Evolutionary drivers of temporal and spatial host use patterns in restio leafhoppers *Cephalelini* (Cicadellidae)

By

Willem Johannes Augustyn

Thesis presented in fulfilment of the requirements for the degree of Doctor of Philosophy in the Faculty of Science at Stellenbosch University

Promoters: Allan George Ellis and Bruce Anderson

December 2015

Declaration

By submitting this thesis/dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Abstract

Understanding how divergent selection results in the evolution of reproductive isolation (i.e. speciation) is an important goal in evolutionary biology. Populations of herbivorous insects using different host plant species can experience divergent selection from multiple selective pressures which can rapidly lead to speciation.

Restio leafhoppers are a group of herbivorous insect species occurring within the Cape Floristic Region (CFR) of South Africa. They are specialised on different plant species in the Restionaceae family. Throughout my thesis I investigated how bottom-up (i.e. plant chemistry/morphology of host plant species) and top-down (i.e. predation and competition) factors drive specialisation and divergence in restio leafhoppers. I also investigated interspecific competition as an important determinant of restio leafhopper community structure.

In chapter 2 I quantified host specificity of restio leafhopper species within a local community for 24 months. I found that restio leafhopper species are highly host specific and potentially synchronised with the growth phases of their host plants.

In chapter 3 I used a network metric, modularity, to determine whether host plant partitioning in a restio leafhopper community is non-random (i.e. driven by a deterministic process). This metric allows the identification of the components underlying host plant partitioning (modules). I then performed experiments to show that modules, and therefore host plant partitioning, can mostly be explained by preference and performance relationships (i.e. bottom-up factors).

In chapter 4 I used null models to test whether niche partitioning in restio leafhopper communities is a general pattern across the landscape. I found non-random niche

partitioning, which results from strong host specificity, in all investigated restio leafhopper communities. In addition, I performed binary host choice experiments in the presence and absence of interspecific competition, but found no evidence that interspecific competition narrows host preferences. These findings suggest that host specificity, the cause of niche partitioning, is likely shaped over evolutionary time.

Sampling multiple interaction networks across the CFR, in chapter 5, I tested whether restio leafhopper populations are more host specific in species rich communities and regions in the CFR than in species poor communities and regions. I found no positive relationship between restio leafhopper species richness and host specificity at any scale. These findings suggest that specialisation is not driven by interspecific competition.

In chapter 6 I investigated host shifts in *Cephalelus uncinatus*. *C. uncinatus* has a broader distribution than any single restio species that it can use; suggesting that host plant related divergence may result from geographic range expansion. I found that allopatric and parapatric populations, but not sympatric individuals, using different host plants have divergent host preferences. I also found evidence for morphological divergence in traits related to predator avoidance in population pairs that exhibit divergent host preferences.

My findings emphasise the importance of both bottom-up and top-down factors, with the exception of interspecific competition, as determinants of specialisation and divergence in restio leafhoppers. I find no evidence that interspecific competition is an important force structuring restio leafhopper communities. Instead, strongly niche partitioned community structure appears to emerge from the speciation process.

Opsomming

Die wyse waarop uiteenlopende seleksie lei tot die evolusie van seksuele isolasie (n.l. spesiasie) is ‘n belangrike vraag in evolutionêre biologie. Plantetende insekpopulasieses wat verskillende gasheerplante gebruik kan onder uinteenlopende veelvoudige seleksie wees en vinnig spesiasie ondergaan.

Restio-blaarspringers is ‘n groep plantetende insekspesies wat gespesialiseerd is op verskillende plantspesies in die restio familie. In my tesis ondersoek ek die onder-op (n.l. plantchemie en morfologie) en bo-af seleksiekragte (n.l. predasie en kompetisie) wat lei tot gasheerspesialisasie en -spesiasie in restio-blaarspringers. Ek ondersoek ook die belangrikheid van tussen-spesieskompetisie in gemeenskapsorganisasie.

In hoofstuk 2 het ek gasheerspesialisasie gekwantifiseer in ‘n klein restio-blaarspringergemeenskap oor 24 maande. Ek het gevind dat restio-blaarspringers hoogs gasheerspesifiek is en moontlik met die groeifase van hul hoofgasheerplante gesinchroniseerd is.

Ek het in hoofstuk 3 ‘n netwerkmetriek, modulariteit, gebruik om te bepaal of restio-blaarspringers se gasheerverdeling nie-stokasties is (n.l. deur deterministiese prosesse veroorsaak is). Hierdie metriek laat ‘n mens toe om die komponente van gasheerverdeling (modules) te identifiseer. Deur middel van eksperimente het ek bepaal dat modules, en dus gasheerverdeling, deur gasheervoorkeur en prestasie (onder-op prossesse) verduidelik kan word.

In hoofstuk 4 het ek ondersoek of gasheerverdeling algemeen is. Ek het deur middel van nulmodelle gewys dat gasheerverdeling algemeen is en veroorsaak is deur sterk gasheerspesifiekheid. Ek het ook voorkeureksperimente uitgevoer in die

teenwoordigheid en afwesigheid van tussen-spesies kompetisie. Hier het ek geen teken gevind dat huidige tussen-spesies kompetisie gasheervoorkeur beïnvloed nie. My bevindinge in hierdie hoofstuk stel dus voor dat spesialisasie, die oorsaak van gasheerverdeling, oor evolutionêre tyd gevorm word.

In hoofstuk 5 het ek ondersoek of populasies van restio-blaarspringers meer gasheerspesifiek is in restio-blaarspringerspesies ryke gemeenskappe en streke as populasies in spesies-arm gemeenskappe en streke. Ek het geen positiewe korrelasie tussen spesiesrykheid en gasheerspesialisasie gevind nie. Dit dui daarop aan dat gasheerspesialisasie, en dus gasheerverdeling, nie deur tussen-spesies kompetisie veroorsaak word nie.

In hoofstuk 6 het ek gasheerplantgekoppelde divergensie ondersoek in *Cephalelus uncinatus*. *C. uncinatus* se verspreiding is breër as enige restio-spesies wat dit kan gebruik. Dit stel voor dat verspreidingvergroting gasheerverskuiwing mag veroorsaak. Ek het gevind dat populasies wat verskillende plante in allopatrie en parapatrie gebruik uiteenlopende gasheerkeuses maak, maar insekte wat verskillende plante in sympatrie gebruik wys nie ontwrigtende gasheerkeuses nie. Die populasies wat uiteenlopende gasheerkeuses getoon het, het ook verskillende morfologiese teen-predasie eienskappe getoon. Dit dui daarop aan dat predasie belangrik mag wees vir spesiasie in restio-blaarspringers.

Die bevindinge van my tesis dui daarop aan dat beide onder-op en bo-af seleksie belangrik is vir gasheer-spesialisasie in divergensie. Nietemin, tussen-spesies kompetisie is nie ‘n belangrike bron vir spesialisasie, divergensie of gemeenskapsorganisasie nie. Gemeenskapsorganisasie is klaarblyklik slegs ‘n gevolg van die spesiasie proses.

Acknowledgements

I would like to thank Allan Ellis and Bruce Anderson, my supervisors, for excellent advice during the last few years. Allan has been an inspiring mentor since before I started my postgraduate studies. Captivating lectures by Bruce, during my second undergraduate year, triggered my interest in biological interactions.

My thanks go to Hannes Wiese and Jurene Kemp, my fellow insecteers, for help in the field and important conversations on natural history and statistics. I would also like to thank everybody from room 1019 for the good vibration we’ve created over the last four years. The passion Ethan Newman, whom I’ve journeyed with since our first undergraduate year, shows for natural history has inspired me greatly.

Lastly, I would like to thank Marion Carrier and my family for years of love and support, and for understanding the importance of what I love to do. Marion and my mother, Betta, helped me with fieldwork. General advice on how to approach a PhD from both Marion and my father, Johann, helped tremendously.

The South African Biosystematics Initiative (SABI) provided personal and project funding. I received additional personal funding via the Stellenbosch University postgraduate merit bursary. I also received an overseas conference travel grant from the Stellenbosch University Botany and Zoology department.

Table of contents

List of Tables

List of Figures

Chapter 1

Introduction

Understanding how natural selection leads to the evolution of reproductive isolation has been an important goal in evolutionary biology since the establishment of Ernst Mayr’s biological species concept (Schluter 2000a, Nosil 2008, MacColl 2011). The main principle is that populations experiencing different selection pressures (divergent selection) are likely to accumulate morphological and genetic differences making them reproductively incompatible (Rundle and Nosil 2005, Nosil et al. 2009). This process is now called ecological speciation (Rundle and Nosil 2005). Traditionally there was a strong emphasis on the geographic mode (sympatry versus allopatry) under which ecological speciation occurs (Bush 1975, Fitzpatrick et al. 2009). Specifically, it was thought that sympatric speciation does not occur in nature and that all speciation was allopatric (reviewed in Nosil 2008). This is because gene flow homogenises differences accumulated between closely located populations (Bolnick and Nosil 2007). However, a more recent shift in thinking now suggests that divergence can occur in the face of gene flow, provided divergent selection is strong enough (Via 2012). In addition, the physical proximity of diverging populations permits interactions between populations, like competition for resources or sexual interference, which can promote divergence (Schluter 2000b, Nosil 2013). Therefore, instead of viewing geographic closeness between populations as necessarily detrimental to evolutionary divergence, focus has shifted to the fact that different selection pressures operate in sympatry than in allopatry (Rundle and Nosil 2005).

Biotic interactions are believed to be important selection pressures that can drive rapid evolutionary change (Thompson 1998, Schemske et al. 2009). In almost all well known cases of vertebrate adaptive radiations, for example stickleback fish (Schluter 1994), cichlid fish (Seehausen and Schluter 2004), anole lizards (Losos 1992) and Darwin's finches (Grant and Grant 2006), interspecific competition has a key role in driving evolutionary change. However, in the case of Hawaiian honeycreepers, of which some species are nectar feeders, rapid speciation has been attributed to their evolutionary flexibility to adapt to a variety of feeding niches (Lovette et al. 2002). In the case of flowering plants, rapid evolution is primarily thought to have resulted from their mutualistic relationships with pollinators (Dilcher 2000). A common way in which plants are thought to undergo ecological speciation is through pollinator shifts. That is, a population loses its ancestral pollinator whilst adapting to another pollinator resulting in reproductive isolation from its ancestral population (Johnson et al. 1998, Whittall and Hodges 2007). A similar process, shifting host plants, is thought to have led to herbivorous insect diversity, which makes up approximately half of eukaryotic life on the planet (Hardy and Otto 2014).

Ecological speciation in herbivorous insects is thought to occur when populations undergo host shifts (Ehrlich and Raven 1964, Hardy and Otto 2014). As plant chemistry can differ dramatically between plant species, plant chemistry was initially thought be the primary divergent selection pressure leading to reproductive isolation in herbivores (Ehrlich and Raven 1964, Després et al. 2007). However, experimental work often fails to detect trade-offs related to plant chemistry. Specifically, insects often do not prefer the host plants on which they physiologically perform best (Futuyma and Moreno 1988, but see Gripenberg et al. 2010). For this reason Forister et al. (2012) pointed out that interspecific interactions additional to insect-plant interactions should be taken into account when trying to understand host shifts. Studies on walking stick insects emphasise this point: populations of *Timema cristinae* that use different host plants have diverged little in their ability to detoxify plant chemicals, but are strongly adapted to colour match their respective host plants in response to predation (Nosil and Crespi 2006). However, different species of *Timema* have diverged in both their ability to detoxify plant chemicals and are adapted to match the backgrounds of their respective host plants (Nosil and Sandoval 2008). This suggests that a single selective pressure may lead to divergence, but multiple selection pressures are required to drive complete (or near complete) reproductive isolation (Nosil and Sandoval 2008, Nosil et al. 2009).

