecified function giving the population change per time, \(N_i\) and \(N_j\) are respectively the number of individuals of species \(i, j\) at time \(t\), and \(r_i, K_i, \alpha_{ij}, \beta_{ij}, \ldots\) are parameters whose values are independent of time (see Table 5-1). To fit cross-sectional field data, eqn 5-1 can be transformed into:

\[
\ln \frac{N_i(t+1)}{N_i(t)} = r \left( 1 - \frac{N_i}{K_i} \right) - r \alpha_{ij} \left( \frac{N_j}{K_i} \right) + \ldots
\]  

(5-2)

This can be expressed to give a generalized linear model:

\[
\ln N_i(t+1) = \ln N_i(t) + AX_1 + BX_2 + \ldots
\]  

(5-3)

where \(\ln N_i(t)\) is an offset term (not an intercept), and \(A = r, X_1 = (1 - N_i/K_i), B = r\alpha_{ij}, X_2 = N_j/K_i\). I used patch size as the carrying capacity \(K_i\) (see Appendix 5-2 for justification).

Population dynamics in different patches were analysed simultaneously. The first month’s
5. Spatial dynamics of spore-feeding beetles

immigration data were removed as the monthly population increase rates could not be calculated. I selected a best-fit model from the six working models in terms of Akaike’s information criteria (AIC), and then obtained competitive coefficient \( \alpha = B/A \) (eqn 5-4) and its estimated variance (eqn 5-5) for each species by the delta method based on Taylor series expansions (Oehlert 1992):

\[
\begin{align*}
\mathbb{E}\left( \frac{B}{A} \right) & \approx \frac{\mathbb{E}(B)}{\mathbb{E}(A)} - \frac{1}{\mathbb{E}(A)^2} \text{Cov}(A, B) + \frac{\mathbb{E}(B)}{\mathbb{E}(A)^3} \text{Var}(A) \\
\text{Var}\left( \frac{B}{A} \right) & \approx \frac{\mathbb{E}(B)^2}{\mathbb{E}(A)^4} \text{Var}(A) + \frac{1}{\mathbb{E}(A)^2} \text{Var}(B) - \frac{2\mathbb{E}(B)}{\mathbb{E}(A)^3} \text{Cov}(A, B)
\end{align*}
\] (5-4)

(5-5)

I constructed a competitive interaction web of the three spore-feeders, using point estimates of the best-fit models. The difference in population growth rates \( r \) from separate models was tested pairwise among three species by t tests, applying a sequential Bonferroni correction \( (p = 0.05/3) \). This allowed me to establish the competitive rank order of the beetles from local dynamics.
5. Spatial dynamics of spore-feeding beetles

Table 5-1. Six working models of local beetle population dynamics (cited from Ayala et al. 1973).
Species $i$ is the focal species while species $j, k$ are its competitors. Parameters: $r_i$ is the intrinsic rate of population increase of the focal species $i$; $N_i$ is the population density of species $i$; $K_i$ is the carrying capacity of species $i$; $\alpha_{ij}$ is the per capita competitive effect of species $j$ on species $i$; $\beta_{ij}$ is the density-dependent per capita competitive effect (e.g., interference competition). For biological interpretation of parameters, see Ayala et al. (1973).

<table>
<thead>
<tr>
<th>Model Number</th>
<th>Function</th>
<th>Number of parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$r_i \frac{N_i}{K_i} (K_i - N_i - \alpha_{ij} N_j - \alpha_{ik} N_k)$</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>$r_i \frac{N_i}{\log(K_i)} (\log(K_i) - \log(N_i) - \alpha_{ij} \log(N_j) - \alpha_{ik} \log(N_k))$</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>$r_i \frac{N_i}{K_i^{1/2}} (K_i^{1/2} - N_i^{1/2} - \alpha_{ij} \frac{N_j}{K_i^{1/2}} - \alpha_{ik} \frac{N_k}{K_i^{1/2}})$</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>$r_i \frac{N_i}{K_i} (K_i - N_i - \alpha_{ij} N_j - \alpha_{ik} N_k - \beta_{ij} N_i N_j - \beta_{ik} N_i N_k)$</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>$r_i \frac{N_i}{K_i} (K_i - N_i - \alpha_{ij} N_j - \alpha_{ik} N_k - \beta_{ij} N_i^2)$</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>$r_i \frac{N_i}{K_i} (K_i - N_i - \alpha_{ij} N_j - \alpha_{ik} N_k - \beta_{ij} N_j^2 - \beta_{ik} N_k^2)$</td>
<td>6</td>
</tr>
</tbody>
</table>

Extinction–colonisation dynamics. I examined the times of extinction and colonisation of *Z. maculifer* and *Holopsis* sp. 2 larval populations in the presence versus absence of *Holopsis* sp. 1 larvae in the prior month. I performed a binomial test to test for increased or decreased likelihood of extinction and colonisation in the presence and absence of competitors. Months where a patch included no beetle larvae were excluded from analysis. I also compared mean persistence times of *Z. maculifer* between ‘*Holopsis* sp. 1-dominant patches’ and ‘*Z. maculifer*-dominant patches’. A ‘*Holopsis* sp. 1-dominant patch’ was defined as a patch where *Holopsis* sp. 1 became more abundant than *Z. maculifer* at least once at any period of the year. Persistence time was quantified by M/I, i.e., the total number of months the focal species persisted (M), divided by the number of immigration trials (I) of the species. Such persistence time can be systematically biased against the ‘real’ persistence times in the absence of information before and after the survey period, but is valid for comparative...
5. Spatial dynamics of spore-feeding beetles

purposes within the same dataset. Since the species present at the starting month of the survey (November 2007) immigrated into the patch at some point in previous months (October 2007), I counted this as an immigration \((I =1)\). Patches observed for twelve months were used for analysis. Because of the rarity of Holopsis sp. 2, I could not define the standardized persistence time or ‘Holopsis sp. 2-dominant patches’ for this species, and hence no persistence time analysis was conducted.

**Patch attributes.** I assessed whether the mean larval abundance of the three beetle species at the patch-level depends on patch productivity (spores day\(^{-1}\)cm\(^{-2}\)) and/or patch size (cm\(^2\)). General linear models were developed for each species with explanatory variables of patch size and/or patch productivity, with an identity link and a Gaussian error term. The mean value of each patch represented one data point. I used only G. applanatum patches that were observed for more than four months, and excluded from analysis the patches where the focal species was absent. Because spore-feeding beetle densities differed between the two plots, I used a factor “plot” (Cascade Kauri or Upper Huia Dam) as a covariate. To test for species sorting over environmental gradients, I checked the correlations between the two patch attributes and environmental variables (temperature, relative humidity, light intensity and height above ground), using the November 2007 dataset from Cascade Kauri and Upper Huia Dam.

**Spatial synchrony.** I examined how the degree of spatial synchrony of population dynamics was associated with interpatch distance for each beetle species. The degree of spatial synchrony was quantified by the product–moment correlation coefficient \(\rho_{ij}\) (lag-zero cross correlation, Holyoak 1996) for each pair of conspecific populations between the patch \(i\) and \(j\), which overlapped at least five months, using the dataset from Cascade Kauri. Population densities were \(\ln(x+1)\)-transformed beforehand. To examine the correlation between spatial isolation and habitat similarity, I conducted Mantel tests, using November 2007 dataset.
5. Spatial dynamics of spore-feeding beetles

Factors considered were: patch size, temperature, humidity, light intensity and height above ground.

Wind tunnel experiment and morphological characters. Differences were tested among the three beetle species for the occurrence of flight, time to take-off, flight direction at different wind speeds, hindwing length, weight, presence of flight muscle by Kruskal Wallis rank sum test with Scheffe’s multiple comparison (Casella and Berger 2002) or $\chi^2$ test. Individuals that escaped during the experiment (four Z. maculifer and one Holopsis sp. 1) were removed from the analysis of physiological correlates. Analyses were implemented in R 2.9.1 (R Development Core Team 2009).

Results

Overview

The spore-feeding beetle community was numerically dominated by Holopsis sp. 1 (n = 38,733, larvae). Zearagytodes maculifer (n = 11,731) and Holopsis sp. 2 (n = 2,059) were less abundant. Patch occupancies over the year showed that Z. maculifer was widespread (79.3 % ± 13.3, mean ± SD), whereas the Holopsis species were restricted to a smaller proportion of patches; Holopsis sp. 1, 27.7 % ± 10.7 and Holopsis sp. 2, 21.1 % ± 8.5. The two plots (Cascade Kauri and Upper Huia Dam) differed significantly in relative beetle abundance patterns based on the cumulative abundance of larva ($\chi^2 = 15106.75$, df = 2, p <0 .0001); Holopsis sp. 1 was far less abundant in Upper Huia Dam (n = 119) than in Cascade Kauri (n = 38,614). While G. applanatum supported 99.9% of the spore-feeding beetle community in terms of larval abundance, G. australe hosted a total of 47 Z. maculifer larvae and no Holopsis species. Only six Z. maculifer and one Holopsis adult were observed in G. australe. Zearagytodes maculifer did not have very strong seasonality or generation patterns, as both adults and larvae consistently co-occurred. The two Holopsis species both had peak larval abundance in late summer to autumn (February to March), whereas Z. maculifer larval
5. Spatial dynamics of spore-feeding beetles

abundance peaked in winter (Fig. 5-3).

**Figure 5-3.** Mean (+/− 1 SE) density of the *Ganoderma*-spore-feeding beetles (a) adults and (b) larvae, and spore released for 24 h. Months are shown with a one-letter code starting from N (November 2007) and finishing in O (October 2008).
5. Spatial dynamics of spore-feeding beetles

**Table 5-2.** Best-fit local spore-feeder dynamics models. The model number, structure and parameters refer to Table 5-1. Species codes: *z*– *Z. maculifer*, *h1*– *Holopsis* sp.1, and *h2*– *Holopsis* sp.2. See Appendix 5-2 for full models.

<table>
<thead>
<tr>
<th>Species</th>
<th>Model</th>
<th>Parameter</th>
<th>Coefficient</th>
<th>SE</th>
<th>t</th>
<th>p</th>
<th>Residual deviance</th>
<th>df</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Z. maculifer</em></td>
<td>5</td>
<td>$r_z$</td>
<td>0.232</td>
<td>0.136</td>
<td>1.692</td>
<td>0.093</td>
<td>287.15</td>
<td>160</td>
<td>562.87</td>
</tr>
<tr>
<td>(n = 163)</td>
<td></td>
<td>$r_z \alpha_{h1}$</td>
<td>-0.147</td>
<td>0.061</td>
<td>-2.398</td>
<td>0.017</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$r_z \beta_{iz}$</td>
<td>-0.002</td>
<td>0.001</td>
<td>-2.476</td>
<td>0.014</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Holopsis sp.</em></td>
<td>1</td>
<td>$r_{h1}$</td>
<td>1.627</td>
<td>0.712</td>
<td>2.287</td>
<td>0.025</td>
<td>327.35</td>
<td>66</td>
<td>305.84</td>
</tr>
<tr>
<td>(n = 68)</td>
<td></td>
<td>$r_{h1} \alpha_{h1}$</td>
<td>-1.342</td>
<td>0.525</td>
<td>-2.555</td>
<td>0.013</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Holopsis sp.</em></td>
<td>2</td>
<td>$r_{h2}$</td>
<td>-0.598</td>
<td>0.316</td>
<td>-1.893</td>
<td>0.067</td>
<td>79.895</td>
<td>33</td>
<td>143.48</td>
</tr>
<tr>
<td>(n = 37)</td>
<td></td>
<td>$r_{h2} \alpha_{h2h1}$</td>
<td>0.714</td>
<td>0.238</td>
<td>2.997</td>
<td>0.005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$r_{h2} \beta_{h2z}$</td>
<td>-0.118</td>
<td>0.038</td>
<td>-3.128</td>
<td>0.004</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$r_{h2} \beta_{h2h1}$</td>
<td>-0.014</td>
<td>0.006</td>
<td>-2.119</td>
<td>0.042</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Null deviances are: *Zearagytodes maculifer*, 316.81 on 163 df; *Holopsis* sp. 1, 366.79 on 68 df; *Holopsis* sp. 2, 136.034 on 37 df.
5. Spatial dynamics of spore-feeding beetles

**Local beetle dynamics**

Table 5-2 summarizes the parameter estimates of the best-fit models for local dynamics, and the full models are presented in Appendix 5-2. Fig. 5-4 depicts the network of per capita competitive interactions that are statistically significant based on the parameter estimates in Table 5-2. Three intriguing patterns were revealed. Firstly, *Holopsis* sp. 1 displayed a higher intrinsic population growth rate compared to *Holopsis* sp. 2 (*t* = 2.88, df = 89.31, *p* < 0.01), although not significantly higher than *Z. maculifer* (*t* = 1.94, df = 71.98, *p* = 0.056). No significant difference in population growth rates was found between *Z. maculifer* and *Holopsis* sp. 2. Secondly, the interspecific competition between *Holopsis* sp. 1 and *Z. maculifer* was strong, and the per capita competitive effect of *Z. maculifer* (−0.913) was nearly symmetrical to that of *Holopsis* sp. 1 (−0.810). Thirdly, *Holopsis* sp. 2 was asymmetrically affected by *Holopsis* sp. 1 (−1.482), but there seemed to be a weak but positive indirect effect when the two competitors *Z. maculifer* and *Holopsis* sp. 1 were present. *Zearagytodes maculifer* exhibited weak density-dependent competition (−0.014).
5. Spatial dynamics of spore-feeding beetles

Figure 5-4. Quantitative competitive interaction web of the three spore-feeders. The values tagged on arrows indicate per capita strengths of competitive interactions, followed by their standard deviations in brackets (see Table 5-2 for the best-fit models). Black straight arrows indicate significant exploitative interactions (α), while grey curved arrows indicate interference competition (−) or facilitation (+) (i.e., β, density-dependent per capita competitive effects).