In contrast to predation, the role of interspecific competition (both as a selective pressure in the evolution of herbivorous insects and as an ecological mechanism structuring insect herbivore communities) has been controversial (Lawton and Strong 1981, Denno et al. 1995). An early argument was that predation keeps population numbers of herbivorous insects below levels that allow interspecific resource competition to occur (Lawton and Strong 1981). Nonetheless, there is evidence that interspecific competition occurs in herbivorous insects (Denno et al. 1995, Kaplan and Denno 2007). However, herbivorous insects often do not exhibit niche partitioning even when there is evidence that interspecific competition occurs (or is suspected to occur) (e.g. McClure and Price 1975, 1976, Rathcke 1976, Hochkirch et al. 2007, Tack et al. 2009, Hochkirch and Gröning 2012). Also, there is not a single study providing evidence that a host shift has occurred as a result of ecological character displacement (an evolved resource shift in response to interspecific competition) in herbivorous insects (supplementary material in Stuart and Losos (2013)). Nonetheless, indirect evidence suggests that interspecific competition may drive host specialisation in herbivorous insects. Ricklefs and Marquis (2012), for example, showed that host plant niches (measured in terms of leaf traits) increase towards the tropics, but much less drastically than the number of insect species. For this reason they argue that insects in the tropics should be more specialised than insects in temperate regions to avoid competition resulting from niche overlap. Therefore, the recent finding that herbivorous insects across the globe increase in host specificity towards the tropics suggests that interspecific competition may be an important driver of host specialisation in insects (Forister et al. 2014).

The Cape Floristic Region (CFR) in South Africa is characterised by plant species richness comparable to that of the wet tropics (Goldblatt 1997), resulting from high spatial turnover in plant species composition (i.e. beta diversity) (Cowling and Rundel 1996). For this reason there has been strong emphasis on allopatric speciation and non-adaptive speciation in the CFR (Ellis et al. 2014). However, the relatively small number of studies focusing on ecological speciation in the CFR consistently find evidence that spatially heterogeneous selection pressures (e.g. soil type) drive ecological divergence between plant populations (Ellis et al. 2014). This is also true for the only rigorously studied case of ecological speciation in animals in this biome (Ellis et al. 2014): Two ecotypes of Cape dwarf chameleons appear to have consistently evolved into either a brown morph associated with shrub vegetation or a green morph associated with forest vegetation (Hopkins and Tolley 2011, Herrel et al. 2011). Surprisingly, despite being model systems for testing ecological speciation (Funk et al. 2002), there are no studies in the CFR that have investigated host plant linked ecological divergence in herbivorous insects.

Unlike plants, herbivorous insect diversity and diversification has received very little attention in the CFR. A prevalent perception is that insect species richness within the

CFR is lower than expected for such a plant species rich region (Johnson 1992, Giliomee 2003). Although recent studies suggest that insect species richness in Fynbos, the main vegetation type in the CFR, is comparable to other biomes in South Africa (Procheş and Cowling 2006, Procheş et al. 2009), no studies suggest that insect diversity in the CFR is high. Also, contrary to most thinking about insect diversity (i.e. that ecological speciation is important) insect diversity in the CFR has largely been ascribed to low extinction rates and vicariance (Picker and Samways 1996). It is thus assumed that insects in the CFR are ancient and have diverged largely as a result of the lack of gene flow between populations because of spatial isolation.

The African Restionaceae family (restios) is one of the most ancient and species rich plant clades in the CFR. Its origin has been estimated at about 65 million years ago and it consists of about 350 extent species (Linder et al. 2003). Because of its dominance, it is also one of the definitive components of Fynbos, the main vegetation type in the CFR. Restios are reed like in appearance with intermittent nodes. At these nodes are dry leaf sheaths that drop off in some species (Fig 1.1). Restio leafhoppers (Cicadellidae, Cephalelini), one of the dominant herbivores on restios (Kemp 2014), appear to mimic these leaf sheaths (Osborn 1903) (Fig 1.2). They are able to complete their entire life cycle on their host plants, and are mostly flightless (a fraction of females develop wings) (personal observation, Davies 1986). Currently there are 21 described restio leafhopper species that are restricted to restios (Prendini 1997). Most restio leafhopper species are restricted to one of two sub tribes of the restio family; the Restioneae (restios with seeds) and the Willdenowieae (restios with nuts) (Wiese 2014). Interestingly, the radiation of restio leafhoppers appears to be recent and they did not co diversify with restios (Wiese 2014). Also, restio leafhopper species are broadly distributed across the CFR and have largely overlapping distributions (personal observation, Wiese 2014). This suggests that restio leafhoppers are not ancient and probably did not diversify through vicariance. Therefore, restio leafhopper diversification likely resulted from ecological speciation.

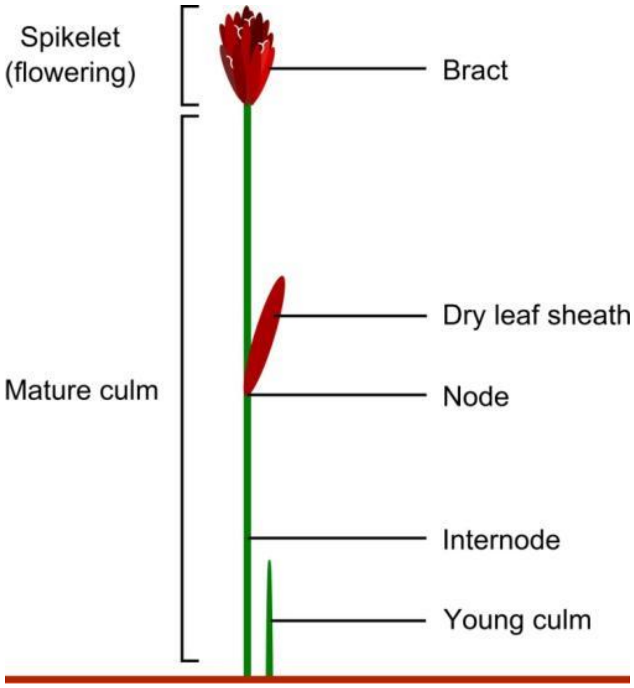


Fig 1.1: The aboveground morphology of restios.

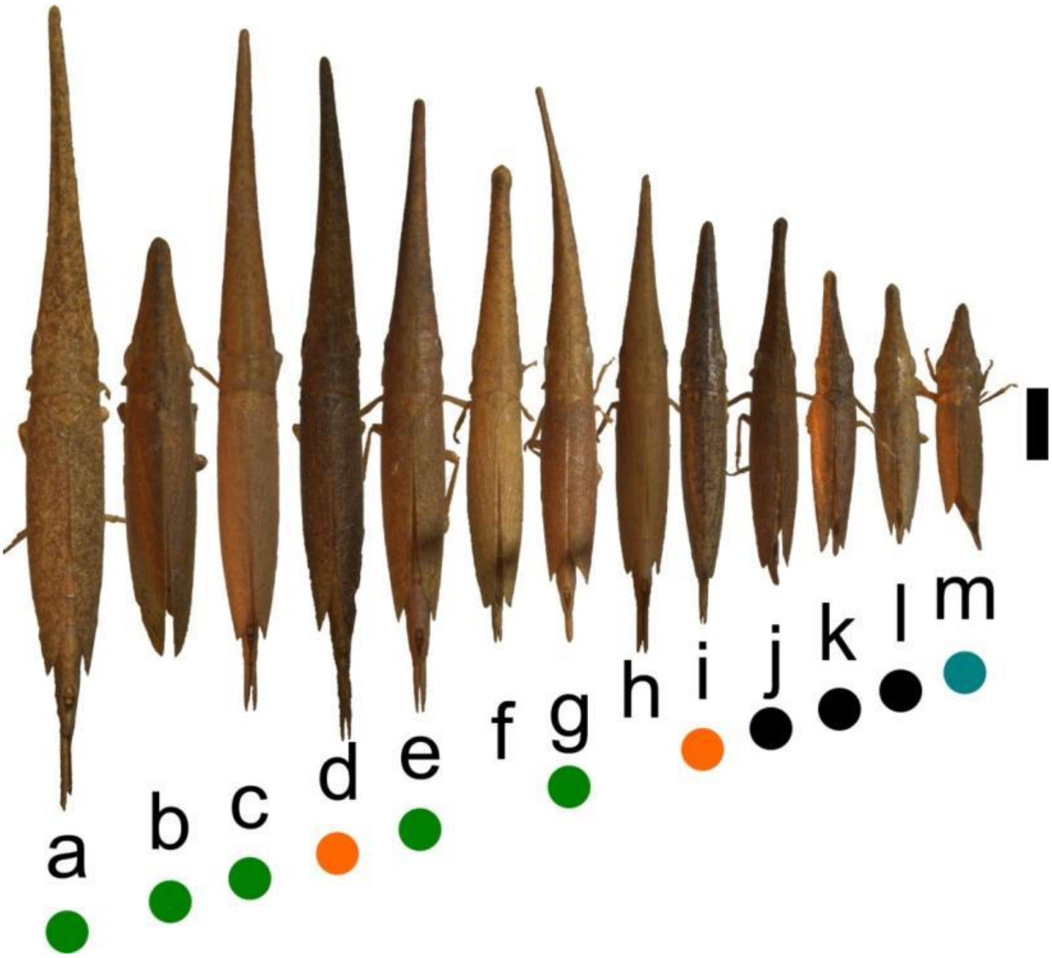
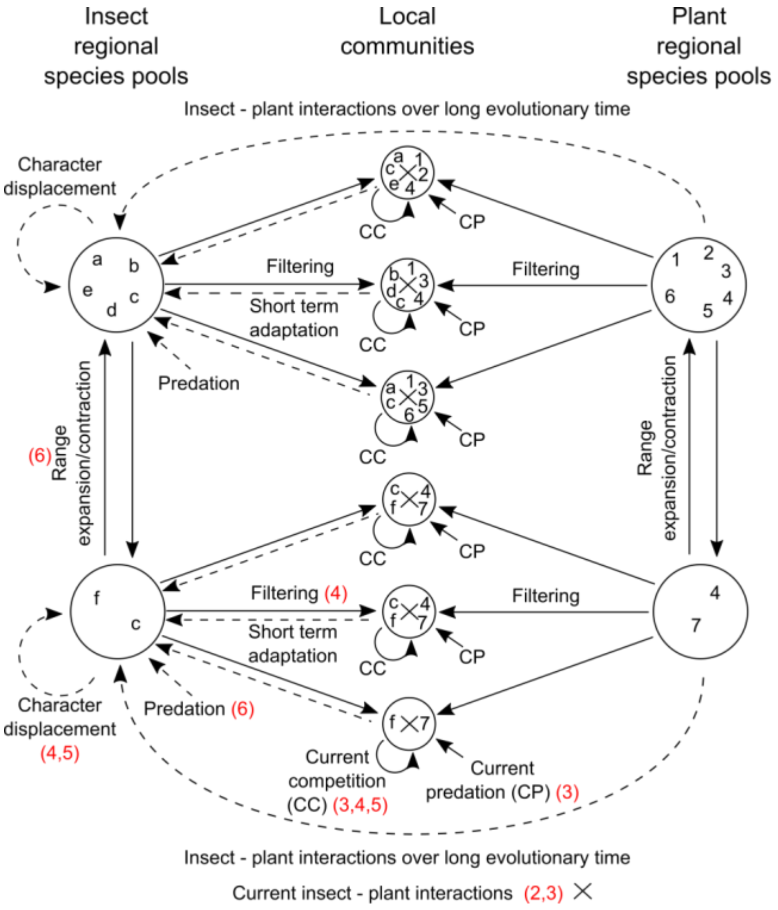


Figure 1.2. A haphazard selection of restio leafhopper species roughly arranged from large to small: a) *Cephalelus angustatus*, b) *C. nov. sp. 2*, c) *C. brevipilus*, d) *C. nivenus*, e) *C. uncinatus*, f) *C. appendiculatus*, g) *C. attenuatus*, h) *C. pickeri*, i) *C. bicoloratus*, j) *C. rawsonia*, k) *C. linderi*, l) *C. campbelli*, m) *Duospina capensis*. Species from the same clades, identified by Wiese (2014) with mitochondrial (Co1) and nuclear (H3) DNA, are indicated with dots of the same colour (the DNA of f and h were not sequenced). The scale bar represents 1 mm. Large bodied restio leafhopper species appear to use restios with thick culms. Crown length (nose-like structure) is possibly involved in restio sheath mimicry. Ovipositor length is possibly related to sheath length because restio leafhoppers slide their ovipositor underneath restio sheaths when ovipositing. Note the short crown and ovipositor of *C. nov. sp. 2* (b). *C. nov. sp. 2* was collected from *Elegia elephantine*: a very large restio species which sheds its leaf sheaths.

Throughout this thesis I explore the contemporary and historic ecological causes of host specialisation in restio leafhoppers. I place emphasis on the direct interactions between restio leafhoppers and restios as food, their interactions with possible competitors, and their predators. These interactions, which I address over five research chapters throughout my thesis, are all studied within the community context (Fig 1.3).



Chapter 2: Specialised host-use and phenophase tracking in restio leafhoppers (Cicadellidae: Cephalelini) in the Cape Floristic Region

Chapter 3:Evolutionary processes, and not contemporary competition, explain niche partitioning in a plant-herbivore community network

Chapter 4: Strongly niche partitioned restio leafhopper communities are not structured by current interspecific competition

Chapter 5:Negative relationship between species richness and host specificity in a clade of specialised herbivorous insects

Chapter 6:Spatial heterogeneity in plant distributions drives host-shifts in *Cephalelus uncinatus*, a broadly distributed restio leafhopper species

Figure 1.3: An insect-plant community framework for insect specialisation and speciation partly tested throughout my thesis. Chapters that address particular components of the framework are indicated with red numbers on the diagram. Large circles on the left represent regional species pools of insects and those on the right represent regional species pools of plants. Small circles represent local insect – plant communities that are, in part, random assemblages from insect and plant regional species pools. Local insect assemblages are, however, also determined by the plant species (i.e. habitat filtering) and insect species (i.e. competitive exclusion) that are present in local communities. Current competition and predation within local communities cause insects to use only a subset of plants that they would use in the absence of competition and predation. Further, over long evolutionary time scales, evolutionary feedback from competition and predation within many local communities leads to host specialisation and host-shifts (i.e. character displacement). Direct interactions between insects and plants also affect specialisation over evolutionary time (i.e. insects do not become specialised to host plants that are not common in local communities across space and time). Divergent host plant adaptation that plays out within regional species pools (through disruptive selection) may lead to sympatric speciation, in which case regional species pool composition and richness changes. Allopatric speciation (between regions), however, lead to geographic range reduction (of an insect species), and changes in species composition in one region, but does not increase species richness within regional species pools. Furthermore, range expansion from one region to another (possibly leading to secondary contact between species that speciated allopatrically) leads to changes in both insect species composition and species richness within the region expanded to. Increased regional species richness leads to more completely filled plant niches within local communities, forcing insects to specialise. There is therefore a continuous feedback between speciation and specialisation.

Background and objectives of research chapters

Chapter 2

Higher host specificity in herbivorous insects is thought to translate into higher herbivore species richness (Erwin 1982). Specifically, the inherent tendency of herbivores to be host specific is thought to have led to their high species richness (Winkler and Mitter 2008). However, despite the emphasis on the importance of diet breadth in understanding insect species richness and diversification it has received little attention in the CFR. As it appears that insect diversity in the CFR is not as high as expected for such a plant species rich region (Johnson 1992, Giliomee 2003), it is possible that conditions in the CFR favour generalists. One aspect that is thought to profoundly affect insect ecology in the CFR is the generally low leaf nitrogen of fynbos plants (Cottrell 1985). Considering the exceptionally low nitrogen levels of restios (Herppich et al. 2002), I hypothesised that restio leafhoppers might be generalists that temporally switch between different restio hosts as restios go through nutrient rich and poor phenological cycles. Therefore I performed monthly surveys of the host use patterns of a single restio leafhopper community over a two year period.

Chapter 3

Communities of organisms often exhibit niche partitioning. Niche partitioning can, however, be driven by a variety of processes (Schluter 1984), although interspecific competition is generally the favoured explanation (Dayan and Simberloff 2005). It is difficult to identify the causes of niche partitioning, and even the broad mechanisms such as whether the pattern is driven by current ecological (Connell 1961) or historical processes (Moen and Wiens 2009) have seldom been investigated. In herbivorous insects, strong linkage between host preference and performance suggests that insects are strongly specialised on their host plants (Gripenberg et al. 2010). That is, their preference is determined by their host plant as a food source, and has not recently been modified by predation or interspecific competition. In this chapter I use a network analysis to determine whether a community of restio leafhoppers exhibit modularity, a measure of niche partitioning. This approach also allows us to identify modules (groups of interacting restio leafhopper and restio species). Using knowledge of module membership I experimentally test whether niche partitioning in a restio leafhopper community is determined by preference-performance linkage. If so, it would suggest that niche partitioning in restio leafhopper communities results from evolutionary processes or competitive exclusion.

Chapter 4

Ecological character displacement (ECD) is an evolved response to interspecific competition that leads to a reduction in resource use overlap (Stuart and Losos 2013). When this process occurs between multiple community members it is termed community-wide ECD (Dayan and Simberloff 2005). Patterns of ECD can, however, also be caused by competitive exclusion (Gause et al. 1934, Hardin 1960, Stuart and Losos 2013). Competitive exclusion is the displacement of one species by another that uses the same resources. Here I investigate multiple restio leafhopper communities and use a null model approach to test whether they exhibit evolved community-wide ECD-like host use patterns. I also investigate the co-occurrence patterns of restio leafhopper species to test whether species using similar restio species do not co-occur. Lastly I perform experiments to test whether host preference is determined by current competition in the form of interference competition or whether host preference stays unaltered. Unaltered host preference would provide additional evidence that niche partitioning emerges as a result of evolutionary and not current ecological processes.

Chapter 5

The link between species richness and specialisation is a common pattern in nature, but it is difficult to determine whether species richness leads to specificity or vice versa. In pollination systems, species richness is thought to drive specialisation through character displacement (Armbruster and Muchhala 2009). This paradigm also features in the understanding of latitudinal trends in herbivorous insect host specialisation. Recently, it was determined that in most herbivorous insect feeding guilds across the globe, tropical species are more host specific than those in temperate regions (Forister et al. 2014). Although latitude is a good proxy for species richness, tropical regions have very different biogeographic histories than temperate regions. Therefore, species richness and host specificity may independently be related to biogeographic history (e.g. time since last glaciation) and not to each other. By restricting analyses to restio leafhoppers, species richness can easily be quantified (as opposed to whole herbivore assemblage richness). Also, as restio leafhoppers are restricted to the CFR, I control for large differences in biogeographic history (as opposed to studies comparing e.g. temperate and tropical rain forests). This allows me to assess whether species richness drives local host specificity in restio leafhopper populations in a more direct way. A positive relationship between species richness and host specificity might suggest that interspecific competition drives host specialisation in restio leafhoppers.

Chapter 6

Spatial heterogeneity in selection pressures is thought to drive ecological speciation in the CFR (Ellis et al. 2014). With the exception of pollination systems there are, however, a paucity of studies testing this hypothesis (Ellis et al. 2014). There are also no studies providing any evidence for ecological speciation in herbivorous insects in the CFR. Here I ask whether population pairs in *Cephalelus uncinatus*, a broadly distributed restio leafhoppers species, associated with different restio species have diverged in allopatry, parapatry and sympatry. Specifically, I test whether restio leafhoppers have diverged in host preference. As restio leafhoppers are thought to mimic the sheaths of restios (Osborn 1903) I also ask whether traits possibly linked to predation have diverged with preference. Particularly, I ask whether restio leafhoppers have diverged in body size and colour. I model body colour in Cape dwarf chameleon vision to test whether the colour of restio leafhoppers matches the leaf sheaths of the restios that they prefer.

Chapter 2

Specialised host-use and phenophase tracking in restio leafhoppers, *Cephalelus* (Cicadellidae: Cephalelini), in the Cape Floristic Region

Published: Augustyn, W. J., B. Anderson, M. Stiller, and A. G. Ellis. 2013. Specialised host-use and phenophase tracking in restio leafhoppers (Cicadellidae: Cephalelini) in the Cape Floristic Region. Journal of Insect Conservation 17:1267–1274.

Abstract

The Cape Floristic Region (CFR) has exceptionally high plant diversity, but because there are so few studies on insect diversity and diet breadth, little is known about the relationship between plant and insect diversity. One possibility is that plant and insect diversity in the CFR are linked through host specialisation. Alternatively, the nutrient-poor soils of the CFR may favour generalist feeding strategies with insects tracking the favourable phenophases of a variety of host plants. I studied *Cephalelus*, a genus of leafhoppers apparently specialised on the Restionaceae, a diverse and dominant plant family in the CFR. I examined patterns of *Cephalelus* host association at a single site during a 24 month field survey to determine whether *Cephalelus* diversity is related to the partitioning of host plant or temporal niches; or whether *Cephalelus* tracks the most nutritive phenophases of restios by temporal host-switching. Seven *Cephalelus* species were recorded which varied in their seasonal abundance patterns. The majority of these species exhibited specialised host use on different Restionaceae species, with the exception of *C. pickeri*. This species specialised on two host plants. The populations of two dominant species, *C. pickeri* and *C. uncinatus*, tracked the phenology of their primary host plants but not of the Restionaceae in general. To conclude; I find no evidence for host-switching or generalism in *Cephalelus*. Instead, they appear to be host-specialised, suggesting coupling between their diversity and that of their host plants; the Restionaceae in the CFR.

Introduction

More than half of all globally described species are insects (Mayhew 2007) of which the majority are herbivorous (Strong et al. 1984). “Arms races” between insects and plants are thought to be a key driver of herbivorous insect diversity and one of the consequences of these races is extreme host specialization (Ehrlich and Raven 1964, Mitter et al. 1988, but see Nyman et al. 2010). Besides being a mechanism that drives insect diversity, the degree of specialisation exhibited by insects can have implications for conservation (Hughes et al. 2000). Most notably, the loss of key plant species is likely to have cascading effects on the diversity of phytophagous insects.

The Cape Floristic Region (CFR) is a biodiversity hotspot with plant diversity comparable to that of the tropics (Goldblatt 1997). However, little is known about insect diversity in the CFR, and in contrast to plant diversity, early workers suggested that insect diversity in this biome is low in general (Johnson 1992); or low relative to the high plant diversity (Giliomee 2003). An early exception was a study by Wright and Samways (1998) which suggested high diversity in galling-insects. Recent studies have shown that insect diversity in the CFR may be comparable to that of other biomes in South Africa (Procheş and Cowling 2006). Kuhlmann (2009) also found exceptionally high levels of both bee endemism and diversity in the CFR. Pryke and Samways (2008) found little seasonal variation in abundance and species richness for insects on foliage. However, none of the above mentioned studies explicitly test if herbivorous insect diversity is coupled to plant diversity through host specialisation. Some of the earliest studies on Cape insect diversity investigated the ecology of insects on proteas. For example Coetzee et al. (1997) found that sclerophylly (hard thick leaves) might be a more important anti-herbivore defence than phenolic compounds like tannins, and they also documented extremely low levels of leaf nitrogen (less than 1% on average) that decreased with leaf age.

Insect host use is often linked to the nutrient content of plants (Joern et al. 2012), which is in turn linked to soil nutrients. In this regard plants and soils of the Fynbos are known to have exceptionally low levels of nitrogen (Mooney and Gulmon 1982, Richards et al. 1997). However, nutrients such as phosphorous and nitrogen are known to fluctuate temporally within a plant as the plant passes through different stages of its life cycle (phenophases) (Klein 1990). Consequently, one way of coping with generally low plant nutrients is to be a generalist, capable of switching between different host plants as their nutrient levels fluctuate. If this were the case, I would expect insects to exhibit preferences for different plant species at different times of the year. Alternatively herbivorous insects may exhibit specialised host use patterns as a result of adaptations to specific plant defences. Cottrell (1985) suggested that sucking insects, because of various feeding adaptations, should be able to deal well with the low leaf nitrogen content of fynbos plants. Therefore it is reasonable to expect this group to exhibit specialised host use patterns.

The African Restionaceae (hereafter called restios) is a highly diverse monophyletic clade which comprises about 350 species, and only 10 of these occur outside the CFR (Linder 2003). It is one of the diagnostic components of fynbos and is one of the oldest clades in the CFR, with its origin estimated at about 65 million years ago (Linder et al. 2003). Restios are generally reed-like in appearance with regular nodes. At these nodes there are often dried out sheaths that drop off in some species. Restios are dioecious and male and female plants can be dimorphic in their general morphology as well as in their reproductive structures. Different species of restios flower at different times of the year (e.g. Linder 2001), especially when occurring in sympatry (personal observation). It has also been shown that secondary metabolites fluctuate with the seasonal phenology of restios (Glyphis and Puttick 1988).

Cephalelus Percheron (Cicadellidae: Cephalelini) is a genus of specialised herbivorous sucking insects that is known to only occur on restios (Davies 1986, Prendini 1995). The Cephalelini tribe shows a typical Gondwanan distribution pattern, parallel to that of their host plants, suggesting great antiquity of the relationship (Prendini 1995). Currently South African Cephalelini comprises 21 species (Davies 1988, Prendini 1995, Prendini and Linder 1998). All *Cephalelus* are characterized by a small, slender body and have a diagnostic elongated crown that resembles the bracts and dried out leaf sheaths (Fig 2.1) of restios. Prendini (1995) established that *Cephalelus*, in all life stages, occurs on restios throughout the year. Preliminary results on host use by Prendini (1995) suggested that different species of restio leafhoppers use few host plant species in the restio family.