Extinction–colonisation dynamics

During the study, eight patches were destroyed (four by disturbance, two of them probably as a result of human interference, and the other four by unknown factors), and four were created. Patch destruction explained only 13.6% of the 44 beetle population extinctions observed. The patch transition analysis found relatively few cases of extinctions and colonisations, but found that *Z. maculifer* was more likely to go extinct in the presence of *Holopsis* sp. 1 than its absence; the binomial probability of obtaining eight or more extinctions from 56 trials, when the expected extinction rate is 7/137, is only 0.0073. There was no increase in the extinction
5. Spatial dynamics of spore-feeding beetles

Probabilities of *Holopsis* sp. 2 when *Holopsis* sp. 1 was present (binomial test, p= 0.829).

Colonisation by *Z. maculifer* and *Holopsis* sp. 2 was more likely to occur when *Holopsis* sp. 1 is already present (binomial test, p= 0.017 and 0.024) (Table 5-3), suggesting the potential for facilitated colonisation.

Furthermore, the mean persistence time of *Z. maculifer* was significantly greater in the absence of *Holopsis* sp. 1 than in its presence (Fig. 5-5). There was no significant difference in mean density of *Z. maculifer* between *Zearagytodes*-dominant patches and *Holopsis* sp.1-dominant patches (t = –1.14, df = 6.48, p = 0.29). Hence, the decreased persistence time of *Z. maculifer* in *Holopsis* sp.1-dominant patches is not explicable solely by demographic stochasticity in the low density of *Z. maculifer* populations. Thus, competitive exclusion of *Z. maculifer* by *Holopsis* sp. 1 probably explains the patch-transition and population-persistence patterns. Although transient coexistence was the norm between these two species, competitive displacement by *Holopsis* sp. 1 might occur slowly and inexorably as a result of its superior spore-feeding strategy and resultant higher population growth rate.

### Table 5-3. Extinction–colonisation dynamics of *Zearagytodes maculifer* and *Holopsis* sp. 2 in the presence and absence of *Holopsis* sp. 1 in the previous month. The first two rows and latter two rows were paired respectively and applied to a one-sided binomial test in each of the two *Holopsis* species.

<table>
<thead>
<tr>
<th>Zearagytodes maculifer</th>
<th>Holopsis sp. 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>present</td>
</tr>
<tr>
<td>presence → presence</td>
<td>48</td>
</tr>
<tr>
<td>presence → absence</td>
<td>8</td>
</tr>
<tr>
<td>absence → presence</td>
<td>8</td>
</tr>
<tr>
<td>absence → absence</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Holopsis sp. 2</th>
<th>Holopsis sp. 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>present</td>
</tr>
<tr>
<td>presence → presence</td>
<td>18</td>
</tr>
<tr>
<td>presence → absence</td>
<td>10</td>
</tr>
<tr>
<td>absence → presence</td>
<td>9</td>
</tr>
<tr>
<td>absence → absence</td>
<td>35</td>
</tr>
</tbody>
</table>
5. Spatial dynamics of spore-feeding beetles

**Figure 5-5.** Mean population persistence times per immigration trial of *Zearagytodes maculifer* populations in *Z. maculifer*-dominant patches (n = 12, patches) and *Holopsis* sp. 1-dominant patches (n = 6). Error bars show + 1 SE. The patch types significantly differed: t-test, t = 2.72, df = 9.72, p < 0.05.

**Patch attributes**

Patch size showed a marked effect on local beetle communities, whereas the effects of patch productivity were unclear (Fig. 5-6). Both *Z. maculifer* and *Holopsis* sp. 1 populations increased in density with increasing patch size, whereas the *Holopsis* sp. 2 population density was inversely related to patch size (Fig. 5-6). The significant interaction between patch productivity and location in *Holopsis* sp. 1 was due to the difference in its abundance between Cascade Kauri and Upper Huia Dam (see also Appendix 5-3). There was no correlation between patch productivity and patch size ($r^2 = -0.076$, $p > 0.70$). Patch productivity did not differ between the two plots ($t = 0.357$, df = 11, $p = 0.728$), but the mean patch size was significantly larger at Cascade Kauri than at Upper Huia Dam ($t = 2.468$, df =
5. Spatial dynamics of spore-feeding beetles

20, \( p = 0.023 \). Patch size was not related to any environmental variables: temperature (Pearson’s \( r = 0.0677, p > 0.75 \)), humidity (\( r = -0.24, p > 0.25 \)), canopy cover (\( r = 0.179, p > 0.40 \)), height above ground (\( r = 0.136, p > 0.50 \)).

Spatial synchrony

*Zearagytodes maculifer* populations synchronized (\( \rho = 0.4–0.7 \)) within the interpatch distances of 50 m, while the two *Holopsis* spp. showed no clear patterns (Fig. 7). Also, *Z. maculifer* showed a high variability in the degree of synchrony in distant patches (>100 m). Interpatch distance was not correlated with any kind of habitat similarity: patch size (Mantel test statistic \( r = 0.18, p > 0.10 \)), environmental variables such as temperature (\( r = 0.15, p > 0.20 \)), humidity (\( r = -0.12, p > 0.70 \)), light intensity (\( r = 0.30, p > 0.05 \)), or ground height (\( r = 0.02, p > 0.30 \)). Together these results suggest that the observed spatial synchrony cannot be attributed to habitat similarity. The large spatial extent of *Z. maculifer* population synchrony across patches therefore suggests that multiple patches can act functionally like a single patch for *Z. maculifer*, perhaps because of the high mobility (or agility) of adults: adults may frequently take off and move across patches, effectively dynamically coupling closely spaced patches.
5. Spatial dynamics of spore-feeding beetles

Figure 5-6. The effects of patch size (cm$^2$) and productivity (spores density per unit area day$^{-1}$) on the three spore-feeding beetle densities (log, mean ± 1 SE). Cascade Kauri patches are shown as open circles with a solid line while Upper Huia Dam patches are open triangles with a dotted line.
5. Spatial dynamics of spore-feeding beetles

**Figure 5-7.** Spatial synchrony $\rho$ versus interpatch distance (m) relationship. The shaded area in grey shows that *Z. maculifer* retained high spatial synchrony at least within 50m, whereas the two *Holopsis* spp. exhibited no clear patterns in spatial synchrony.

**Wind tunnel experiment**

A total of 104 flight trials were conducted. In 80% of trials, *Z. maculifer* took flight, while none of the two *Holopsis* spp. did (Table 5-4). I found no sign for unfolding wings for *Holopsis* spp. that generally walked on the edges of a platform for 20 mins. For *Z. maculifer*, greater wind speed reduced the occurrence of take-offs ($\chi^2 = 6.4247$, df =1, $p = 0.011$), marginally increased the time until take-offs (Welch’s t = $-2.041$, df = 16.75, $p = 0.057$), but did not affect the direction of flight ($\chi^2 = 2.735$, df = 3, $p > 0.40$). In 64% of the trials *Z. maculifer* took-off downwind and in 19.3% of the trials *Z. maculifer* took-off upwind but eventually turned downwind, despite the presence of upwind lure (host fungus *Ganoderma*). The first and second flight did not differ for the occurrence of flight ($\chi^2 = 0.910$, df = 1, $p = 0.34$) or the time until take-offs (Welch’s t = $-0.0447$, df = 30.91, $p > 0.95$). The location of platforms did not significantly affect the occurrence of flights (Kruskal-Wallis $\chi^2 = 5.033$, df = 3, $p > 0.15$) or the time until take-offs ($\chi^2 = 1.234$, df = 3, $p > 0.70$).
5. Spatial dynamics of spore-feeding beetles

Morphological characters
Dissection showed that 94% of Z. maculifer developed flight muscles fully (82.4%) or partially (12%), whilst 83.3% of Holopsis sp. 1 and 100% of Holopsis sp. 2 had reduced flight muscles (Table 5-4). Forty-two percent of females of Holopsis sp. 2 possessed 1–10 eggs. The eggs were large compared to its body size, occasionally occupying 3/4 of the volume of the entire body. Holopsis sp. 1 possessed significantly shorter hindwings than Z. maculifer (Kruskal Wallis $\chi^2 = 36.449$, df =1, $p < 0.0001$) and Holopsis sp. 2 (Kruskal Wallis $\chi^2 = 6.143$, df =1, $p = 0.046$) (Table 5-4). The two Holopsis species did not differ in weight, but were lighter than Z. maculifer ($p < 0.001$). I found no differences between sexes for hindwing length (Kruskal-Wallis $\chi^2 = 1.646$, df = 1, $p > 0.15$), weight (Kruskal Wallis $\chi^2 = 1.245$, df = 1, $p > 0.25$) and flight muscles ($\chi^2 = 0.887$, df = 2, $p > 0.60$).

Table 5-4. Dispersal-related parameters (SE) of the three beetle species by wind tunnel experiments and dissection of flight muscle and wings, and the measurement of weight (mg).

<table>
<thead>
<tr>
<th></th>
<th>Z. maculifer</th>
<th>Holopsis sp.1</th>
<th>Holopsis sp. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flight occurrence</td>
<td>33/41</td>
<td>0/25</td>
<td>0/38</td>
</tr>
<tr>
<td>Time to take-offs (s)</td>
<td>213.9 (38.3)</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Flight muscle</td>
<td>14/17</td>
<td>2/12</td>
<td>0/19</td>
</tr>
<tr>
<td>Hindwing length (mm)</td>
<td>1.668 (0.044)</td>
<td>0.964 (0.025)</td>
<td>1.128 (0.020)</td>
</tr>
<tr>
<td>Weight (mg)</td>
<td>11.06 (0.710)</td>
<td>3.462 (0.253)</td>
<td>4.833 (0.224)</td>
</tr>
</tbody>
</table>

++ indicates no sign for take-off for 20 min. Standard errors are shown in brackets.

Discussion
The cross-sectional datasets of the spore-feeding beetle community revealed two apparently conflicting patterns: relatively frequent extinction events, and transient patch-level coexistence with species composition and relative abundance changing dramatically over time. I conclude that CC dynamics exist in a somewhat modified form (e.g., competitive equivalents, patch size, population synchrony), and are made more complicated by the various pieces of biology that influence spatial dynamics. This conclusion follows from two key results. First, Z. maculifer may escape from competition with Holopsis sp. 1 by means of
5. Spatial dynamics of spore-feeding beetles

its higher mobility at small spatial scales (clusters of patches) and also at larger spatial scales by means of its widespread distribution. Both the results of the wind-tunnel experiment and the measurement of hindwing lengths were concordant with the hypothesis that *Z. maculifer* is a stronger or more frequent disperser than the two *Holopsis* species. Despite its frequent take-offs, *Z. maculifer* hardly resisted against even a gentle wind, suggesting that they disperse like ‘air plankton’.