Like other herbivorous insects, *Cephalelus* might be able to detect nutritional changes in a restio community and consequently shift between the most nutritious hosts, a strategy which might be favoured in nutrient poor systems like the CFR. Alternatively, *Cephalelus* might have evolved strong host specialisation in which case exploitation of less suitable hosts would be minimal. In this study I distinguish between these two possibilities by answering the following specific questions:

1) Can more than one restio leafhopper species co-occur within a restio community?

2) Are restio leafhoppers using particular hosts more (or less) frequently than expected from the relative abundance of restio species in the community, suggesting specialisation?

3) Are restio leafhoppers switching hosts as the favourability (in terms of nutrition) of a plant host changes, or are they synchronised with the phenology of a single host species?

4) Does the sex of the restio influence host use by restio leafhoppers?

5) Do male and female leafhoppers exhibit similar host use patterns?

Materials and Methods

Cephalelus sampling

To assess temporal host-use patterns of restio leafhoppers, monthly surveys were conducted in Pringle Bay (-34.3259, 18.8401) from February 2010 to January 2012 at a single 2500m^2 site that encompassed a single restio dominated plant community. As restio communities exhibit high levels of species turnover across habitat gradients (Linder and Vlok 1991, Araya et al. 2011) I confined my sampling to a single habitat at the bottom of a valley with a homogenous supply of groundwater.

Cephalelus were sampled on 20 individual male and 20 female plants of five dominant restio species at the site each month (i.e. 200 host plants sampled per monthly survey). One additional restio species was present at the site (*Willdenowia* sp.), but its abundance was too low to allow sampling. When male and female plants became indistinguishable during non-flowering phenophases, 40 individual unsexed plants were sampled. Different restio species always remained easily distinguishable from each other. All *Cephalelus* were sampled by walking along transects and beating individual plants by hand into a fine mesh beat net. This way I made sure not to sample the same individual plant twice per sampling interval. Sampling effort was standardised by beating each individual plant ten times. Nymphs and adults of *Cephalelus* were recorded. Adult *Cephalelus* were preserved in alcohol or pinned. Male *Cephalelus* were identified by dissecting out their genitalia and matching them with type specimens (Stellenbosch University Conservation Ecology & Entomology) and the species descriptions of Davies (1988) and Prendini (1997). Females were then matched with male *Cephalelus* through external morphology, comparing them to type specimens and my own collection from broad geographical surveys of the Cephalelini. Both male and female *Cephalelus* at my site could be identified without ambiguity using this approach. Nymphs (which were unidentifiable) were counted and released. Voucher material was deposited in the insect collection of the Department of Conservation Ecology & Entomology (Stellenbosch University) and in the National Collection of Insects (ARC-PPRI, Queenswood, Pretoria).

Host availability

Cover of the five dominant Restionaceae species at the site: *Hypodiscus aristatus* (Thunb.) Krauss, *Elegia filacea* Mast., *Nevillea obtusissima* (Steud.) Linder, *Thamnochortus lucens* Poir., and *Staberoha vaginata* (Thunb.) Pillans, 1925; was estimated along nine randomly-placed 40m transects across the study site. Cover for each species was estimated as the total length of the canopy of individual plants that were intersected by the transect tape. Plant voucher specimens are held at the Department of Botany and Zoology, Stellenbosch University.

I hypothesised that if a *Cephalelus* species was a generalist it would use all the available host plants in proportion to their relative abundances in the community. However, if the *Cephalelus* species specialised or avoided certain plants, I expected their relative host-use frequencies to be disproportional to the relative abundances of the host plants. To test this, I compared the abundances of *C. pickeri* Prendini, 1997, *C. uncinatus* Davies, 1988, *C. rawsonia* Davies, 1988 and *C. attenuatus* Davies, 1988 adults on all the host species at the site to the relative abundances of the hosts using Chi square tests (following Neu et al. 1974). I then calculated Bonferroni corrected statistics to determine which plant species were used more or less frequently than expected from their relative abundances. Abundances of other *Cephalelus* species were too low for analysis.

Host phenology

Each month plant phenophase (flowering and presence/absence of new growth) was recorded for 20 individual plants (10 males and 10 females) of each species. The phenology of a plant was recorded directly after it was beaten for restio leafhoppers. *E. filacea* males and females were indistinguishable for 6 months of each year. Restios were assigned the "flowering" phenophase when fertile anthers or stigmas were detected. When plants were not flowering any longer, and not growing new culms, the “no new growth” phenophase was assigned. The “new growth” phenophase was assigned to plants when they sprouted new culms at the base.

To test whether *Cephalelus* tracked the phenophases of their host plants, I asked whether disproportionally more or fewer *Cephalelus* individuals were present during a particular phenophase, and whether this pattern was consistent for *C. pickeri* and *C. uncinatus,* the most abundant species at the site. I used Chi-squared tests to compare the observed use to the expected use for all phenophases. As some phenophases stretched over more sampling intervals than others, expected values were adjusted so that more observations were expected during longer phenophases. All analyses in this manuscript were conducted in R version 2.9.0 (R Development Core Team 2009, Vienna, Austria).

Plant sex

Hypodiscus aristatus was used to test whether *Cephalelus* used male and female plants equally. Other host plants were not used, as there were either too few sampling intervals where males and females were distinguishable, or too few sampling intervals with any insect observations. Chi-squared tests were used to determine whether male and female plants were being used equally by *Cephalelus* adults and by nymphs.

Insect sex

Male and female *Cephalelus* were easily distinguished, as females are larger than males and have long ovipositors (Fig 2.1). The host species on which each individual was caught was recorded during each sampling interval. I used both *C. pickeri* and *C. uncinatus* to test whether male and female *Cephalelus* individuals have the same host preferences. I calculated the proportion of males and females caught on each host plant, and then compared the proportions using Chi-squared tests.

Results

Adult *Cephalelus* occurrence

During a 24-month sampling period at a single site, seven species in the genus *Cephalelus* were recorded (Table 2.1). Two species, *C. pickeri* and *C. uncinatus* were abundant, whereas the other 5 species were encountered less frequently. Four of the *Cephalelus* species predominantly occurred on a single host plant species. *Cephalelus pickeri* mainly occurred on *E. filacea*, *C. uncinatus* and *C. angustatus* Evans, 1947 on *H. aristatus*, and *C. rawsonia* on *S. vaginata*. *Cephalelus appendiculatus* Davies, 1988 and *C. attenuatus* exhibited a more generalist pattern, occurring with equal probability on 3 and 4 hosts respectively.

Although low numbers of adult individuals of most species were present throughout the year, *Cephalelus* species did exhibit clear and variable temporal abundance peaks (Table 2.2). Species either peaked in abundance in late summer / winter (*C. pickeri*, *C. rawsonia*, *C. attenuatus*, *C. angustatus* and *C. campbelli* Davies, 1988) or in spring / early summer (*C. uncinatus* and *C. appendiculatus* Davies 1988). The two most abundant species; *C. pickeri* and *C. uncinatus*; peaked at different times of the year (Table 2.1 and 2.2).

Host availability

All of the four analysed species of *Cephalelus* displayed host use patterns which differed significantly from the expectation based on the relative abundances of restio species in the community (*C. pickeri*: $\chi^2_4 = 21.95$, $p < 0.01$, *C. uncinatus*: $\chi^2_4 = 1073.83$, $p < 0.01$, *C. rawsonia*: $\chi^2_4 = 65.47$, $p < 0.01$ and *C. attenuatus* $\chi^2_4 = 81.78$, $p < 0.01$; Figure 2.2). Significant insect-host over-utilization indicative of specialisation was as follows; *C. uncinatus* on *H. aristatus*, *C. rawsonia* on *S. vaginata* and *C. attenuatus* on *T. lucens*. Even though *C. pickeri* mostly occurred on *E. filacea* (Figure 2.2), it over-utilized both *E. filacea* and *H. aristatus*.

Host phenology

Despite the fact that restio species had different flowering peaks and thus likely differed in nutritional quality across the seasons, adult *Cephalelus* did not switch hosts in response to host phenophase (Figure 2.3). *Cephalelus pickeri* consistently occurred on *E. filacea* and *C. uncinatus* on *H. aristatus*. Adult *C. pickeri* peaked in abundance on *E. filacea* during the old growth phenophase (Fig 2.3a. $\chi^2_2 = 155.93$, $p < 0.01$). The same was found for *C. uncinatus* adults on *H. aristatus* (Fig 2.3b. $\chi^2_2 = 168.53$, $p < 0.01$). The abundance of nymphs on *E. filacea* peaked during the flowering phenophase (Fig 2.3c. $\chi^2_2 = 304.78$, $p <0.01$), whereas on *H. aristatus*, nymphs peaked in abundance during the new growth phase (Fig 2.3c. $\chi^2_2 = 49.49$, $p < 0.01$).

Plant and insect sex

Female *H. aristatus* plants were used more often, by all *Cephalelus* species, than male plants ($\chi^2_1 = 4.68$, $p < 0.05$). Nymphs used male and female plants equally. For both *C. pickeri* ($\chi^2_3 = 0.09$, $p = 0.99$) and *C. uncinatus* ($\chi^2_4 = 0.10$, $p = 0.99$), I found that males and females had similar host use patterns (Fig. 2.4).

Discussion

In this study I show that *Cephalelus* leafhoppers in the nutrient poor CFR exhibit host-use patterns indicative of host-plant specialisation, although species vary in their level of specialization. I rule out seasonal host-switching to track nutritive plant phenophases as a strategy for coping with limited nutrients. Instead taxa within communities tend to be specialised on one or a few host plant species and exhibit strong preference hierarchies that even extend to the level of plant sex.

Co-existence

As closely related organisms are thought to compete strongly (e.g. Slingsby and Verboom 2006), it is remarkable that seven species of *Cephalelus* can co-exist in a single community. I identified two mechanisms (not necessarily mutually exclusive) which may allow co-existence: Firstly *Cephalelus* adults of different species show different seasonal peaks; and thus different species that use the same host plants can avoid competition by phenological separation (e.g. *C. angustatus* and *C. uncinatus)*. Secondly, different *Cephalelus* species can occur at the same time, but avoid competition by using different host plants (e.g. *C. pickeri*, *C. rawsonia* and *C. attenuatus)*. Niche partitioning along temporal (e.g. Després and Jaeger 1999) and host species (e.g. Bush 1969a) axes in this genus may allow several species to co-exist.

Host synchronization

Specialised insects have been shown to be tied to the phenology of their host plant (e.g. Gould and Sweet 2000). I provide evidence suggesting that the two most abundant *Cephalelus* species at my site may also be synchronised with the phenology of their host plants. Nymphs peaked while the culms of their host plants were growing, and adults peaked on mature culms. Although it is unknown whether host phenology synchronisation maximises larval performance (Wennström and Hjulström 2010) and/or adult performance (Scheirs et al. 2000) in *Cephalelus*, this may be a mechanism which allows them to maximise nutrient uptake from their hosts in the nutrient impoverished CFR. The synchronization of insect reproductive cycles with plant phenology may also facilitate the specialisation of restio leafhoppers to different host plants.

Host sex

Besides being able to distinguish between different host species, insects are known to specialise on, or to prefer, specific plant phenotypes of the same host species. In other similar systems it has been shown that insects are biased towards using a certain host plant sex, usually the male plants (Boecklen et al. 1990, Jing and Coley 1990, Boecklen and Hoffman 1993). My finding that *Cephalelus* exhibits a bias for females of *H. aristatus* contrasts with this trend, but other examples where female plants are preferred have been reported (Cornelissen and Stiling 2005). Why this bias exists remains unclear, although one possibility is that female plants of *H. aristatus* are provisioning large seeds during the old growth phenophase when *Cephalelus* are most abundant on them, and insects may be attracted to plants in which large amounts of nutrients are being shifted.

Insect sex

Host plants are often chosen by female insects to maximise larval (nymph) performance (Wennström and Hjulström 2010), which may result in divergent host use patterns in males and females. In *Cephalelus*, however, I found that males and females have the same host preferences. This suggests that both nymphs and adults may have specific food requirements and that adult performance is also maximised by host choice. However, further testing is necessary to determine how host choice affects fitness.