Second, the two *Holopsis* spp. coexist by spatial niche partitioning along a patch-size gradient. I typically observed two types of patch-level community states in large patches: the *Zearagytodes*-dominated patches and the *Holopsis* sp. 1–dominated patches. In contrast, *Holopsis* sp. 2 may be able to proliferate in small patches, until the patch grows larger and allows *Holopsis* sp. 1, with its lower probability of colonization, to dominate the patch. A space-for-time substitution therefore suggests that the two *Holopsis* spp. can coexist by successional niche partitioning along the patch-size gradient. Alternatively, *Holopsis* sp. 2 may have competitive advantage only in small patches, even though I have no mechanistic evidence to support such mechanisms of competitive advantage related to patch size. No clear environmental gradients along patch productivity (Chapter 2) or patch size indicated that species sorting over patch size per se is not very likely. I could not find any differences in dispersal-related parameters between the two *Holopsis* spp., so the reasons for their reluctance to take-off—absence of flight muscles, behavioural factors, or both—remain unclear. Although the spore-feeder community did not show a simple linear competitive and dispersal hierarchy, my data collectively suggest that the three spore-feeding beetles exploit subtle differences in spatial niches to coexist in patchy environments.

Coexistence mechanisms other than CC trade-off may also play some, even if minor, role in the spore-feeding beetle community. First, resource partitioning is the coexistence mechanism by which consumer species use different resource species. Although the three
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species predominantly occurred on *G. applanatum*, some *Z. maculifer* occurred on *G. australis*, and these individuals may evade competition with the two *Holopsis* species.

Second, seasonal change in abundance may be important. *Holopsis* spp. declined in abundance in winter, whereas *Z. maculifer* reproduced in the winter. *Holopsis* larvae became inactive on cold days (<12°C), whereas *Z. maculifer* larvae were active in such weather (K.K., personal obs.). This pattern may create a temporal refuge for *Z. maculifer* from *Holopsis* sp. 1; the doubled population density of *Z. maculifer* in winter might be partially due to temporal competitive release from *Holopsis* sp. 1 (Fig. 3). Seasonality alone cannot explain the observed extinction-colonization dynamics, however, because population extinctions in winter accounted for only 11.4% of the extinction events. Third, the negative density dependence in *Z. maculifer* may prevent it from driving *Holopsis* sp. 1 extinct, thus contributing to pairwise species coexistence.

I certainly need further mechanistic evidence on beetle dispersal to demonstrate the existence of CC trade-offs beyond demonstrating at least that a CC trade-off is driving spatial dynamics. Some community patterns remain partially unexplained by currently available information. For example, why did the *Holopsis* spp. not clearly display spatial population synchrony? Perhaps their dispersal behaviour is more similar to the fungivorous Tenebrionidae beetles, which employ three distinct types of dispersal or movements: frequent short-distance movements by walking around a log, movements of adults across logs (Heatwole and Heatwole 1968, Jonsell et al. 2003), and infrequent long-distance dispersal that takes place only during a few weeks in summer (Jonsell et al. 2003). Field studies have shown that dispersal abilities of other mycophagous beetles are rather good up to a few kilometres (Jonsell et al. 1999, Komonen 2008), and I therefore think that the low patch occupancy patterns for *Holopsis* spp. resulted from dispersal behaviour resulting in low colonization probability, not from limitation of dispersal by weak flight ability. Remarkably,
5. Spatial dynamics of spore-feeding beetles

the results of our take-off experiment are similar to those of Jonsson (2003), who studied two
tenebrionid beetles in a field take-off experiment and found that the hypothesized inferior
colonizer did not take off at all. Colonization patterns of beetles on polypore sporocarps are
affected not only by interpatch distance and habitat-matrix characteristics (Jonsell et al. 1999)
but also by microhabitat preferences of beetles (Komonen and Kouki 2005, Komonen 2008).
These factors were not controlled for in our study and could have biased our findings against
detection of spatial synchrony.

I acknowledge that observational studies on local dynamics or patch dynamics
provide weak evidence without experimental manipulation. In the present study, the local
dynamics of competition with Z. maculifer and Holopsis sp. 2 by Holopsis sp. 1 are based on
simplified assumptions, and experimental studies are obviously required for direct
demonstration of interspecific competition. In addition, the model-fitting necessarily ignores
immigration by adults. For example, the intrinsic rate of population growth in Holopsis sp. 2
was unrealistically negative despite its actual persistence, suggesting that immigration by
adults might rescue Holopsis sp. 2 from extinction. However, one can nonetheless learn much
from the simultaneous examination of local dynamics, extinction-colonization dynamics,
spatial synchrony, and patch attributes. Modelling local dynamics plays an integral part in
understanding the competitive rank order and the network of competitive interactions, and
analyses of patch attributes showed that patch size operates as an axis of spatial niche
partitioning. The combined evidence from the extinction-colonization data and persistence
time found not only transient coexistence but also that local competitive displacement may
occur slowly and inexorably.

My study offers the first evidence for CC dynamics in insect communities on long-
lived bracket fungi. The results clearly contrast with those from mushroom-feeding insect
communities that compete for ephemeral resources and can coexist by spatial aggregation:
spatial aggregation of insect competitors can provide probabilistic spatial refuge for an inferior competitor, where intraspecific competition outweighs interspecific competition (Shorrocks et al. 1979, Toda et al. 1999, Takahashi et al. 2005). In ephemeral mushrooms, competitive interactions at the level of sporocarps are so ephemeral as to persist for only a single generation of insects, whereas perennial bracket fungi allow multiple generations to persist on a sporocarp and to compete with each other until an inferior competitor is excluded. In addition, many mycophagous dwell inside sporocarps, eventually demolishing them, and are likely to be food-limited (Kadowaki 2010), whereas spore-feeding insects are exposed, can range only over a restricted area of hymenial surface, and are likely to be space-limited. Such different persistence of a resource patch and distinct modes of competition might result in, for example, a difference in the effects of patch size on competitive coexistence: in contrast to the probabilistic spatial refuge for inferior competitors offered by large patches with low competitor density in ephemeral mushrooms (Toda et al. 1999), my results suggest that small patches offer deterministic spatial refuge for an inferior competitor in long-lived bracket fungi, where a trade-off between competition and colonization ability drives dynamics. Such cross-system comparisons may therefore extend our ability to understand complicating biological factors that drive empirical patterns to deviate from those predicted by simple CC dynamics.

The present study demonstrated that competitive coexistence of the spore-feeding beetles occurred not solely but in large part through two types of spatial niche partitioning, each of which explains the pairwise coexistence of competitors but not community-wide coexistence. Much of the debate about spatial coexistence has been directed at how metacommunity structure modifies local dynamics, but further work is necessary to reveal how local dynamics build up to influence metacommunity dynamics. Importantly, future studies must predict what kind of factors (e.g., species’ traits) are related to the types of CC
5. Spatial dynamics of spore-feeding beetles

dynamics and must investigate parameters that control local population dynamics and the network of local competitive interactions to elucidate long-term impacts of CC trade-offs on species coexistence. Such approaches, more broadly, offer a crucial step toward unravelling the mutual feedbacks between local dynamics and the metacommunity structure.
5. Spatial dynamics of spore-feeding beetles

Appendix 5-1. Wind tunnel experiment

Figure A5-1. Spatial arrangements of the working station of the wind tunnel used for take-off experiments. Wind blows in the direction indicated by bold arrows. A voltage-regulated fan forced air from the room through glass-fiber and charcoal filters into the tunnel. Two metal screens (1 mm) were used to reduce the turbulence and smooth the flow before entering the working section of the tunnel. The first metal screen was perforated with 10-mm-diameter holes; the second, with 5-mm-diameter holes. The downwind end of the tunnel was closed with grey mosquito netting curtain. Temperature was set to 22 ± 2 °C; relative humidity 36 ± 2 %; light intensity 12.1–12.9 lux.

Figure A5-2. A photograph of the wind tunnel.
Appendix 5-2. The assumptions behind local beetle dynamics analysis

I used larval density for estimating population parameters. Logically, larval density can decrease not only by competition but also by leaving the patch for pupation. If most of the extinction events of larval populations occurred because of the generation gaps, then my estimates may not reflect the effects of competition. However, I think the possibility of this is very small. Although the egg-to-pupa development time of *Z. maculifer* is about four weeks (Kadowaki et al. 2011, Chapter 3), beetles tended to breed repeatedly within the patch in overlapping generations as long as a patch was continually suitable. Furthermore, larval populations are not directly affected by density-dependent emigration that could potentially occur in adult populations. Therefore, larval density can be used as an advantageous indicator of patch-level populations for modelling local dynamics, even though there are several crucial ecological factors that were not included in the models (e.g., the age structure of populations, and recruitment of adults).

I initially had no knowledge of the carrying capacity of a patch, so I explored which factor (spore density per unit area or patch size) operated better as an indicator of carrying capacity. I found that patch size always gave a better fit to the model, rather than highly fluctuating spore density (patch productivity) (Table A5-1).
5. Spatial dynamics of spore-feeding beetles

Table A5-1. Six fitted working models of local spore-feeding beetle dynamics. See Table 5-2 for model formulae.

(a) *Zearagytodes maculifer*

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameter</th>
<th>Coefficient</th>
<th>SE</th>
<th>(t)</th>
<th>(P)</th>
<th>Residual deviance</th>
<th>df</th>
<th>AIC</th>
<th>(\Delta)AIC</th>
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5. Spatial dynamics of spore-feeding beetles

\[
\begin{align*}
\hat{\alpha}_{h1} & = -0.321 \\
\hat{\alpha}_{h2} & = -0.682 \\
\hat{\beta}_{h11} & = 0.000062 \\
\hat{\beta}_{h20} & = 0.00396
\end{align*}
\]

(b) *Holopsis* sp. 1

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<tr>
<th>Model</th>
<th>Parameter</th>
<th>Coefficient</th>
<th>SE</th>
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<th>Residual deviance</th>
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<td>0.0011</td>
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5. Spatial dynamics of spore-feeding beetles

\[
\begin{align*}
r_{h1}a_{h2} & \quad -0.546 & 0.715 & -0.764 & 0.448 \\
r_{h1}b_{h1} & \quad 0.000076 & 0.000074 & 1.022 & 0.311 \\
\end{align*}
\]

6

\[
\begin{align*}
r_{h1} & \quad 0.141 & 0.109 & 1.294 & 0.200 & 341.57 & 63 & 147.33 & 7.09 \\
r_{h1}a_{h2} & \quad -1.929 & 1.316 & -1.463 & 0.148 \\
r_{h1}b_{h1} & \quad 0.089 & 2.398 & 0.037 & 0.970 \\
r_{h1}b_{zz} & \quad 0.006 & 0.005 & 1.164 & 0.249 \\
r_{h1}b_{h2} & \quad -0.0032 & 0.014 & -0.227 & 0.822 \\
\end{align*}
\]

(c) Holopsis sp. 2

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameters</th>
<th>Coefficient</th>
<th>SE</th>
<th>( t )</th>
<th>( P )</th>
<th>Residual deviance</th>
<th>df</th>
<th>AIC</th>
<th>( \Delta \text{AIC} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( r_{h2} )</td>
<td>-0.440</td>
<td>0.439</td>
<td>-1.002</td>
<td>0.323</td>
<td>121.02</td>
<td>34</td>
<td>156.85</td>
<td>11.56</td>
</tr>
<tr>
<td></td>
<td>( r_{h2}a_{h2} )</td>
<td>-0.764</td>
<td>0.731</td>
<td>-1.045</td>
<td>0.304</td>
<td>90.131</td>
<td>34</td>
<td>145.94</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>( r_{h2}b_{h2} )</td>
<td>0.085</td>
<td>0.114</td>
<td>0.744</td>
<td>0.462</td>
<td>90.131</td>
<td>34</td>
<td>145.94</td>
<td>0.65</td>
</tr>
<tr>
<td>2</td>
<td>( r_{h2} )</td>
<td>1.829</td>
<td>0.925</td>
<td>1.978</td>
<td>0.056</td>
<td>90.131</td>
<td>34</td>
<td>145.94</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>( r_{h2}a_{h2} )</td>
<td>-2.898</td>
<td>0.807</td>
<td>-3.592</td>
<td>0.001</td>
<td>90.131</td>
<td>34</td>
<td>145.94</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>( r_{h2}b_{h2} )</td>
<td>0.118</td>
<td>0.450</td>
<td>0.262</td>
<td>0.795</td>
<td>90.131</td>
<td>34</td>
<td>145.94</td>
<td>0.65</td>
</tr>
<tr>
<td>3</td>
<td>( r_{h2} )</td>
<td>-0.678</td>
<td>0.579</td>
<td>-1.171</td>
<td>0.250</td>
<td>120.08</td>
<td>34</td>
<td>156.56</td>
<td>11.27</td>
</tr>
<tr>
<td></td>
<td>( r_{h2}a_{h2} )</td>
<td>-0.056</td>
<td>0.069</td>
<td>-0.810</td>
<td>0.424</td>
<td>120.08</td>
<td>34</td>
<td>156.56</td>
<td>11.27</td>
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<tr>
<td></td>
<td>( r_{h2}b_{h2} )</td>
<td>0.0059</td>
<td>0.0068</td>
<td>0.862</td>
<td>0.395</td>
<td>120.08</td>
<td>34</td>
<td>156.56</td>
<td>11.27</td>
</tr>
<tr>
<td>4</td>
<td>( r_{h2} )</td>
<td>-0.677</td>
<td>0.372</td>
<td>-1.818</td>
<td>0.078</td>
<td>79.47</td>
<td>32</td>
<td>145.29</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>( r_{h2}a_{h2} )</td>
<td>0.291</td>
<td>0.703</td>
<td>0.414</td>
<td>0.682</td>
<td>79.47</td>
<td>32</td>
<td>145.29</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>( r_{h2}b_{h2} )</td>
<td>0.714</td>
<td>0.241</td>
<td>2.961</td>
<td>0.006</td>
<td>79.47</td>
<td>32</td>
<td>145.29</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>( r_{h2}b_{h2} )</td>
<td>-0.127</td>
<td>0.044</td>
<td>-2.890</td>
<td>0.007</td>
<td>79.47</td>
<td>32</td>
<td>145.29</td>
<td>0.00</td>
</tr>
</tbody>
</table>
5. Spatial dynamics of spore-feeding beetles

\[
\begin{array}{cccccc}
& r_{h2} & r_{h2a_h22} & r_{h2a_h22} & r_{h2h_{h2h}} & r_{h2h_{h2h}} \\
5 & -0.013 & 0.007 & -2.037 & 0.050 & \\
& -0.981 & 0.437 & -2.247 & 0.031 & 95.7 & 33 & 150.16 & 4.87 \\
& -0.235 & 0.684 & -0.344 & 0.733 & \\
& 0.144 & 0.105 & 1.367 & 0.181 & \\
& -0.010 & 0.003 & -2.955 & 0.006 & \\
6 & -0.146 & 0.480 & -0.305 & 0.762 & 108.18 & 32 & 156.7 & 11.41 \\
& -328.000 & 148.000 & -2.218 & 0.034 & \\
& 0.065 & 0.273 & 0.236 & 0.815 & \\
& 0.012 & 0.006 & 1.949 & 0.060 & \\
& 0.0000057 & 0.000079 & 0.072 & 0.943 & \\
\end{array}
\]

Null deviances are; *Zearagytodes maculifer*, 316.81 on 163 df; *Holopsis* sp. 1, 366.79 on 68 df; *Holopsis* sp. 2, 136.034 on 37 df.
5. Spatial dynamics of spore-feeding beetles

An alternative approach to establishing competitive relationships of beetles would be to use model 2 as a general-purpose model that was the best or second-best for all three species. This makes comparisons of parameter estimates more robust, since the competitive effects of *Holopsis* sp. 1 in Fig. 5-4. must have been underestimated (i.e. $a_{ij}$ is the coefficient to log ($N_j$) in *Holopsis* sp. 1 whereas $N_j$ in *Z. maculifer* and *Holopsis* sp. 2). However, I prioritized to choose the most parsimonious models in each species in order to avoid missing any biologically meaningful components in model selection. Even though the presence of any parameter is treated equally in penalizing candidate models, some components like $r$ (intrinsic population growth rate) or the strength of density dependence obviously have more theoretical significance than other competition terms when inferring the competitive network of species based on population dynamics. This is perhaps a conceptual problem that occurs when linking population dynamics models and model selection approaches; for example, *a priori* weighting the selection of specific terms in models may be controversial.
Appendix 5-3. Detailed results of patch attribute analysis

Table A5-2. Effects of (a) patch productivity, (b) patch size on population densities of the three spore-feeding beetles. Patch size is the total pore surface area of a sporocarp, and patch productivity is per area spore density, and both are ln-transformed before analysis. The plot includes two levels: Cascade Kauri (baseline) and Upper Huia Dam.

(a) Patch productivity

<table>
<thead>
<tr>
<th>Species</th>
<th>Variable</th>
<th>Coefficient</th>
<th>SE</th>
<th>t</th>
<th>P</th>
<th>Residual deviance</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z. maculifer</td>
<td>Intercept</td>
<td>0.720</td>
<td>1.587</td>
<td>0.454</td>
<td>0.655</td>
<td>19.5</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Patch productivity</td>
<td>0.133</td>
<td>0.103</td>
<td>1.286</td>
<td>0.212</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patch prod. × Plot</td>
<td>−0.009</td>
<td>0.024</td>
<td>−0.376</td>
<td>0.711</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Holopsis sp.

<table>
<thead>
<tr>
<th></th>
<th>Intercept</th>
<th>10.700</th>
<th>6.764</th>
<th>1.585</th>
<th>0.139</th>
<th>17.5</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Patch productivity</td>
<td>−0.249</td>
<td>0.413</td>
<td>−0.604</td>
<td>0.557</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patch prod. × Plot</td>
<td>−0.180</td>
<td>0.037</td>
<td>−4.828</td>
<td>0.0004</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Holopsis sp.

<table>
<thead>
<tr>
<th></th>
<th>Intercept</th>
<th>2.511</th>
<th>3.685</th>
<th>0.681</th>
<th>0.506</th>
<th>23.1</th>
<th>15</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>Patch productivity</td>
<td>−0.075</td>
<td>0.220</td>
<td>−0.341</td>
<td>0.738</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patch prod. × Plot</td>
<td>−0.007</td>
<td>0.036</td>
<td>−0.207</td>
<td>0.839</td>
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<td></td>
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</tbody>
</table>

(b) Patch size

<table>
<thead>
<tr>
<th>Species</th>
<th>Variable</th>
<th>Coefficient</th>
<th>SE</th>
<th>t</th>
<th>P</th>
<th>Residual deviance</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z. maculifer</td>
<td>Intercept</td>
<td>1.405</td>
<td>0.468</td>
<td>3.00</td>
<td>0.007</td>
<td>15.2</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Patch size</td>
<td>0.777</td>
<td>0.511</td>
<td>1.521</td>
<td>0.143</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patch size × Plot</td>
<td>0.317</td>
<td>0.370</td>
<td>0.856</td>
<td>0.402</td>
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</tbody>
</table>

Holopsis sp.

<table>
<thead>
<tr>
<th></th>
<th>Intercept</th>
<th>1.255</th>
<th>1.047</th>
<th>1.199</th>
<th>0.254</th>
<th>22.4</th>
<th>12</th>
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<tr>
<td>1</td>
<td>Patch size</td>
<td>4.287</td>
<td>1.016</td>
<td>4.218</td>
<td>0.001</td>
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</tr>
<tr>
<td></td>
<td>Patch size × Plot</td>
<td>−2.591</td>
<td>0.772</td>
<td>−3.355</td>
<td>0.006</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Holopsis sp.

<table>
<thead>
<tr>
<th></th>
<th>Intercept</th>
<th>−0.663</th>
<th>0.718</th>
<th>−0.924</th>
<th>0.370</th>
<th>15.98</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1/Patch size</td>
<td>3.098</td>
<td>1.273</td>
<td>2.433</td>
<td>0.028</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/Patch size × Plot</td>
<td>−0.937</td>
<td>0.518</td>
<td>−1.809</td>
<td>0.091</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Chapter 6 No evidence for a *Ganoderma* spore dispersal mutualism in an obligate spore-feeding beetle *Zearagytodes maculifer*

PLATE 6. Moire reserve, the Waitakere City (study site, left) and the dead logs with *Ganoderma* sporocarps (right) used for experimental study (*Photos: K. Kadowaki*).
6. Spore dispersal mutualism

**Introduction**

Over the past century fungus–insect interactions have fascinated biologists for their ecological diversity and complexity. In terms of ecological diversity, a diverse range of phylogenetic groups are involved from mushroom-feeding beetles and flies (Hanski 1989), leaf-cutting ants and their fungal gardens (Bass and Cherrett 1995; Mueller et al. 2001), Scolytinae and Platypodinae weevils and ambrosia fungi in their mycangia (Batra 1963; Kajimura and Hijii 1992), wood wasps (Chrystal 2009), termites and sclerotium-forming fungi that mimic termite eggs (Matsuura et al. 2000; Matsuura 2006) and ambrosia galls (Bissett and Borkent 1988). Their ecological complexity arises out of their “interaction types” (antagonists, neutralists, mutualists), with varied “fidelity” in their associations (obligate vs. facultative) (Cooke 1977). While there is a rich body of evidence for “fidelity” in fungus–insect associations (e.g., diffusive, nested, compartmentalized associations; Lawrence 1973; Ståhls et al. 1989; Leschen 1990; Orledge and Reynolds 2005; Epps and Arnold 2010), in contrast, “interaction type” has been largely anecdotal and unexplored in fungus–insect studies, mainly because quantifying the extent to which insects influence fungal fitness is often challenging. Consequently, little is understood about specific ecological costs and benefits that drive fungus–insect interactions.

Fungal spore dispersal by insects is analogous to seed dispersal or pollination mutualisms in plant–animal interactions (Fulton 1889; Ingold 1953; Parker and Bultman 1991). Insects transport fungal spores or spermatia in return for nutritional rewards of gleba and fungal nectar and/or for mycelium to feed their offspring, while phytophagous animals disseminate seeds or cross-pollinate flowers for rewards of fruits, pollen and/or nectar. *Phorbia phrenione* (Séguy) (Anthomyiidae) adult flies “pollinate” plant-pathogenic ascomycete fungi *Epichloë* (Fr.) Tul. and C. Tul. by transporting spermatia between self-incompatible fungal stromata, and subsequent fly larvae consume perithecia that produce
6. Spore dispersal mutualism

fungal propagules (i.e., ascospores) (Parker and Bultman 1991; Bultman et al. 1995). Another example of insect-mediated transfer of sexual spores was documented in rust fungi *Puccinia graminis* Pers. (Craigie 1927) and *P. monoica* Arth. (Roy 1993). On the other hand, there are numerous examples of asexual spore dispersal studies in fungus–insect literature (i.e. “seed dispersal” type studies; Ingold 1953; Harrington 1980; Vega and Blackwell 2005). For example, bark beetles may serve as vectors for the dispersal of spores and mycelia, thus enhancing colonisation of the cambium by the fungal partner (Paine et al. 1997; Six et al. 2003; Harrington 2005).