Conclusion

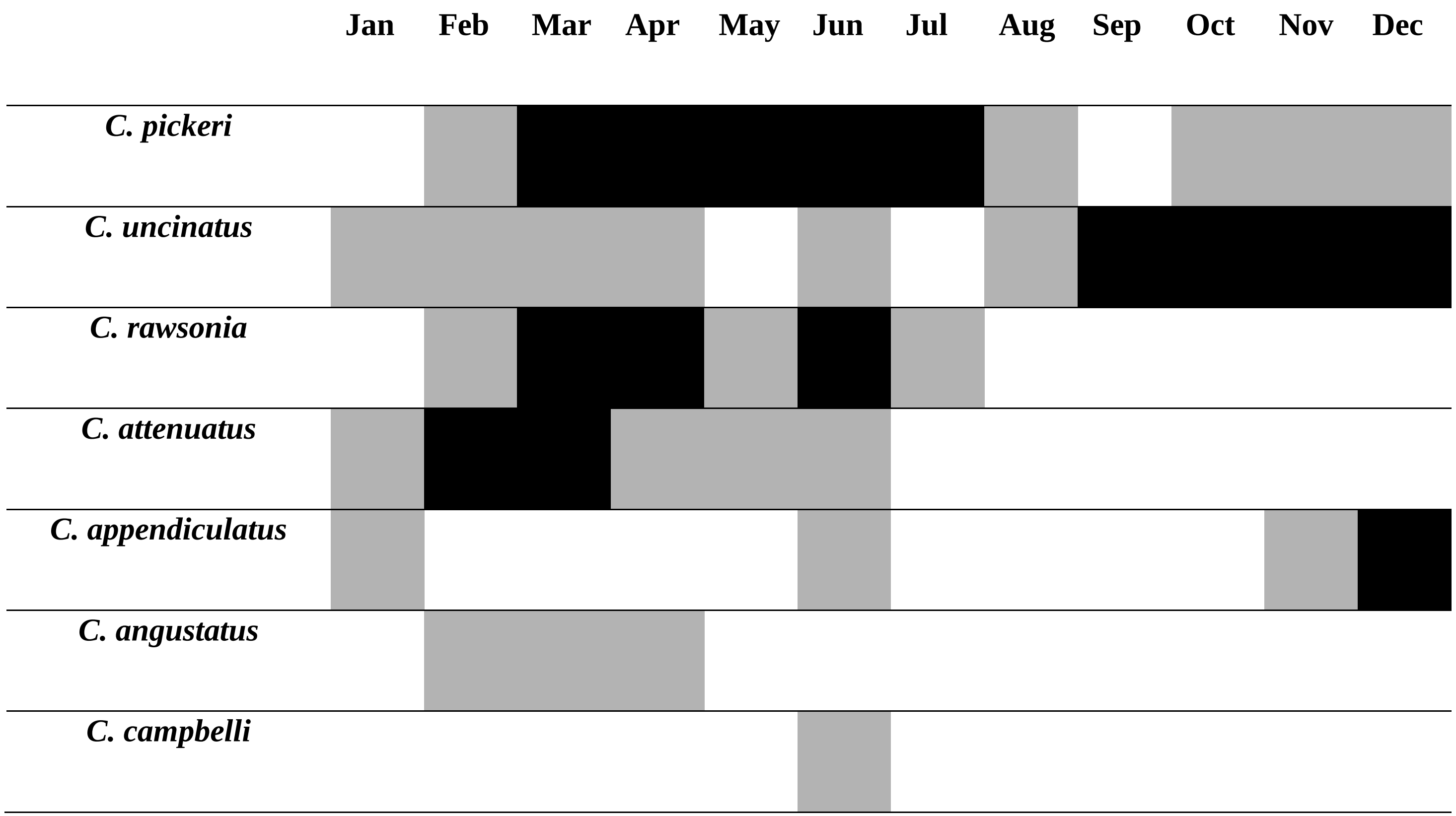
Besides studies on *Protea* herbivores (e.g. Wright and Samways 1999, Roets et al. 2009) this is the first study that quantitatively investigates the ecology of an assemblage of herbivorous insects in the CFR. My finding that insects in the genus *Cephalelus* tend to be specialised on one or a few host plant species at the local community scale demonstrates that herbivorous insects can be host-specific in the nutrient depleted CFR. However, investigations of specialisation on a broader spatial scale are necessary to elucidate the role of local adaptation in determining local-level host-use patterns.

Tables

Table 2.1: All the *Cephalelus* species caught at a single site over a 24-month period. The plant species sampled were: *Elegia filacea*, *Staberoha vaginata*, *Hypodiscus aristatus*, *Thamnochortus lucens* and *Nevillea obtusissima*. Only adult *Cephalelus* were identified at the species level. * species with adequate sample sizes for statistical analyses of host use, + species with adequate abundances for analysis of host synchronization.

	Host Plant Species					
Insect Species	***E. filacea***	***S. vaginata***	***H. aristatus***	***T. lucens***	***N. obtusissima***	**Total**
C. pickeri*+	92	21	19	0	3	135
C. uncinatus*+	3	4	83	4	2	96
C. rawsonia*	6	16	1	1	2	26
C. attenuatus*	6	4	3	5	0	18
C. appendiculatus	4	3	3	0	0	10
C. angustatus	0	0	5	0	0	5
C. campbelli	0	0	1	0	0	1
Total Adults	111	48	115	11	7	292
Total Nymphs	151	39	167	15	9	381

Table 2.2: Temporal occurrences for all *Cephalelus* species recorded at the site. Colours indicate total abundance of Cephalelus caught over a two year sampling period during each calendar month. White = 0 individuals, grey = 1-3 individuals and black = 4-54 individuals.



	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
C. pickeri	□	▨	■	■	■	■	■	▨	□	▨	▨	▨
C. uncinatus	▨	▨	▨	▨	□	▨	□	▨	■	■	■	■
C. rawsonia	□	▨	■	■	▨	■	▨	□	□	□	□	□
C. attenuatus	▨	■	■	▨	▨	▨	□	□	□	□	□	□
C. appendiculatus	▨	□	□	□	□	▨	□	□	□	□	▨	■
C. angustatus	□	▨	▨	▨	□	□	□	□	□	□	□	□
C. campbelli	□	□	□	□	□	▨	□	□	□	□	□	□

Figures

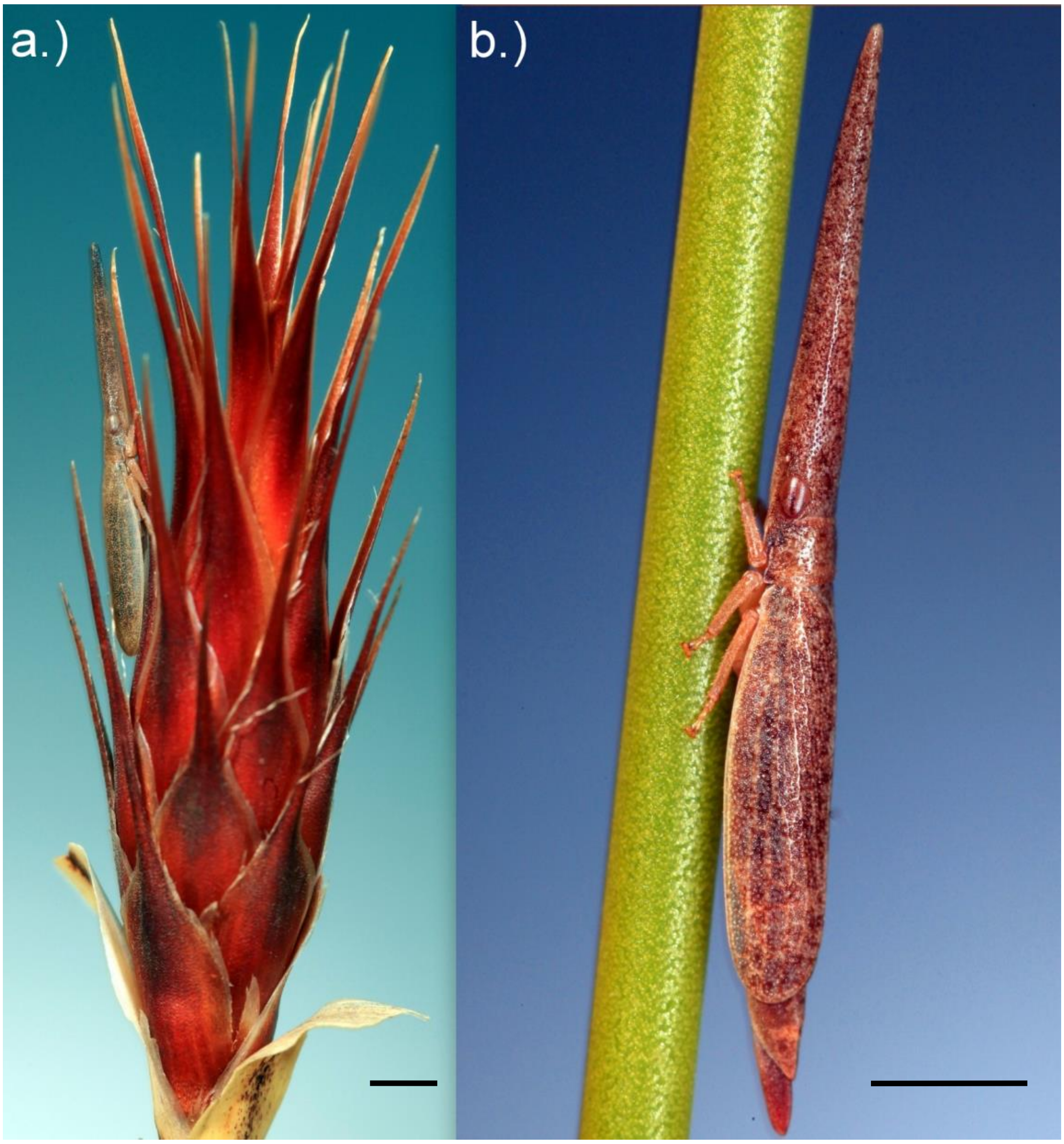


Figure 2.1: a) *Cephalelus uncinatus* male on an inflorescence bract of female *Hypodiscus aristatus*. b) A flightless *Cephalelus uncinatus* female on culm of *Hypodiscus aristatus.* Scale bars represent 2mm.

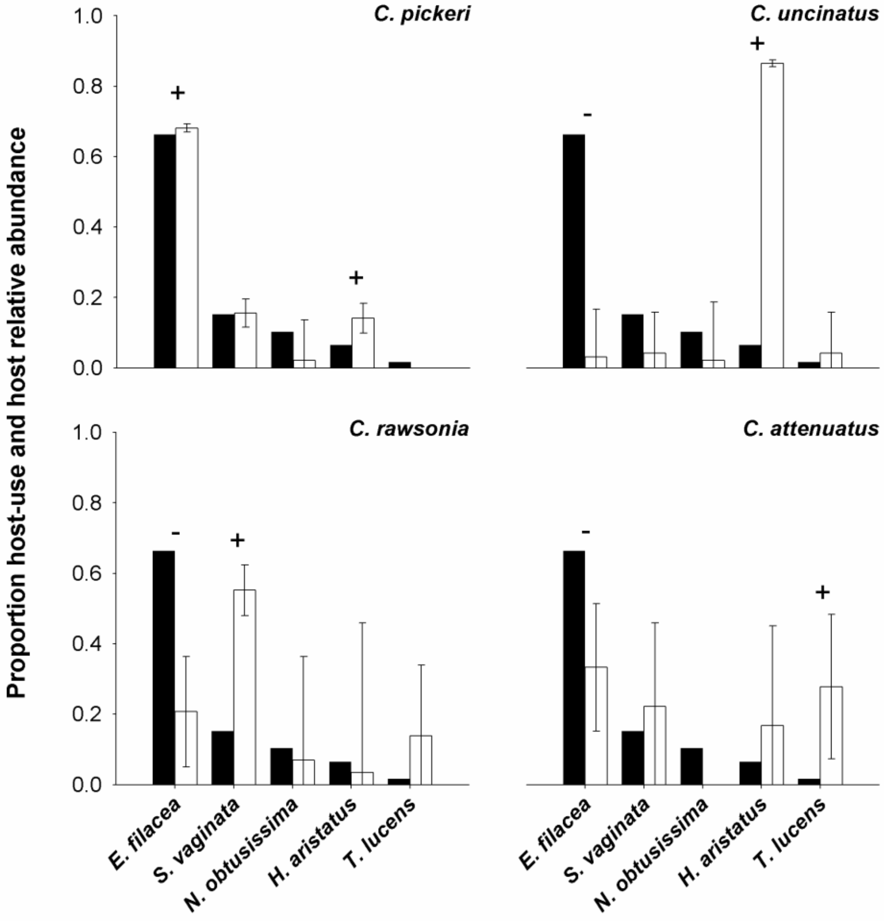


Figure 2.2: Host specialisation analysis for *C. pickeri*, *C. uncinatus*, *C. rawsonia* and *C. attenuatus* with Bonferroni corrected 95% CI. Black bars indicate the relative abundances of the host plants. White bars indicate host-use. Plusses (+) indicate significant over-utilization indicative of host specialisation. Minuses (-) indicate significant avoidance.