An insect is a mutualist to a fungus if the insect consumes some spores and disperses others and the consequence is that the fitness of that fungus is higher than it would have been in the absence of the interaction with the insect. Insect-mediated spore dispersal consists of the following stepwise processes: (1) attraction of potential insect vectors, (2) transportation of spores into new habitats or substrates, and (3) successful spore germination. Previous studies have often focused either on the first or second process, whereas the third step of spore germination has rarely been examined by experimentation (Table 1) and I highlight the more important studies here. Fulton (1889) was perhaps the first to experimentally demonstrate that the passage of spores of the stinkhorn *Phallus impudicus* L. through the digestive canal of the insects does not interfere with their viability. Gordon (1938) and Talbot (1952) showed that passage through digestive tracts of arthropods might delay spore germination in the rust fungus *Ustilago avenae* (Pers.) Rostr. and wood decay fungus *Serpula lacrymans* (Wulfen) J. Schröt., respectively. More recently, Tuno (1998) demonstrated that stinkhorn *Dictyophora duplicata* Fisch (Phallaceae) spores consumed by gleba-feeding flies (Drosophilidae and Muscidae) retain the equivalent level of germination capability as controls.
6. Spore dispersal mutualism

**Table 6-1. Spore dispersal mutualism studies in the fungus–insect literature.**

<table>
<thead>
<tr>
<th>Year</th>
<th>Author(s)</th>
<th>Description</th>
<th>Types</th>
<th>Steps</th>
</tr>
</thead>
<tbody>
<tr>
<td>1616</td>
<td>Hadrianus Junius</td>
<td>Observation on <em>Phallus impudicus</em> attracting flies via gleba</td>
<td>S</td>
<td>1</td>
</tr>
<tr>
<td>1760</td>
<td>Schäffer</td>
<td>Observation on <em>Phallus</em> attracting flies</td>
<td>S</td>
<td>1</td>
</tr>
<tr>
<td>1872</td>
<td>Giraud</td>
<td>Observation of insect attraction by plant-pathogen <em>Epichloë</em></td>
<td>P</td>
<td>1</td>
</tr>
<tr>
<td>1875</td>
<td>Cooke and Berkeley</td>
<td>First proposal of spore dispersal mutualism hypothesis (in <em>Phallus</em> [=<em>Phalloides]</em>)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1889</td>
<td>Fulton</td>
<td><strong>First experimental demonstration of <em>Phalloides</em> spore germination via fly excrement</strong></td>
<td>S</td>
<td>1, 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Observation on deliquescent <em>Coprinus fomentarius</em> attracting flies</td>
<td>S</td>
<td>1</td>
</tr>
<tr>
<td>1927</td>
<td>Craigie</td>
<td>Discovery of sexual reproduction of rust fungi and attraction of insects for transferring sexual spores in <em>Puccinia graminis</em>. Proposal of fungal “pollination” mutualism hypothesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1938</td>
<td>Gordon</td>
<td>Demonstrated that passage through the digestive tract of a beetle, <em>Cartodere filum</em>, delays spore germination of <em>Ustilago avenae</em></td>
<td>S</td>
<td>3</td>
</tr>
<tr>
<td>1952</td>
<td>Talbot</td>
<td>Demonstrated that passage through the digestive tract of woodlice delays spore germination of <em>Merulius lacrymans</em></td>
<td>S</td>
<td>3</td>
</tr>
<tr>
<td>1980</td>
<td>Harrington</td>
<td>Rejected spore dispersal hypothesis by insects in <em>Cryptoporus volvatus</em></td>
<td>S</td>
<td>2</td>
</tr>
<tr>
<td>1982</td>
<td>Nuss</td>
<td>Discovery of two spore types in <em>Ganoderma</em> in relation to its germination strategy Proposal of spore dispersal mutualism hypothesis in <em>Ganoderma</em>–flies system</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1991</td>
<td>Parker and Bultman</td>
<td><strong>Discovery of an obligate “pollination” mutualism in <em>Epichloë</em>–flies system. Fly adults transfer sexual spores while offspring feed on perithecia</strong></td>
<td>P</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>1993</td>
<td>Roy</td>
<td>Experimental demonstration of insect-mediated transfer of sexual spores in <em>Puccinia monoica</em></td>
<td>P</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>1998</td>
<td>Tuno</td>
<td><strong>Support for insect-mediated spore dispersal by a spore germination experiment in <em>Dictyophora</em> and flies</strong></td>
<td>S</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>1999</td>
<td>Tuno</td>
<td>Suggestive evidence for spore transfer of <em>Ganoderma applanatum</em> by drosophilid flies</td>
<td>S</td>
<td>1, 2</td>
</tr>
<tr>
<td>2005</td>
<td>Lilleskov and Bruns</td>
<td>Seedling inoculation demonstrated the germination capability of <em>Tomentella subilacina</em> spores consumed by a millipede <em>Harpaphe haydeniana</em></td>
<td>S</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>2010</td>
<td>Nakamori and Suzuki</td>
<td>Observation of broken spores by a collembolan, <em>Ceratophysella denisana</em></td>
<td>S</td>
<td>1, 2, 3</td>
</tr>
</tbody>
</table>

Bold indicates studies with strong experimental evidence, rather than observational, indirect or circumstantial evidence. S = the study of “seed dispersal” type mutualisms, whereby insects engage in disseminating asexual spores; P = the study of “pollination” type mutualisms, whereby insects cross-fertilize sexual spores. The three steps in insect-mediated spore dispersal are: (1) insect attraction (S and P); (2) transfer of spores by insects (S and P); (3) successful spore germination.
6. Spore dispersal mutualism

germination in new habitats (S) or cross-fertilization of sexual spores (P). I limited myself to the system of mushroom-forming fungi, and therefore some insect groups (leaf-cutting ants, termites, and ambrosia beetles) were excluded from the list.
6. Spore dispersal mutualism

Wood-decaying basidiomycete *Ganoderma* has been said to employ insect-mediated spore dispersal (Nuss 1982; Tuno 1999). Numerous mycologists attest to the difficulty of germinating *Ganoderma* spores (e.g., Aoshima 1954; Bazzalo and Wright 1982; Adaskaveg and Gilbertson 1986). Spore germination rates of *Ganoderma* varied greatly among studies, ranging from less than 0.5% (*G. lucidum* (Curtis) P. Karst), 8−15% (*G. lucidum*; Lu and Chang 1975), to 60−90% (*G. orbiforme* (Fr.) Ryvarden; Ho and Nawawi 1986). Nuss (1982) reported the formation of two types of basidiospores in *G. lucidum*. Smaller proteospores with thinner walls, formed at the beginning of sporulation period, germinate readily (see also Lu and Chang 1975; Chen and Hu 1995). In contrast, spores formed later by the same fruiting body have thicker walls and must first pass through a fly larva gut before germination (Nuss 1982). Tuno (1999) showed that *G. applanatum* spores retain viability even after having passed through the digestive tracts of *Mycodrosophila* Oldenburg.

In this work, I studied an obligate spore-feeding beetle, *Zearagytodes maculifer* Broun (Leiodidae: Coleoptera: Insecta), and its host basidiomycete, *Ganoderma* P.Karst (Ganodermatacea: Basidiomycota), in New Zealand. I performed a spore germination experiment using a factorial design over two independent pairs of sporocarp–beetle populations in Auckland, New Zealand. I addressed three questions. Firstly, does the germination rate of *Ganoderma* spores increase if they are consumed by the beetle? Secondly, does germination rate differ across sporocarps? Thirdly, does temperature affect germination rate, and are there any interactive effects with beetle feeding?

**Material and methods**

**Study organism**

The endemic spore-feeding beetle *Zearagytodes maculifer* is a locally abundant and regionally widespread species on wood-decaying bracket fungi in the genus *Ganoderma* spp. in New Zealand (Kadowaki et al. 2011, Chapter 3). Larvae develop in 4 weeks feeding on
6. Spore dispersal mutualism

spores, and reproduction is aseasonal (Kadowaki et al. 2011). The average number of spores consumed by a larva in its life is estimated to be $7.87 \times 10^4 - 2.98 \times 10^5$ (range) (Kadowaki et al. 2011).

*Ganoderma* cf. *applanatum* (Ganodermataceae: Basidiomycota) is the most common host basidiomycete for *Z. maculifer*. *Ganoderma* produces perennial sporocarps (Buchanan and Wilkie 1995) that produce an astronomical number of spores in a year; mean spore release (day$^{-1}$ cm$^{-2}$ pore surface area) varied from $1.22 \times 10^7$ at minimum to $4.31 \times 10^7$ at maximum and the monthly total spore release amounted to $1.32 \times 10^{10}$ spores for an intermediate-sized sporocarp (pore surface area 195.7 cm$^2$) (Kadowaki et al. 2010, Chapter 2). Spores are uniquely double-walled with an outer thin cell wall (pellicle) and an inner thick and rigid cell wall, typically ovoid, echinulate, and enlarged or truncated at the apex, and measures 9.0–11.0 × 6.0–7.5 µm (Mims and Seabury 1989; Buchanan and Wilkie 1995). Spores germinate readily but in low numbers from fresh spore prints on malt extract agar at ambient conditions (Buchanan and Wilkie 1995), but the germination rate has not been quantified.

**Experimental design and procedures**

I designed a spore germination experiment to study the effects of beetle consumption (control or beetle treatment) and incubation temperature (20, 25, and 30°C) in 2 × 3 factorial experiments, replicated five times, using two independent beetle–sporocarp populations in an array of 60 experimental cultures.

Spores were collected by attaching a plastic plate (3.0 × 4.0 cm) underneath two sporocarps for 24 h in the suburban Moire Reserve in Auckland (36°49'31–33" S, 174°37'48–58" E) from 9 to 10 Feb 2010. The plate was washed with 70% ethanol beforehand to sterilize its surface, and the hymenial surface was gently brushed to remove
6. Spore dispersal mutualism

faecal pellets adhering to it. These spores were used as a control, and also as food for beetles in the experiment. *Zearagytodes maculifer* adults were also collected from each of the two sporocarps of *Ganoderma cf. applanatum* on 9 February 2010. Beetle populations from the two sporocarps were kept separately in plastic containers, supplied with a piece of damp paper towel for 24 h under ambient conditions to clean out their guts. Then, 30 mg of spore mass was provided for each individual on a filter paper in a clean Petri dish (diameter = 3.0 cm, height = 1.5 cm) for 24 h.

The beetle specimens were first dipped into 70% ethanol for 10 s to sterilize their bodies, rinsed in sterilized water (hereafter, water) for 30 s, and then dissected in water under a microscope (×10) to remove the rectum. The extracted recta from 18 individuals from each beetle population were pooled, and gently squeezed for 5 min by a pestle in 1 ml water in Eppendorf tubes with enough pressure to break apart tightly packed *Ganoderma* spores in dissected larval digestive tracts. This preparation method involves a dilemma in examining insect impacts on spores, since it is technically impossible to make a well-mixed and replicated suspension without crushing digestive tracts potentially as well as the spores inside. However, microscopic examinations showed that the potential physical impacts of the preparation on spores are small (Appendix 6-1).

Ideally, one could use beetle faecal pellets for the treatment in experiments. A pilot study showed (1) that the addition of benomyl (0.4 g Benlate® and 20 ml acetone) to media successfully suppressed potential ascomycete contaminants that could have covered the entire culture and prevented me from examining *Ganoderma* spore germination; (2) benomyl did not influence *Ganoderma* spore germination rate itself, although it slowed *Ganoderma* mycelial growth to some extent; and (3) that the spores in faecal pellets exhibited such a high level of fungal and bacterial contamination that even benomyl could not readily control them, while spores from digestive tracts were relatively cleaner. Therefore, I used benomyl–malt
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medium, which consists of 6.25 g malt extract, 10.00 g agar, 500 ml water, and benomyl solution as above.

For controls, spores were directly scraped from each plastic plate by forceps and 20 mg of spore mass was suspended into 1 ml water in Eppendorf tubes. Thirty microlitres of the homogenized suspension were inoculated on medium in each Petri dish (diameter = 90 mm, height = 15 mm) replicated five times, over a total of 60 cultures. All the procedures except beetle dissection were conducted inside a laminar flow cabinet. Cultures were incubated in the dark at 20, 25 and 30°C, and were checked for germination rates on 1, 2, 4, 8, 12 days after inoculation.

Spore germination rates were estimated under a microscope (×100) by directly counting the number of germinating spores among 300 randomly selected spores. Direct counting of germinating spores is more labour intensive but more accurate than counting the number of colonies on cultures. *Ganoderma* spores were distinguished from any contaminant spores by spore size and shape. Spore densities in the source suspensions were estimated by haemocytometer (Neubauer, Hawksley, London), replicated five times and averaged.

**Statistical analysis**

I performed logistic regression analysis to test for the effects of beetle feeding, temperature and sporocarps on mean spore germination rates. The averaged spore germination rates across five replicates were rounded and used as a binomial response variable. Explanatory factors considered were the main effects of treatment (control vs. beetle sample), temperature (transformed into −1, 0, and 1 for 20, 25, and 30°C), sporocarps (transformed into 0, 1), and their second- and third-order interaction terms. The day effect (1, 2, 4, 8, 12 days) was used as an offset term that influences only intercepts, assuming that spore germination rates inherently increase over time. The most parsimonious model was selected by backward
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elimination of all those variables from the full model based on Akaike’s information criterion (AIC). Statistical analyses and graphics were performed in R (R Development Core Team 2010).