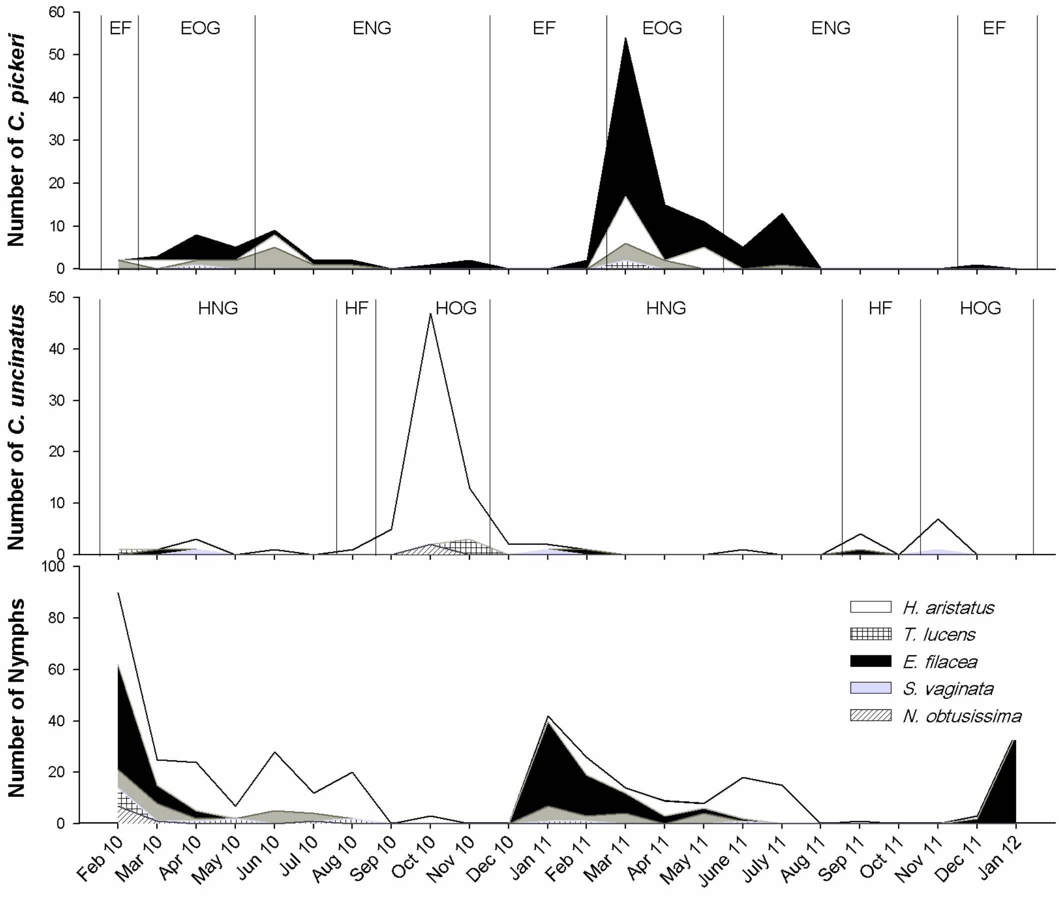


Figure 2.3: Stacked time series showing the temporal host use patterns of the two dominant *Cephalelus* species at the sampling site and for nymphs, which could not be identified to species. Shading under the curves indicates the host species identify, while letters indicate host phenophases (F-flowering, NG – new growth, OG – old growth) of the dominant host species used by *C. pickeri* (E – *E. filacea*) and *C. uncinatus* (H- *H. aristatus*).

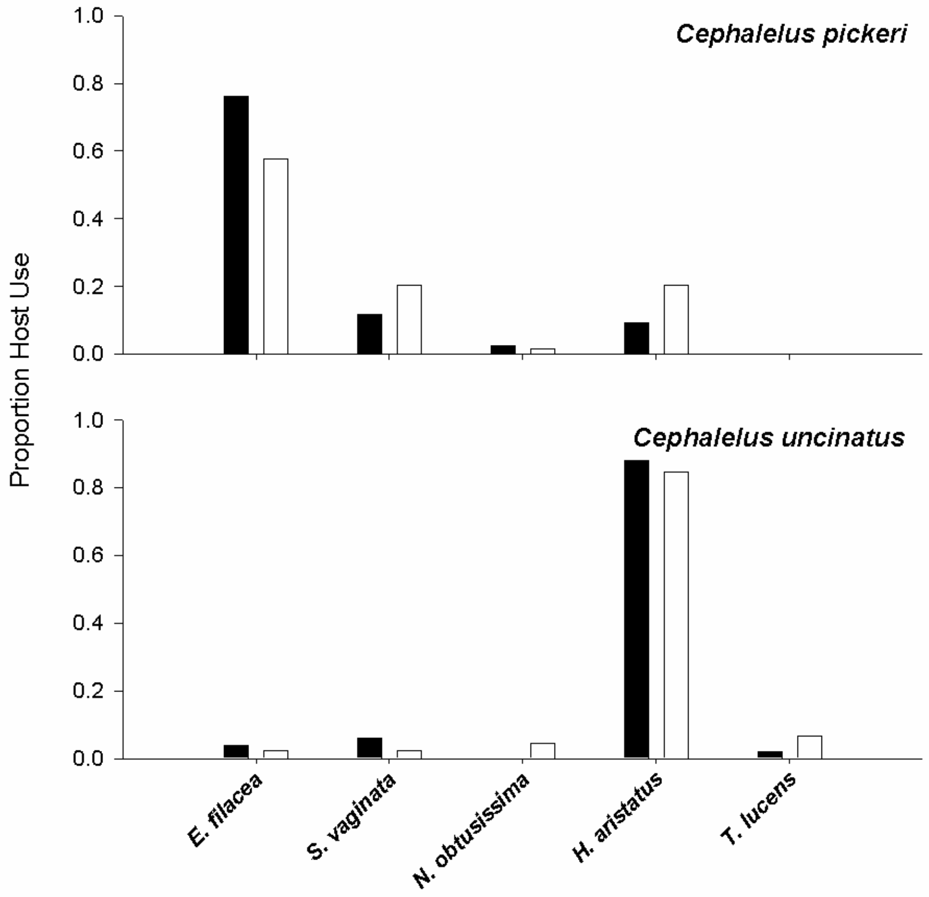


Figure 2.4: Comparison of host preference between males and females of the two dominant *Cephalelus* species. No evidence was found for divergent host use between males (black bars) and females (white bars).

Chapter 3

Evolutionary processes, and not contemporary competition, explain most niche partitioning in a plant-herbivore community network

Under review at Journal of Animal Ecology

Abstract

Patterns of niche partitioning can result from local ecological interactions (i.e. interspecific competition) occurring within a contemporary time frame (realised niche partitioning). Alternatively they may represent the end-product of evolutionary processes acting over longer time frames (fundamental niche partitioning). Niche partitioning is often detected by analysing patterns of resource use within communities, but experiments are rarely conducted to test whether patterns of niche partitioning are ecological or evolved. I studied a community of restio leafhoppers from the genus *Cephalelus*, and their host plants, the Restionaceae (restios). I used network and experimental approaches to determine whether network modularity (a measure of niche partitioning within local communities) results from ecological or evolutionary processes. Using a weighted modularity index for two party networks (e.g. insect - plant) I determined whether the network of this community is modular (i.e. consists of groups of species interacting strongly, with weak interactions between groups). I also aimed to identify specific *Cephalelus* - restio modules (groups).Using knowledge of module membership, I tested whether *Cephalelus* species from two different modules, *C. uncinatus* and *C. pickeri*, prefer and perform better on restios from their own modules versus restios from other modules. These experiments were performed under controlled conditions, eliminating the influences of competition and predation on host choices. The *Cephalelus* – restio community was significantly modular, implying niche partitioning. *Cephalelus* also preferred and performed better on restios from their own modules in the absence of local contemporary factors. I demonstrate the importance of evolutionary processes as drivers of niche partitioning in a local community.

Introduction

Biological communities often exhibit niche partitioning. It is a pattern that is thought to emerge largely as a result of contemporary interspecific competition (i.e. an ecological process) or from divergence in niche use as an evolutionary response to interspecific competition playing out over evolutionary timeframes (i.e. an evolutionary process). Current competition can structure niche use patterns through competitive exclusion when species using the same resources cannot co-exist because one always drives the other to extinction (Gause et al. 1934, Hardin 1960). This mechanism assumes that resource use traits are fixed. Patterns of resource use can, however, also result from direct contemporary interspecific competition that does not lead to competitive exclusion. For example, phenotypic/behaviour plasticity influenced by interspecific competition can change patterns of resource use (Pfennig and Murphy 2000, Harrington et al. 2009). Contemporary processes driving patterns of niche partitioning, excluding competitive exclusion, do so by reducing the fundamental niche to the realised niche (Connell 1961, Arakaki and Tokeshi 2011). For example, the famous experiments conducted by Connell (1961) on barnacles showed that when one barnacle species was manually removed, another species expanded its range of habitat use. Thus the observed pattern under natural conditions resulted from non-overlapping realised niches. Here I will refer to non-overlapping resource use patterns resulting from current competition as realised niche partitioning.

Changes to the fundamental niches of species can result from ecological character displacement (referred to as ECD henceforth), an evolutionary process whereby exploitative competition between species using the same resource causes a reduction in fitness (Schluter 1994). Over time competing species then evolve to use different resources, thereby alleviating interspecific competition (Brown and Wilson 1956, Schluter 1994). When this process occurs between multiple species within a community, it is termed community-wide ECD (Strong et al. 1979). Patterns of niche partitioning can also result from other processes that do not involve interspecific competition (Schluter 1984). Niche partitioning of local communities is, for example, expected if they comprise species that have evolved divergent resource use in allopatry (potentially in the absence of competition) and subsequently expanded their geographic ranges (Stuart and Losos 2013). If field patterns arise purely from ECD (or any other process shaping the niche over evolutionary time), observed niche use should reflect the fundamental niches of organisms. Therefore I will refer to patterns of niche partitioning resulting from evolutionary processes collectively as fundamental niche partitioning.

Niche partitioning has been studied using two broad approaches: 1) studying patterns of resource use in entire communities from which patterns of niche partitioning and underlying mechanisms are inferred or, 2) experimentally assessing preference and performance (niche breadth) of select species, which limits the ability to make inferences about whole communities. Typically, community-wide studies analyse patterns of niche partitioning by constructing species-resource-use matrices. The observed level of niche partitioning of resources (quantified by several different metrics) is then compared to that generated by a null model (e.g. Winemiller and Pianka 1990). Some community wide studies on niche partitioning have, however, made use of both pattern-based and experimental approaches. This is true for studies conducted on anole lizard communities in particular (Losos 1992, Irschick and Losos 1999). In this system it has convincingly been shown that community structure has resulted from ECD using a variety of approaches (Losos 1992, 1998, Irschick and Losos 1999).

In recent years, the study of community structure has been focused on interaction networks (e.g. Rezende et al. 2007, Thompson et al. 2013, Toju et al. 2014). Network approaches, similar to traditional approaches (e.g. Winemiller and Pianka 1990) use indices to quantify community structure and then compare indices to null models (Schleuning et al. 2014). One such metric, modularity, describes the level of niche partitioning within a network. Specifically, modularity describes the extent to which a community comprises groups of strongly interacting species weakly connected to other such groups. For example, in a modular plant-pollinator network, different pollinator species group around specific flower types (Olesen et al. 2007). The advantage of studying niche partitioning patterns in networks is that interactions within whole communities can be analysed. However, community-wide patterns of resource use can contain the signatures of both ecological and evolutionary processes, making it difficult to distinguish the relative roles of particular processes.