**Microscopic analysis**

I compared the external and internal ultrastructure of *Ganoderma* spores between control and egested spores by beetles using scanning electron microscopy (SEM) and transmission electron microscopy (TEM), respectively. Spores directly collected from the field and those in beetle faecal pellets that were not crushed for the germination experiment were prepared using the standard fixation techniques; 2.5% glutaraldehyde for SEM, and 2.5% glutaraldehyde and OsO₄ (osmium tetroxide) fixation for TEM (Mims and Seabury 1989). The SEM samples were dried by a critical point drier (E3000 Series II Critical Point Dryer, Polaron, Watford, UK) and coated with gold. The TEM samples were dehydrated in ethanol (30, 50, 70, 90 and 100%) to acetone, embedded in epoxy resin, and cured at 60°C for 48 h. I cut ultra-thin serial sections 70 nm thick, picked up on copper 200-mesh grids, and stained with uranyl acetate and lead citrate. I observed the outer thin-cell walls (pellicles), inner rigid-cell walls, and the presence of cytoplasm. Samples for microscopic inspection were collected from the same study site on 28 February 2010.

**Results**

I readily confirmed spore germination of *Ganoderma* spores as well as clamp connections on benomyl–malt medium (Fig. 6-1). Spore germination rates were low, ranging from 0.0 to 18.6% when the experiment was terminated after 12 days (Fig. 6-2). Germination started 24–48 h after incubation and germination rates increased rapidly from day 2 (0.12 ± 0.06%, mean ± SE) to day 8 (5.83 ± 2.01%), and reached a plateau at days 8–12 (5.87 ± 2.22%).
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**Figure 6-1.** Germinating *Ganoderma* spores with extending germ tubes. Spores were cultured in benomyl-malt medium. Triangles indicate clamp connections that are formed when two germ tubes from separate spores meet with each other.

Three key results were obtained from the most parsimonious logistic regression model (Table 6-2). Firstly, there was a significant reduction in *Ganoderma* spore germination rates as a result of the beetle’s digestive activity. The large effect size indicates that beetle digestion primarily controlled spore germination, compared with the other factors. Secondly, although the temperature effect was only marginally significant, temperature did influence germination rates, as shown by the significant interaction between temperature and sporocarps. Thirdly, germination rates differed significantly between the two sporocarps.
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Table 6-2. Results of the full model and the most parsimonious logistic regression model testing for the effects of temperature, beetle feeding, and sporocarp identity on mean germination rates of *Ganoderma* spores

<table>
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<th>Coefficient</th>
<th>SE</th>
<th>Z</th>
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</table>

Number of days was used as an offset term. Interaction terms are denoted as ×.

The scanning electron micrographs showed that the outer envelopes of spores (pellicles) were completely removed in faecal spores (Fig. 6-3a–d). A small portion of faecal spores appeared to be cracked or completely broken (Fig. 6-3e, f). The transmission electron micrographs (Fig. 6-4) revealed a clear difference in external spore morphology between intact spores and the spores consumed by beetles. The pellicle of control spores was intact or only partially damaged but the spores retained the full cytoplasm with no cracks on the inner cell walls (Fig. 6-4a), whereas spores egested by beetles no longer possessed pellicles and often had cracks in the inner cell walls (Fig. 6-4b–f). This crack damage caused more than 50% of spores in the beetle faecal spores to significantly lose cytoplasm.
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**Figure 6-2.** Mean (+1 SE) germination rates of *Ganoderma cf. applanatum* spores consumed by *Zearagyctodes maculifer* and control spores at three temperature levels (20, 25, and 30°C) in the two sporocarps (1 and 2) after 12 days.
Figure 6-3. Scanning electron micrographs of intact spores and faecal spores; (a)-(b) intact spores (× 4000, and 8000 respectively); (c)-(f) faecal spores (× 4000, × 8000, × 30000, × 16000). Spores in the faecal pellets were highly damaged (e, f).
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**Figure 6-4.** Transmission electron micrographs of intact and faecal spores; (a) intact spores in controls with full pellicle and cytoplasm (scale bar = 1 µm); (b) broken spores in faecal pellets of beetles (scale = 5 µm); (c) digested spores, some of which contain cytoplasm, while the others are empty husks (scale = 2 µm); (d) digested spores with inner cell walls damaged (triangle indicates a minute crack) and outer thin walls removed (scale = 0.5 µm); (e) and (f) partially broken inner cell walls and the lost cytoplasm outside spores (scale = 1, 2 µm respectively).
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Discussion

The hypothesis that *Z. maculifer* has a mutualistic association with *Ganoderma* cf. *applanatum* was not supported by the experimental analysis, which showed a significant large reduction in germination rate among spores from beetle digestive tracts. Two other causal links were revealed: that the effect of temperature was variable but modulated by the variation between sporocarps, and that spore samples from different sporocarps differed significantly in germination rates. Altogether, I found that *Z. maculifer* is a spore predator on, rather than mutualist with, *Ganoderma* cf. *applanatum*.

The results were in contrast with the spore germination experiment by Tuno (1998), who demonstrated that stinkhorn spores retain spore germination capability even after being digested by generalist mycophagous flies. I found that a specialist spore-feeding beetle *Z. maculifer* suppressed *Ganoderma* spore germination. These contrasting results are unlikely to be explained by differences in spore physical resistance to the digestive activity of insects between stinkhorn and *Ganoderma*, as *Ganoderma* spores have more rigid cell-wall structure than stinkhorn spores (see Tuno et al. 2009). An alternative possibility is differences in the mechanical or chemical properties (e.g., pH, enzymes) of the fly and beetle digestive systems. For example, Lilleskov and Bruns (2005) showed that beetles have a greater ability than flies to damage spores of *Tomentella sublilacina* (Ellis and Holw.) Wakef.

The scanning and transmission electron micrographs showed removal of pellicles, cracks in inner rigid cell walls, and the resultant loss of cytoplasm in digested spores, suggesting that digestive activity by beetles may have a deleterious effect on survival of *Ganoderma* spores. I argue that the loss of cytoplasm caused by beetles is a likely cause for the reduction in *Ganoderma* spore germination rates. It is noteworthy that even a few cracks on the rigid inner cell wall led to a loss of cytoplasm, and therefore spores that appeared only...
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partially damaged under a low resolution binocular microscope (×200–400) could be empty husks.

Apart from Fulton (1889) and Tuno (1998, 1999), few fungus–insect studies have unequivocally demonstrated the effect of insects on fitness-related parameters of fungi. Some suggestive evidence for negative fungivory effect by ciid beetles is reported by Guevara et al. (2000b), who found a negative correlation between Octotemus glabriculus (Gyllenhal) and the functional hymenial surface area of Trametes versicolor (L.:Fr.) Quél. Anecdotal evidence indicates that, for example, the erotylid beetle Gibbifer californicus (Lacordaire) consumes Polyporus adustus (Wild. ex Fr.) to perhaps affect host fitness (Graves and Graves 1966), and the platypezid fly Agathomyia wankowiczi (Schnabl) induces gall formation on the hymenial surface of Ganoderma applanatum (Pers.) Pat. (Kotiranta and Niemelä 1981). I demonstrate by TEM that an obligate spore-feeding beetle decimates Ganoderma spore germination, in contrast to the delaying effects of other arthropods on spore germination (Gordon 1938; Talbot 1952).

The total number of spores consumed by Z. maculifer populations represents a very small percentage (0.1–0.4 %) of the spores discharged, even at the seasonal peak of abundance (Kadowaki et al. 2011, Chapter 3). So does this fact suggest that the effect of Z. maculifer on Ganoderma is neutral? The finding that a fungus beetle can exert a negative impact on spore survival clearly distinguishes myself from the “established” theory that the effects of fungal consumption by flies and beetles are neutral (Cooke 1977; Shaw 1992), no matter how small the relative incidence of the encounter with beetles is. Previous authors have argued for neutral effects simply by observing that the vast majority of spores discharged from a sporocarp are not consumed by insects, without examining the actual effects of insects’ feeding on spores. In this vein, the analogy with plant-herbivore studies can be misleading, because it appears as if a sporocarp were an “individual” of fungal
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populations, rather than a complex of fungal populations, each of which are formed when two germ tubes from spores that have compatible sexes meet with each other. In mutualistic plant-herbivore interactions, gene migration can be mediated through haploid pollen grains in pollination, which is different from the diploid seed movement. Fungal spore dispersal appears to be somewhat an intermediate state between pollination and seed dispersal, in that the destination of spore migration (possibly, dead woods) is not as highly specific as the case of pollination but not as diffuse as seed dispersal. The predation on spores (homokaryotes) on a fungal sporocarp (fungal populations) is distinct from pollen feeding on a flower and seed predator on a plant in unique ways.

An additional finding from my study was that the mean germination rates in controls for *G. cf. applanatum* were much lower than those of Malaysian *G. orbiforme* (Ho and Nawawi 1986), but were similar to *G. lucidum* (Lu and Chang 1975), although the timing of *G. cf. applanatum* may be typical for *Ganoderma* species. For example, *G. orbiforme* germination starts 30 h after incubation on various media (Ho and Nawawi 1986), while *G. cf. applanatum* germination started 24 h after incubation, and increased until 8 days. Increasing temperature decreased spore germination, but its effect may be complex and largely depends on the sporocarps used for study. For example, Ho and Nawawi (1986) showed that warm (25−30°C) temperatures were favourable for spore germination in a tropical species *G. orbiforme*, whereas moderate to high germination rates at 20−25°C were favourable in a temperate species *G. cf. applanatum* in this study. Any temperature effect, however, is likely to be overridden by the effect of beetle feeding.

Variation between sporocarps can be an important factor in spore germination studies, with sporocarps likely having inherently variable germination capability, and variable response to temperature regimes. Kadowaki et al. (2010, Chapter 2) revealed huge spatial variability in the spore release dynamics of *G. cf. applanatum*, and, suggesting that the
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reproduction of *Ganoderma* spp. appears to be highly variable across sporocarps. What causes the variation in spore release and germination capability in terms of fungal reproductive biology is an open question. Lu and Chang (1975) suggested that *G. lucidum* spores formed at the end of the sporulation period had less-well-formed chitin in the inner cell wall, and germinated more readily.

This study marks the first reported experiment to evaluate the relative importance of insect feeding and temperature on spore germination rates. Although I have not directly measured the relative importance of alternative spore dispersal strategies, i.e., wind dispersal vs. insect-mediated dispersal, the results are concordant with the traditional views held by mycologists that wind dispersal plays a primary role in fungal spore dispersal (Ingold 1953). Much can be gained by extending experiments to various insect and fungal taxa, as the experimental method used here would be generally applicable to other basidiomycete–insect communities. Essentially, a mutualistic interaction between a fungus and an insect does not always require increased germination rates after consumption of the fungus by the insect, but higher reproductive success of the fungus in the presence of the insect, even if many of the individual spores die in the process of being consumed. From the fungus’s point of view, the loss of some spores may be an acceptable cost in return for the targeted dispersal of non-ingested spores to suitable habitats. The important future challenges would be to narrow down key dispersal agents by short-term spore germination experiments, and then to estimate the integrated fitness of fungi by removal experiments of those dispersal agents.
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**Appendix 6-1. The effect of the preparation method on *Ganoderma* spores**

I examined the potential physical impact of crushing digestive tracts on *Ganoderma* spores. I made control spores (collected from field, and washed into water) and treatment spores (spores from field that are crushed directly in an Eppendorf tube with a pestle for 5 min), and counted the proportion of damaged spores. Both spore samples were fixed using standard fixing techniques described in the main text. I examined if the direction of bias by crushing coincides with the predicted effect of beetle feeding under test. Note that the treatment by direct crushing considers the ‘worst’ case one can think of in terms of the possible physical damages, since spores in the germination experiments were squeezed indirectly inside digestive tracts. I used $\chi^2$ test to test for the difference in the extent of physical damage between the control and the treatment by quantifying the number of partially or fully damaged over randomly selected 300 spores under scanning electron microscopy ($\times 4000$). The result showed that both control and treatment spores contained partially damaged spores (15.7% and 21.3% respectively). My observation was in accordance with Mims and Seabury (1989) who showed partial removal of pellicles from spores that are simply collected from the field. In the present case, partial removal of pellicles was likely to be caused or amplified in centrifuges that gather spores that tend to instantly disperse in water in the fixing procedure. There was no significant difference in the proportion of spores partially damaged in pellicles ($\chi^2 = 2.2126$, df = 1, p = 0.1369). This suggests that crushing spores in digestive tracts of beetles indirectly by a pestle was unlikely to cause significant impacts on spores than direct crushing as I demonstrated here.
Figure A6-1. Scanning electron micrographs of *Ganoderma* spores. (a) control spores ($\times 4000$), (b) treatment spores ($\times 4000$), (c) control spores ($\times 8000$), (d) treatment spores ($\times 8000$). Spores were equipped with hilar appendixes that were originally attached to basidia, but the pellicles were occasionally stripped.
Chapter 7 General discussion

Plate 7. The kauri-dominated native forest in the Waitakere Ranges, Auckland (top and bottom left), and the saproxylic habitat that fosters diverse insect communities (right) (Photos: K. Kadowaki).
7. General Discussion

This dissertation presents an overall context and several detailed analyses of fungus–insect ecology, all related to factors influential in shaping host fungus–consumer insect interactions, fungus–insect mutualisms, and insect competition in the wood-decaying bracket fungi *Ganoderma* and associated spore-feeding beetles. The wood-decaying basidiomycetes of the genus *Ganoderma* produce perennial fruiting bodies (hereafter sporocarps) that provide habitat for three beetle species, *Zearagytodes maculifer* (Leiodidae) and two *Holopsis* species. Both larvae and adult beetles graze on spores discharged from the hymenial surface. There has been little detailed work on the ecology of spore-feeding beetles globally, and none in New Zealand; thus, I began by describing several aspects of the natural history of the system (Chapter 1: Study System). In this chapter, I briefly summarize the key findings of my research (Chapters 2–6) and place them in a broader ecological context, discuss limitations of the studies so far, and highlight the still unexplored domains of research and priorities for future progress.