Experimental approaches typically only focus on a small subset of species within the community, but have the advantage of potentially revealing the mechanisms driving patterns of niche partitioning. Connell (1961), for example, showed experimentally that competition for space drove patterns of realised niche partitioning. Although not specifically designed to do so, experiments testing preference for and performance on particular resources are often conducted in the absence of competition, thus providing insight into the extent to which interspecific competition determines realised niche partitioning. For example, Thompson (1993) experimentally tested whether butterflies have evolved preferences for the host plants that they use in the field. When combined with field patterns of community structure, experiments like Thompson's can be useful for understanding whether community structure is determined by evolutionary or ecological processes: If patterns of niche partitioning result from processes that can be experimentally demonstrated in the absence of contemporary competition (i.e. the realised niche remains the same in the presence or absence of interspecific competition), then pattern is the end product of evolutionary processes alone. If niche partitioning patterns mismatch experimental findings in the absence of interspecific competition (i.e. the realised niche is different in the presence and absence of interspecific competition), then both ecological processes and evolutionary processes are probably playing a role in structuring communities. Lastly, if pattern is not explained by experiments in the absence of interspecific competition, resource partitioning is likely entirely the result of ecological processes.

Here I adopt this two-pronged approach – I first look at the niche partitioning pattern in the context of a local community and then experimentally test whether resource use remains unchanged in the absence of interspecific competition. In particular, I use this approach to study niche partitioning in a local community of herbivorous insects from the genus *Cephalelus* (tribe Ulopinae Cephalelini).*Cephalelus* are specialised on the Restionaceae family (Davies 1988, Prendini 1997). Previously, Augustyn et al. (2013) found that different *Cephalelus* species were using different restios within a local community, suggesting niche partitioning. However, it is unclear whether *Cephalelus* are exhibiting fundamental or realised niche partitioning. *Cephalelus* may, for example, like in other herbivorous insects, exhibit interspecific aggression (McLain 1981, Raupp et al. 1986, Flamm et al. 1987). This may drive species of *Cephalelus* to use host plants that they do not prefer or perform best on.

To test this, I first constructed a local community network and identified specific *Cephalelus* – restio modules contributing to the overall modularity (a measure of niche partitioning) of the local community. By means of preference and performance experiments conducted in the absence of interspecific competition I then test whether module membership can be explained by preference and performance relationships. I ask the following questions: 1) Is a local *Cephalelus* – restio community modular, suggesting that either fundamental and/or realised niche partitioning play a role in structuring the *Cephalelus* community? 2) Do *Cephalelus* species, in the absence of interspecific competition, prefer and perform better on restios with which they share a module opposed to restios that they do not share a module with? If they do, this suggests that fundamental niche partitioning plays a role in structuring hopper communities. 3) Do host use patterns in the field match experimental findings entirely, suggesting that within module interactions are also determined by evolutionary processes? Where possible, host use by *Cephalelus* was investigated separately for females and males to determine whether host use and its underlying determinants differ between the sexes.

Materials and methods

Field survey and network analysis

To construct a *Cephalelus* – restio network, I conducted monthly surveys at Pringle bay (34.3259 S, 18.8401 E) at a single 2500m^2 site. During each monthly sampling interval, from February 2010 until January 2012, 40 individual plants of five restio species (*Hypodiscus aristatus*, *Elegia filacea*, *Nevillea obtusissima*, *Thamnochortus lucens*, and *Staberoha vaginata)* were sampled for *Cephalelus*. A single individual of *Willdenowia glomerata* was present at the site, but was not sampled. This amounts to 200 plants sampled every month. Sampling was standardised, by beating individual plants ten times into a fine-meshed beat net. Adult *Cephalelus* were then preserved in alcohol for identification in the laboratory. Following the approach outlined in Augustyn et al. (2013), I identified male insects by dissecting their genitalia and then linking them with females through external morphology. I also estimated the relative cover of restios by means of 10 randomly placed 40m transects.

As Augustyn et al. (2013) did not find temporal host-switching, and because only 292 adult *Cephalelus* were captured, I lumped data from all months for network analysis. Because differences between the sexes of the most abundant *Cephalelus* species (*C. uncinatus* and *C. pickeri*) were the focus of further experiments in this study, sexes of these species were treated separately in the network analyses. This allowed me to test whether females and males of the same species belong to the same modules and whether females and males both exhibit the same level of specificity. *Cephalelus* were destructively sampled, eliminating the possibility of pseudo replication (i.e. sampling the host use of an individual more than once). The species used in this study are listed in Table 3.1.

I used the QuaBiMo algorithm (Dormann and Strauss 2014) to calculate modularity. Currently it is the only algorithm that uses quantitative interactions between species to identify modules in bipartite networks. It uses an iterative process to find the most modular arrangement of species in an association dendrogram, with branches weighted by the strength of the interactions. This means that distinction is made between weak and strong interactions. Modularity defined as Q, was determined as the highest value after 10^6 permutations. Allowing more permutations and rerunning the analysis did not change Q. After finding the most modular arrangement of my network, I tested it for significance against a null model with 1000 randomisations. Null model Q values were calculated for each random matrix, and transformed to z-scores, against which the z-score of the observed Q value was tested.

For comparison with other published networks, I also calculated H2’ (Blüthgen et al. 2006), which is highly correlated with Q (see Dormann and Strauss 2014). H2’ is a network wide measure of specialisation and niche partitioning which is based on Shannon entropy (similar to Shannon's diversity index). To compare the level of specialisation of individual species and sexes within my network, I calculated d’. This metric is based on the same principles as H2’, and shares similar properties such as robustness against the number of interactions detected within a network (Blüthgen et al. 2006). It is calculated as the frequency distribution of resource use of *Cephalelus* species compared to the relative availability of restios (estimated by means of proportion cover). The relative proportions of restios used to calculate d’ were in descending order: *Elegia filacea* – 0.55, *Staberoha vaginata* – 0.24, *Nevillea obtusissima* – 0.13, *Thamnochortus lucens* – 0.05 and *Hypodiscus aristatus* – 0.03. All network analyses were performed in the R package Bipartite.

Preference experiments

Dual host choice experiments were used to test whether host preference explains module membership. For dual host choice experiments, I used the most utilised restio species from each identified *Cephalelus* - restio module; these were: *Hypodiscus aristatus*, the only restio module member in the *Cephalelus uncinatus* module (red module, Fig 3.1), *Elegia filacea*, the only restio module member of the *Cephalelus pickeri* module (blue module, Fig.3.1), and *Staberoha vaginata*, the primary host of *Cephalelus rawsonia* (yellow module, Fig 3.1). Although *C. rawsonia* was the third most abundant *Cephalelus* species in the community, it was not common enough to be used in experiments. Thus experiments tested host preferences of *C. pickeri* (blue module, Fig 3.1) and *C. uncinatus* (red module, Fig 3.1) across the preferred restio species in each module (*Hypodiscus* – red module, *Elegia* – blue module, *Staberoha* – yellow module, Fig 3.1). As *Cephalelus* are hemimetabolous (larvae resemble adults and typically have the same feeding biology as adults), adult feeding preference should reflect larval feeding preference, and they should respond similarly to factors like plant chemistry. For this reason, instead of quantifying female oviposition preference, I conducted dual perching choice experiments for males and females of both species. An additional advantage of this approach is that it allowed us to compare the preferences of the sexes within species. Both species and sexes were offered all combinations of *Hypodiscus*, *Staberoha* and *Elegia*.

Cephalelus uncinatus and *C. pickeri* were collected in the field from the two host plants in their modules. During collecting, insects were individually placed into clean vials, and kept cool in a cooling box to prevent them from injuring themselves or overheating. After collecting, both insects and plant cuttings were kept overnight in a fridge at 10 °C. Experiments were always initiated the day following collection from the field. I collected restio cuttings haphazardly from the same site after each collecting session. Experiments on *C. uncinatus* caught from *Hypodiscus* were initiated on 5, 11, and 26 October, 10 and 29 November, and 13 and 21 December 2011, when it was most abundant. For *Cephalelus pickeri* caught from *Elegia*, experiments were initiated on 20 March, 24 April, and 8 May 2013, when it was most abundant.

Insects were randomly separated into three experimental groups which were each cycled between three different preference experiments: *Hypodiscus* vs. *Elegia*, *Hypodiscus* vs. *Staberoha* or *Elegia* vs. *Staberoha*. To avoid competitive or sexual interactions, individual insects were dropped alone into 740 ml preserve jars with one 135 mm cutting of each restio species. Culms were placed in the jar so that they were touching. To keep restio cuttings fresh, I attached 0.6 ml vials with distilled water to the bottom of each cutting. I prevented fogging of jars by replacing lids with fine gauze. Insects could easily move over the glass surface of the jars, and from one culm to another. Preliminary three hourly observations of preference revealed that the majority of insects stayed on the same culm after 12 hours. Therefore host preference was recorded after 12 hours and the experiment terminated. Experimental arenas were then reset with fresh restio culms before cycling each experimental group of insects onto a new choice combination. Each individual insect was thus exposed to all three combinations of restios over three days. After all experiments were completed, insects were sexed and preference was analysed separately for males and females.

I analysed host preference data using separate binomial generalised linear models (GLM) with log link functions for each of the six experiments (i.e. three choice experiments per *Cephalelus* species). The response variable was always a binary choice for the restio species most frequently used in the field (indicated in the upper bar of Figures 3.2a and b for each experiment). Each model tested for the role of the independent variable, sex, in determining host choice. For each GLM I back transformed and plotted GLM estimates of means and 95% CIs on a scale ranging from 0 to 1. If 95% CIs did not overlap with a 0.5 preference, a preference for one of the compared restio species was assigned. All GLMs were implemented in the base GLM package in R version 3.

Performance Experiments

To test whether the host preferences of adult *C. uncinatus* and *C. pickeri* are linked with evolutionary adaptation (e.g. in response to unidentified plant chemistry/structural defences) I measured insect survival on different host plant species from two different modules (*Hypodiscus* and *Elegia*). These plants were caged to exclude predators and competitors (i.e. contemporary ecological factors) in the field. Survival is an accepted surrogate for performance in the insect preference-performance literature (Gripenberg et al. 2010), and also potentially a more direct measure of fitness than, for example, body mass (see Stockhoff 1991). I also tested whether females and males respond similarly to host plants (i.e. evolved factors). I used a vacuum sampler to suck all arthropods (potential competitors and predators) off individual restio plants prior to caging them with a very fine (0.2 mm) mesh. It should be noted that I do not have complete certainty that I excluded small parasitoids like strepsipterans and wasps that lay their eggs on the host plants. However, I visually detected and excluded field-caught *Cephalelus* infested by strepsipterans and wasps prior to introducing them to the cages. Strepsipterans were visible as black “warts” on the outside of the abdomen, and a wasp infection as a distinctive darkening of the abdomen. Field-caught *Cephalelus* were introduced to cages directly after catching and inspecting them for parasites. For each *Cephalelus* species, five adult males and five females were introduced to either a caged *Hypodiscus* or *Elegia* plant in the field. Eight replicate plants of each species were caged, and thus 140 individuals of each insect species were used. After 20 days, cages were reopened and insects were vacuum sampled off the caged plants. Vacuuming continued until no further insects could be removed from the plants, and all survivors were counted. Controlling for additional hatching of eggs was not needed as newly hatched nymphs would not reach adulthood over the time span of the experiment. Also, no nymphs were caught during these experiments. These experiments were performed – once for *C. uncinatus* (16 October – 5 November 2013) and once for *C. pickeri* (9-29 April 2013). One cage containing *Hypodiscus* was damaged whilst testing *C. uncinatus* survival and was excluded from the experiment.

To test whether adult survival differs between host plants and sexes, I used binomial generalized linear mixed effect models (GLMMs) with log link functions. Each species was analysed with a separate GLMM with individual caged plant as a random factor. For each GLMM, I included insect sex and host species as independent variables, together with an interaction term between sex and host species. Both GLMMs were implemented in the R package lme4.