**Summary of main findings**

In Chapter 1, I set out to identify trends and knowledge gaps in fungus–insect ecology, which remains somewhat of a mystery in terrestrial ecology. I surveyed 477 published articles and book chapters from 1800 to the present (2010) and examined two main facets of insect–fungal associations: fungal habitat types and focal ecosystem roles. The review identified two major types of insect fungal habitats (ephemeral mushrooms and long-lasting bracket fungi) and five ecosystem roles of fungus–insect communities (host fungus–consumer insect, insect–fungus mutualism, fungus–insect competition, insect competition, and insect predator–prey interactions). Many studies of long-lasting bracket fungus systems have simply observed and described fungus–insect associations without reference to theoretical predictions, whereas studies of ephemeral mushroom systems have been dominated by experimental analysis. Few studies have quantified the effects of insects on fungal fitness, the roles of
competition or trophic interactions on insect population and community dynamics, or the integrated effects of multiple ecosystem roles in shaping fungus–insect communities. The knowledge gaps identified in the coverage of fungus–insect studies highlight two future approaches: quantification of fungus–insect interactions, and a multilateral examination of fungus–insect communities in terms of ecosystem roles.

Chapter 2 investigated how environmental variables shape spore release dynamics and the magnitude of seasonal periodicity and synchrony in the basidiomycetes of the genus *Ganoderma*. I measured spore density and its associated environmental variables for 30 sporocarps of two *Ganoderma* species at monthly intervals for a year in the Waitakere Ranges, New Zealand. Results showed that the rate of spore release per square centimetre of sporocarp was not related to temperature, relative humidity, light intensity, height above ground, or sporocarp size. Model selection revealed a significant but weak seasonal periodicity and no clear spatial synchrony across different sporocarps in spore release by *Ganoderma*. I argue that either the high amplitude in daily fluctuation of spore release overrides any monthly patterns, or undetermined physiochemical processes within the substrate might create what appears to be spatial asynchrony in spore release periodicity.

Chapter 3 examined spore consumption and aspects of the life history of *Zearagytodes maculifer* (Coleoptera: Leiodidae) on the bracket fungi *Ganoderma cf. applanatum* and *G. austral e*. Eggs, the four larval instars, and their durations were described. The 25-day development time (egg to adult) that I observed is short compared to development times of other mycophagous beetles inhabiting bracket fungi. The numbers of larvae and adult beetles found on hosts were only weakly associated with season, and both larvae and adults were active in winter. The rate of spore consumption did not depend on larval instar or host fungus species. However, fewer larvae and adult beetles were found per sporocarp on *G. austral e* than on *G. cf. applanatum*. Ingested spores of *G. austral e* appeared
to be less easily broken. The impact of *Z. maculifer* on host fitness appeared to be neutral, as larvae consumed a very small proportion of discharged spores.

Chapter 4 was dedicated to a little-studied aspect of life history traits in fungus–insect studies: ontogeny. I compared the allometric relationships of larval morphology between two species of sympatric fungus beetle in the genus *Holopsis* (Coleoptera: Cuculoidea): *Holopsis* sp. 1 has a long rostrum that it inserts into pore tubes of bracket fungi, whereas *Holopsis* sp. 2 lacks a long rostrum. Four measurements were taken from 311 field-collected specimens: body length, body width, head size, and rostrum length. I used a new statistical method (normal mixture models) to identify the number of instars (i.e., components) and examined the ontogenetic allometries of *Holopsis* species. Normal mixture models identified four instars in both *Holopsis* species, but *Holopsis* sp. 1 possessed a developmental polymorphism; some individuals possessed a disproportionately longer rostrum and narrower head in the fourth instar, whereas the others had a shorter rostrum and a wider head. Dissection of larval digestive tracts showed that *Holopsis* sp. 1 consumed a small number (2%) of immature spores, whereas *Holopsis* sp. 2 consumed only mature spores. However, I found no effect of rostrum length on the proportion of immature spores in the *Holopsis* sp. 1 gut, suggesting no clear evidence for foraging polymorphism in *Holopsis* sp.1. This study revealed that ontogenetic allometry differed significantly between the two *Holopsis* species, suggesting the potential importance of ontogenetic niche divergence.

Chapter 5 provided a rigorous quantitative analysis of dynamics of the spore-feeding beetle community. I investigated the role of competition–colonisation dynamics in the competitive coexistence of the three beetle species. One species, *Holopsis* sp. 1, was a pore tube specialist, whereas the other two, *Zearagytodes maculifer* and *Holopsis* sp. 2, were surface grazers. I surveyed larval and adult abundance of the three species, daily spore release per square centimetre (patch productivity), pore surface area (patch size), and environmental
variables over 30 sporocarp patches at monthly intervals from 2007 to 2008. I compared flight behaviour and the associated physiological traits of the three competitors in a wind tunnel experiment. A simultaneous examination of (1) local dynamics, (2) patch transitions in local community states and population persistence times, (3) effects of patch attributes, and (4) spatial synchrony from time series data found (1) the competitive equivalence of *Holopsis* sp. 1 to *Z. maculifer*, and the superiority to *Holopsis* sp. 2; (2) a reduced population persistence time of *Z. maculifer* because of the dominance of *Holopsis* sp. 1, possibly via the superior feeding strategy of the latter and the resultant exceedingly high population growth rate; (3) the dominance of *Z. maculifer* and *Holopsis* sp. 1 in larger, longer-lived patches and of *Holopsis* sp. 2 in smaller, shorter-lived patches; and (4) that local populations of *Z. maculifer* had a greater spatial extent than did those of *Holopsis* spp. A take-off experiment showed that *Z. maculifer* flew readily, whereas *Holopsis* spp. never did. The combined evidence indicates that the coexistence of spore-feeding beetles may occur via two types of spatial niche partitioning, each of which explains the pairwise coexistence of competitors, but not the community-level coexistence: *Z. maculifer* evade the competition with *Holopsis* sp. 1 by being a frequent or a strong flier, whereas *Holopsis* sp. 2 may evade competition by partitioning a patch size gradient, as it only dominates in smaller-sized and newly emergent patches until the arrival of *Holopsis* sp. 1.

In Chapter 6, I described an experiment that tested whether the obligate spore-feeding beetle *Zearagytodes maculifer* has a mutualistic relationship with its host bracket fungus *Ganoderma* cf. *applanatum* via spore dispersal. I hypothesized that the *Ganoderma* spore germination rate is increased via beetle digestive activity and is dependent on temperature and sporocarp identity. Spore germination rates were examined in $2 \times 3$ factorial experiments (spores consumed by beetles or not $\times$ temperature, 20, 25, and 30°C), replicated five times, using two independent pairs of sporocarp-beetle populations in an array of 60 experimental
7. General Discussion

cultures. Analysis showed a significant reduction in germination rate associated with beetle feeding. The effect of temperature was modulated by the effect of individual sporocarp and was overridden by the beetle effect. Microscopic analysis revealed that spores from beetle faecal pellets exhibited extensive damage to their thin outer walls (pellicles) and thick inner walls, as well as significant loss of cytoplasm, while control spores were intact. The overall evidence argued against the spore dispersal mutualism hypothesis, suggesting that Z. maculifer can potentially exert a negative, if vanishingly small, fitness effect on its host fungus G. cf. applanatum.

![Diagram](image)

**Figure 7-1.** The causal links revealed in the *Ganoderma* and spore-feeding beetle community. Fig. 1-3 was modified by the main findings in the preceding chapters: very weak host fungus–consumer insect interactions and the predominance of competition among beetles lead to spatial dynamics of the beetle community.

In summary, the multilateral approach provided insights into three key ecosystem roles in the communities of the wood-decaying bracket fungi, *Ganoderma*, and spore-feeding beetles: host fungus–consumer insect interactions, insect–fungus mutualisms, and insect
competition (Fig. 7-1). Spore-feeding beetles have unique lifestyles in various ways. They are surface grazers that are exposed to the external environment on the hymenium, whereas many mycophagous beetles are internal feeders that dwell inside a sporocarp that physically protects them. The three species studied here differed markedly in spore consumption patterns, ontogenetic niches, dispersal behaviour, and population dynamics. Long-lasting bracket fungi offer a spatial arena whereby beetles can make use of only a small proportion of discharged spores, with 99% of discharged spores simply being dispersed by wind. Although there is an extremely abundant resource supply in this tiny ecosystem, the consumer beetles spend much of the day feeding (K.K. pers. obser.), and they compete with each other for limited space on the hymenium surface. The weak responses of the beetles to fluctuations in spore release may indicate that resource levels are generally high enough to sustain the beetles, which require only a small proportion of the discharged spores. All of the available evidence supports weak interactions between host fungi and consumer insects and the principal role of insect competition in shaping the *Ganoderma* and spore-feeding beetle community. Despite the pervasive structuring force of insect competition, species coexistence is likely to occur because *Z. maculifer* and *Holopsis* sp. 1 escape from competition with *Holopsis* sp. 1 via different colonisation strategies. As a strong or frequent disperser, *Z. maculifer* finds empty patches where its competitive equivalent *Holopsis* sp. 1 has not yet arrived; and *Holopsis* sp. 2 is likely to colonise more frequently than *Holopsis* sp. 1, thereby proliferating only in small-sized and shorter-lived sporocarp patches. The three beetle species exploit such subtly different spatial niches to persist in long-lasting and exposed fungal habitats.

These findings have an important implication for the conservation ecology of fungus–insect communities. Past efforts to consider conservation management of insects associated with fungal habitats have been directed to correlative analyses between insect abundance and
environmental variables that point to the importance of the abundance of substrates for fungi and micro- and macro-habitat characteristics. This approach, however, overlooks the role of ecological interactions and the mechanisms that buffer these destabilizing forces of ecological interactions in shaping the current distributions and abundance patterns of insect communities. In the spore-feeding beetle community studied here, competition drives species extinction at the sporocarp level, but the trade-off between competition and colonisation maintains the coexistence of the three beetle species. In such a community, theory predicts that in the face of habitat destruction or random catastrophic disturbance of fungal sporocarps, a superior and abundant competitor would be lost first, accompanied by a substantial time lag, i.e., “extinction debt” (Tilman et al. 1994, Pacala and Rees 1998). The reason that the competitively superior, most abundant species is more vulnerable to extinction is because of its poor colonising ability. It may be somewhat counter-intuitive that the most numerically dominant species, *Holopsis* sp. 1 in this case, would be more vulnerable to habitat destruction than would the rarer species, *Z. maculifer* and *Holopsis* sp. 2. Although I could not determine the spatial scale at which *Holopsis* sp. 1 is dispersal limited, this study offers a cautionary tale that prioritizing the rare and less abundant species (inferior competitors such as *Holopsis* sp. 2 or *Z. maculifer*) as conservation targets in conservation management may lead to extinction of the most abundant species (superior competitors, e.g., *Holopsis* sp. 1). Conservationists may need to consider that numerical dominance can be fleeting in a community controlled solely by competition–colonisation trade-offs (Tilman et al. 1994, Pacala and Rees 1998), and examine not only correlations between abundance and environmental variables but also key ecological factors of competition and coexistence.

**Limitations of the research**

Understanding the importance of competition–colonisation dynamics would have been greatly strengthened by experimental work. Rescue effects via immigration cannot be
understood without the experimental introduction of beetles. For example, my modelling work (Chapter 5) showed that the population growth rate of *Holopsis* sp. 2 was unrealistically negative, suggesting that the current dataset does not account for all relevant factors. It would be interesting to test whether immigration rescues *Holopsis* sp. 2 from extinction at a sporocarp patch. Furthermore, the role of patch size and patch quality in emigration and immigration is not well understood. Interpatch distance may also interact with these patch attributes to influence the metacommunity structure of beetles. Thus, many predictions derived from metapopulation and metacommunity theory remain to be tested. I did not provide rigorous evidence that small sporocarps are always new patches, even though I acknowledge that some sporocarps did not grow much in size during the entire year of study. I could not determine with confidence what constitutes carrying capacity for beetles. The success in using sporocarp size only rather than spore release per centimetre as a measure of carrying capacity in model fitting may be partly due to the key role of space (hymenial surface area) limitation rather than spore density itself in controlling local beetle dynamics. I found that resource levels in general may be high enough to sustain a beetle community that requires only a small portion of discharged spores. Personal observation suggests that spore-feeding beetles spend the vast majority of the day involved in feeding activities, perhaps because spore-feeding is a highly inefficient mode of foraging. Collectively, spore-feeding beetles are likely to be space-limited over the hymenial surface.

Field experiments to measure the colonisation ability of spore-feeding beetles can be logistically prohibitive, as it is extremely labour-intensive to change the spatial arrangement of sporulating fruiting bodies on which beetles can live, and even the translocation of logs with sporocarps can stop spore release, as described below. A more realistic approach would be mark–recapture methods (Nilsson 1997; Starzomski and Bondrup-Nielsen 2002), although working with such small beetles is not easy; alternatively, fluorescent dust marking may
allow investigation of short-term dispersal behaviour (e.g. Corbett and Rosenheim 1996). One could perhaps measure dispersal parameters in flight-mill experiments, even though some fungus beetles are notoriously reluctant flyers (Jonsson 2003), perhaps due to natural selection for infrequent dispersal in highly predictable habitats. Measuring dispersal ability at the right time can be a challenge if the beetles are prepared for reproduction after histolysis (Jonsson 2003). The results of wind tunnel experiments might not have been very relevant to patch colonisation by beetles in my study because none of beetles virtually arrived at a lure of fragments of *Ganoderma* sporocarps. The use of a fresh, living sporocarp would have provided a more realistic attractant to beetles without significantly modifying the chemical composition of volatile emission from *Ganoderma.*

In addition, various aspects of basic life history are missing for *Holopsis* species, and partially for *Z. maculifer:* adult life span, fecundity, larval period, mating and oviposition behaviours, spore consumption rates, and the seasonality of dispersal. These parameters are necessary not only for building realistic local population dynamics models that include factors omitted from my analysis, but also for exploring various types of fitness trade-offs that may affect metacommunity dynamics.

**Challenges in measuring competition**

Two planned experiments did not succeed. First, I aimed to measure the relative importance of exploitative and interference competition among beetle larvae in the laboratory by manipulating the spatial distribution of spore mass in Petri dishes. Prior to the experiment, I attempted to establish methods for rearing spore-feeding beetles by providing larvae with *Ganoderma* spores collected from the field, either on clean damp filter paper or appropriate agar cultures. In both methods, beetle larvae did not feed on spores very much and failed to survive more than 2 weeks. I could not identify the factors that limited the feeding behaviour of the beetles. Collecting living sporocarps from the field was another option; however, the
7. General Discussion

dying sporocarps tended to be covered by mould in two weeks, and then became an unsuitable resource for spore-feeding beetles. Thus, in a third attempt to provide larvae with fresh spores, I used a chainsaw to extract an 8-m-long dead log (in two pieces) that harboured three sporulating *Ganoderma* fruiting bodies from Spragg Bush Walk, the Waitakere Ranges. I transported the two 4-m logs to a small bush fragment at the University of Auckland (Tamaki Campus) using a trailer (Fig. 7-2). The edges of the logs were covered with sphagnum moss to prevent excessive moisture loss and watered with 10 L of tap water every three days. Although all three *Ganoderma* sporocarps remained alive with rejuvenating hyphae, they stopped sporulating after 2 weeks. The changes in microclimate conditions might have triggered the cessation of *Ganoderma* sporulation, as the small bush fragment would have been drier and more exposed to edge effects than the Waitakere Ranges. Another possibility is that fungi are very sensitive to the correct vertical orientation of their pore tubes; my relocation of dead logs might not have simulated the original orientation of *Ganoderma* sporocarps in the Waitakere Ranges. Thus, despite considerable effort, I did not achieve even partial success in establishing adequate conditions for rearing spore-feeding beetles, and was therefore unable to perform laboratory experiments.
Second, I attempted to conduct a field competition experiment by simultaneously introducing two competitor beetles into experimental arenas built on hymenial surfaces. I initially developed a method to set experimental arenas on the hymenial surface of sporocarps (Fig. 7-3). A by-product of this effort was presented in Chapter 4, whereby the larval period of *Z. maculifer* was measured in a field trial. However, the field competition experiment was hindered by the technical difficulty of confining the very minute larvae of *Holopsis* spp. in an experimental arena. Even tightly attached plasticine arenas inevitably created a small gap between the hymenium and the plasticine due to the accumulating effects of daily temperature fluctuations and gravity. Transferred *Holopsis* spp. larvae could not be confined
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to the arena and escaped by crawling over the arena or under small gaps between the plasticine and the hymenia. Not only the small larval size (<1.0 mm in first instar body length), but also such an explorative behaviour of the *Holopsis* spp. larvae prevented me from keeping track of individual larvae in the experimental arenas. When experimenting with larvae proved intractable, I next attempted to introduce adults (both *Zearagytodes* and *Holopsis* spp.) to the arenas to study competition. However, the adults reproduced only occasionally in the artificial arenas, perhaps due to the artificial darkness or modified microclimate created by the mesh. An alternative experimental design would be to use a sporocarp as an experimental unit and to introduce larvae or adults, rather than using highly replicated plasticine arenas; however, due to the limited number of sporocarps in the Waitakere Ranges and the logistical difficulty of moving between an adequate number of replicates, I did not explore this option further. If there had been many replicate sporocarps, it would have been possible to block off access to some of the pore surface area to increase competition for space. Finally, I concluded that it was not possible for me to conduct a field competition experiment.
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Figure 7-3. Experimental arenas used for field competition experiments. These arenas were made of a plastic ring (3 cm diameter) embedded in 10.5 g of plasticine (used to attach the arenas to the rough hymenial surface) and covered with fine fabric mesh (to prevent immigration and emigration of beetles). Artificial factors created by the experimental setup included darker conditions, enhanced humidity inside the arenas, and the accumulation of spores on the mesh, which needed to be replaced regularly (Photo: J. Beggs).

Unfortunately, these two failures, due in large part to limitations of the study organisms (especially Holopsis spp.), resulted in significant changes to the overall structure of the thesis. Rather than focusing on competition and coexistence, I examined various aspects of fungus–insect communities, leading to Chapters 3, 4, and 6. These two failures highlight that a good model system for observational study is not necessarily a good experimental system.
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**Priorities for future study**

The study of fungus–insect ecology has been hampered by the lack of coordinated efforts to exploit the advantages of fungus–insect communities. However, recent theoretical and methodological developments in ecology have improved our ability to identify key ecosystem roles that are most likely to regulate the dynamics of insect communities in fungal habitats. It should be a priority to compare various ecosystem roles between insect communities on ephemeral mushrooms and those on long-lasting bracket fungus systems at multiple levels, ranging from mechanisms that allow insect populations to persist at the sporocarp patch, to the extent to which the dispersal of insect populations affects extinction risks in the region, and eventually, how such factors drive large-scale biodiversity patterns of fungus–insect communities.

The two key approaches addressed in Chapter 1 should continue to be useful to the study of fungus–insect ecology in future decades. First, much work is needed to quantify the potentially diverse impacts of mycophagy on fungal fitness. For example, one could investigate the effects of fungivorous insects on fungal reproductive efforts by examining the biomass of mushroom stipes as a measure of the amount of resources allocated to reproduction by the fungus in mycological cultures (e.g., Schmit 1999). The forms of extant fungus–insect interactions vary in unique ways across systems. It is therefore important to document direct empirical evidence based on case studies, rather than labelling fungus–insect associations using artificial categories (e.g., mycetobionts, mycetophiles; Benick 1952) or qualitative classifications (antagonistic or mutualistic interactions; Cooke 1977).

Second, the multilateral approach provides powerful insights into the study of insect competition and predator–prey interactions. Future studies should combine observational and experimental analyses to examine multiple sources of quantitative information on the types, strengths, and variation of interactions and their effects on long-term ecological dynamics.
7. General Discussion

There is an increasing appreciation of insect diversity in fungal habitats, which has previously been overlooked in conservation ecology (Grove 2002; Komonen 2003). Much of the information gained from the multilateral approach is helpful for conservation management, allowing for accurate predictions and wise choices. Additionally, cross-system comparative analysis is heuristically informative and can have evolutionary implications. A wide range of theories address the influence of temporal stability of fungal habitats on fungus–insect communities explicitly or implicitly, and a meta-analysis of fungus–insect systems that encompasses various subdisciplines may provide novel perspectives on the study of fungus–insect ecology.
Appendix 7-1. Arthropods found on *Ganoderma* spp. in the Waitakere Ranges

Table A7-1. Arthropods found on *Ganoderma* spp. in the Waitakere Ranges. Some of the groups listed below are identified at the species level in Kushel (1990).

<table>
<thead>
<tr>
<th>Order</th>
<th>FAMILY</th>
<th>Species</th>
<th>Ecological category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Araneae</td>
<td>THERIDIIDAE</td>
<td><em>Cryptachaea veruculata</em></td>
<td>Generalist predator</td>
</tr>
<tr>
<td>Araneae</td>
<td>THOMISIDAE</td>
<td><em>Sidymella</em> sp.</td>
<td>Generalist predator</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>ADERIDAE</td>
<td><em>Xylophilus</em> sp.</td>
<td>?</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>CIIDAE</td>
<td><em>Cis</em> spp.</td>
<td>Mycophagous</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>CIIDAE</td>
<td><em>Scolytocis novaezelandiae</em></td>
<td>Mycophagous</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>CLAMBIDAE</td>
<td><em>Clambus</em> sp.</td>
<td>?</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>CORYLOPHIDAE</td>
<td><em>Holopsis</em> sp.</td>
<td>Spore-feeder</td>
</tr>
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<td>ENDOMYCHIDAE</td>
<td><em>Holoparamecus</em> sp.</td>
<td>?</td>
</tr>
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<td><em>Cryptophila</em> sp.</td>
<td>Mycophagous</td>
</tr>
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<td>LATRIDIIDAE</td>
<td><em>Corticariinae</em></td>
<td>Mycophagous</td>
</tr>
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<td>LEIODIDAE</td>
<td><em>Zearagytodes maculifer</em></td>
<td>Spore-feeder</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>MYCETOPOHAGIDAE</td>
<td><em>Triphyllus</em> sp.</td>
<td>Mycophagous</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>STAPHYLINIDAE</td>
<td>ALEOCHARINAE spp.</td>
<td>?</td>
</tr>
<tr>
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<td><em>Scaphisoma hanseni</em></td>
<td>Spore-feeder</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>STAPHYLINIDAE</td>
<td><em>Sepedophilus</em> sp.</td>
<td>?</td>
</tr>
<tr>
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<td>PTILIIDAE</td>
<td></td>
<td>Spore-feeder</td>
</tr>
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<td>Mycophagous</td>
</tr>
<tr>
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<td><em>Ablabus</em> sp.</td>
<td>Mycophagous</td>
</tr>
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<td><em>Bitoma insularis</em></td>
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</tr>
<tr>
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<td>ZOPHERIDAE</td>
<td><em>Pycnomerus</em> sp.</td>
<td>Mycophagous</td>
</tr>
<tr>
<td>Hemiptera</td>
<td>ARADIDAE</td>
<td><em>Woodwardiessa quadrata</em></td>
<td>Mycophagous</td>
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