Survival experiments were also performed on *C. pickeri* nymphs; again survival was compared between *Hypodiscus* and *Elegia*. However, nymphs could not be sexed, so I only tested whether nymphs survived better on their natal host plant, *Elegia* compared to *Hypodiscus*. I caught similar sized nymphs from *Elegia*, and measured them with digital callipers before introducing them to the cages. Nymph sizes did not differ between cages (F = 1.321, df = 9, P = 0.228, Fig S3.1). The experiments were similar to the adult experiments, except that I caged only five plants per species, and added three emergence controls per restio species. These controlled for the possible hatching of *C. pickeri* nymphs, which could have inflated the survival number on *Elegia*. Another modification was that I introduced 20 nymphs per cage, and recorded survival after 12 days (from 9-21 February 2012). To confirm the identity of the nymphs, they were returned to their cages, and after two months survivors were collected as adults and identified. I was unable to conduct this experiment for *C. uncinatus*, as I could not catch enough nymphs of the same size class. I used the same statistical approach to analyse nymph survival, except that sex was not taken into account, and consequently no interaction term was included.

Results

Network analyses

The weighted network was significantly modular (Q = 0.37, z-score = 18.24). Three different modules were identified: The first module consisted of three restio leafhopper species (*C. uncinatus* females and males, *C. angustatus* and *C. campbelli)* associated with a single restio species, *H. aristatus* (Fig 3.1 –red module). The second module consisted of four restio hopper species (*Cephalelus attenuatus*, *C. appendiculatus*, and *C. rawsonia* associated with three restio species (*T. lucens*, *S. vaginata* and *N. obtusissima*) (Fig 3.1 –yellow module). The third module contained only *Cephalelus pickeri* males and females which associated with a single restio species *Elegia filacea* (Fig 3.1 - blue module).

The two most common *Cephalelus* species, *C. uncinatus*, and *C. pickeri* were assigned to modules with only one restio species each, indicating strong host specialisation. Interestingly, male host use patterns appeared slightly more specialised than female host use patterns (Fig 3.1). This was reflected by slightly lower d' (ranges from 0 (compete generalist) to 1 (complete specialist)) values for females compared to males (Table 3.1). The specialisation of the total web (with sexes lumped), measured by H2' was 0.39 (scale similar to d').

Preference experiments

Female and male *Cephalelus* of both *C. uncinatus* and *C. pickeri* exhibited similar trends in host preferences (Fig. 3.2). However, there were some differences in the strengths of their preferences. *C. uncinatus* (Fig. 3.2a) always preferred the host plant (*Hypodiscus)* from its own module, over hosts from other modules, *Elegia* and *Staberoha* (95% CI always higher than 0.5). Males and females did not have different preference strengths for either of these choices (*Hypodiscus* vs. *Elegia,* z = 0.669, P = 0.504, left panel; *Hypodiscus* vs. *Staberoha*, z = 0.684, P = 0.493, centre panel). Given the choice between two hosts from other modules (*Elegia* vs. *Staberoha),* males had a significant preference for *Elegia* (95% CI higher than 0.5), but females had no preference (95% CI overlaps with 0.5). The difference in the strength of preference by male and female *C. uncinatus* between these two non-member plant species was significant (z = 2.658, P = 0.008 right panel). This suggests a non-hierarchical preference for *C. uncinatus* females (*Hypodiscus* >*Elegia* = *Staberoha*), and a hierarchical preference for males (*Hypodiscus* >*Elegia* >*Staberoha*). In *C. pickeri* (Fig. 3.2b) both males and females preferred their module member plant, *Elegia* over a non-member, *Staberoha* (left panel), but males had a stronger preference (z = 2.275, P = 0.023). However, between *Elegia* (member) and *Hypodiscus* (a non-member) (Fig. 3.2b centre), females had a stronger preference for *Elegia* (z= 2.615, P = 0.009), and males had no preference for *Elegia* (member) over *Hypodiscus* (non-member). Males had a significant preference for *Hypodiscus* over *Staberoha*, two non-members, but females did not (Fig. 3.2b left). However there was no significant difference between males and females in their strength of preference (z = 0.463, P = 0.643).As it is, females do not show a hierarchical ranking (*Elegia* > *Hypodiscus* = *Staberoha*), and neither do males (*Elegia* = *Hypodiscus* > *Staberoha).*

Survival

More *C. uncinatus* adults survived on their preferred host plant and module member, *Hypodiscus* than on their less preferred host and non-member, *Elegia* (Host: z =

2.229, P = 0.026, fig 3.3a). Insect sex had no influence on survival in general (Sex: z = 0, P = 1, Fig 3.3a) and both sexes were affected equally by host species identity (Plant X Sex: z= 1.406, P = 0.159, Fig 3.3a). This was also true for *C. pickeri*, where more adults survived on *Elegia* (module member) than on *Hypodiscus* (non-member) (Host: z = 3.568, P < 0.001, Fig. 3.3b). Similar to *C. uncinatus*, insect sex had no influence on survival (Sex: z = 0.624, P = 0.533, Fig. 3.3a), and both sexes were affected similarly by host species identity (Host X Sex: z = 0.158, P = 0.874, Fig 3.3a). Similar to their adult phase, *Cephalelus pickeri* nymphs survived better on *Elegia* than on *Hypodiscus* (Plant: z = 5.211, P < 0.001, Fig 3.3c). When nymphs reached adulthood on *Elegia*, all were positively identified as *C. pickeri*. No nymphs were caught from emergence controls.

Discussion

I find that a local *Cephalelus* - restio community network is modular, and that the degree of modularity is non-random, suggesting that restio niches are partitioned. *Cephalelus* host use (specifically module membership) observed in the field could be explained by preference and performance relationships in the absence of competition or predation. This suggests that the influence of current ecological interactions in generating smaller realized niches does not appear to play a significant role in the niche partitioning patterns observed in this system. Consequently, network structure (and therefore community structure) is most likely an evolved property which is the result of fundamental niche partitioning.

Network structure

Attempting to understand the mechanisms underlying network structure is a relatively new endeavour (Vázquez et al. 2009). Recent studies have shown that the evolutionary history of network members can determine network structure (Ives and Godfray 2006, Rezende et al. 2007, Thompson et al. 2013). One generalisation emerging out of studies investigating determinants of network structure is that network structure is dependent on the type of relationship between interacting partners (Guimarães et al. 2007, Thompson et al. 2013). In particular, non-mutualistic networks should have weaker specificity (and lower modularity) than those of mutualistic networks (Guimarães et al. 2007). This implies that the degree of niche partitioning (or niche overlap) should differ between community types. The metrics that I used in this study, network wide specificity (H2') and modularity (Q) are both measures of niche partitioning (Blüthgen et al. 2006, Dormann and Strauss 2014, Morris et al. 2014). I am not aware of any published quantitative herbivorous insect – plant networks that used the same metrics that I used in this study. However, a recent study by Morris et al. (2014) sourcing many antagonistic networks (parisitoid-insect) across the globe report H2' and Q values which encompass the values observed by my study. Specifically, I report an H2' value of 0.39 which falls within the range of values (0.65 ± 0.31, SD) reported by Morris et al. (2014). Similarly, my Q value of 0.37 is similar to the average of 0.26 (SD 0.19) reported by Morris et al. (2014), which was based on small networks (< 100 celled interaction matrices).

Realised niche partitioning

I argued that if modules could be explained by preference and performance, the *Cephalelus* community in my study likely exhibits evolved or fundamental niche partitioning. It should, however, be mentioned that I did not investigate modules within modules (Dormann and Strauss 2014). Thus, inferences in my study potentially only relate to broad network structure. It is, for example, possible that modules within modules are driven by realised niche partitioning. For example, the yellow module (see Fig 3.1) might be formed because all *Cephalelus* species in that module prefer *S. vaginata*. However, interspecific competition might force *C. attenuatus*, for example, to use *T. lucens*, potentially forming a separate sub module within the larger yellow module. Also, I only investigated the preference and performance relationships of two species within this network and therefore I cannot rule out that host use of other species may be driven by realised niche partitioning.

Although broad host use patterns appear to be structured mainly through evolved, fundamental niche partitioning, current ecological factors appear to account for a minor part of the partitioning observed. For example, the field specificity of restio leafhoppers was not a perfect mirror of the preference experiments: Specifically, in the field, *C. pickeri* males were more host specific than *C. pickeri* females (d' values of males were closer to 1). Males did not use *H. aristatus* (a non-module member restio), whereas females frequently did so. The opposite was true in preference experiments: Males showed no preference between *E. filacea* (sole module-member restio of *C. pickeri*) and *H. aristatus*, whereas females had a strong preference for *E. filacea* over *H. aristatus* (Fig 3.2b). A possible explanation for this, is that females move away from *E. filacea* after having mated, to avoid sexual harassment from males (Gosden and Svensson 2009). Although I can only speculate on the actual mechanism, it nevertheless appears as though some ecological processes may play a small role in shaping network structure.

Fundamental niche partitioning

Despite some incongruence, the choices made by *Cephalelus* largely supported the hypothesis that community structure reflects fundamental niche partitioning. Females of both *C. uncinatus* and *C. pickeri* always preferred module member restio species over non-module member restio species and they showed no secondary preferences. In contrast, both male *C. uncinatus* and *C. pickeri* had secondary preferences, i.e. had preferences between non-module member restios. This suggests that *Cephalelus* females might be choosing restios that maximise larval performance (besides their own performance) (Scheirs et al. 2000, Gripenberg et al. 2010), while males choose restios suitable only for their own nutritional requirements (Scheirs et al. 2000).

While this suggests that preferences likely reflect adaptation to bottom-up factors (e.g. host plant related allelochemicals or nutrients), this does not need to be the case (Bernays and Wcislo 1994). For example, insects may evolve to choose plants that are not the best food plants, but are free from competitors or predators (Courtney 1981, Denno et al. 1990). In this case host preference shifts may evolve first in response to these interactions, followed by adaptation to bottom-up factors. In *Cephalelus*, if interspecific competition drove field host use patterns in contemporary time, I would have expected to see incongruence between field patterns (modules), preference or/and performance. Instead I found that both male and female *C. uncinatus* collected from *H. aristatus* performed better on their preferred host, *H. aristatus,* than on *E. filacea*. Similarly, *C. pickeri* adults collected from *E. filacea* performed better on *E. filacea* than on *H. aristatus*. Also, *C. pickeri* larvae had significantly reduced survival when transferred from *E. filacea* to *H. aristatus* compared to when transferred to *E. filacea*. The reduction of survival shown for *C. pickeri* nymphs, also suggests that the preferences made by *Cephalelus* females might be linked to maximising larval performance (Gripenberg et al. 2010). These findings suggest that, differences in survival can be ascribed to bottom-up factors, i.e. differential adaptation to allelochemicals (Després et al. 2007) or plant nutrition (Carroll et al. 1998). Considering that there is little incongruence between module membership, host preference and performance, my findings suggest that fundamental niche partitioning is unlikely to be the result of recent interspecific competition.

Conclusion

Quantitative network metrics, such as the recently developed weighted modularity for bipartite networks (Dormann and Strauss 2014), allow the exploration of network structure within small networks such as the one in my study. Studying a small bipartite network allowed me to conduct experiments to test whether network structure is determined by fundamental or realised niche partitioning. I found that preference and performance were linked to modules within a *Cephalelus* – restio network, suggesting that modularity arose as a result of fundamental niche partitioning. This suggests that modularity in this system is primarily an evolved product and is not very strongly influenced by contemporary ecological factors. In addition to ECD, another plausible evolutionary mechanism which may generate niche partitioning is allopatric divergence and secondary contact: Species may adapt to different resources in allopatry and then maintain those adaptations after colonising the same geographic region (Stuart and Losos 2013). The resulting evolutionary niche partitioning pattern can therefore result from ecological speciation, initiated or finished in allopatry, followed by secondary contact (Rundle and Nosil 2005).

Tables

Table 3.1: Numbers of all *Cephalelus* species and sexes of *C. uncinatus* and *C. pickeri* caught over the 24 month sampling period. Specificity values (d') from network analysis are shown in the last column. The metric ranges from 0 -1 (complete generalist – complete specialist).

Insect Species	Host species					Total	d'
	H. aristatus	*E. filacea*	*S. vaginata*	*T. lucens*	*N. obtusissima*		
C. uncinatus (F)	39	1	1	3	2	46	0.76
C. uncinatus(M)	44	2	3	1	0	50	0.79
C. pickeri (F)	12	34	12	0	1	59	0.60
C. pickeri (M)	7	58	9	0	2	76	0.90
C. rawsonia	1	6	16	1	2	26	0.10
C. attenuatus	3	6	4	4	0	17	0.15
C. appendiculatus	3	4	3	0	0	10	0.16
C. angustatus	5	0	0	0	0	5	1
C. campbelli	1	0	0	0	0	1	1

Figures

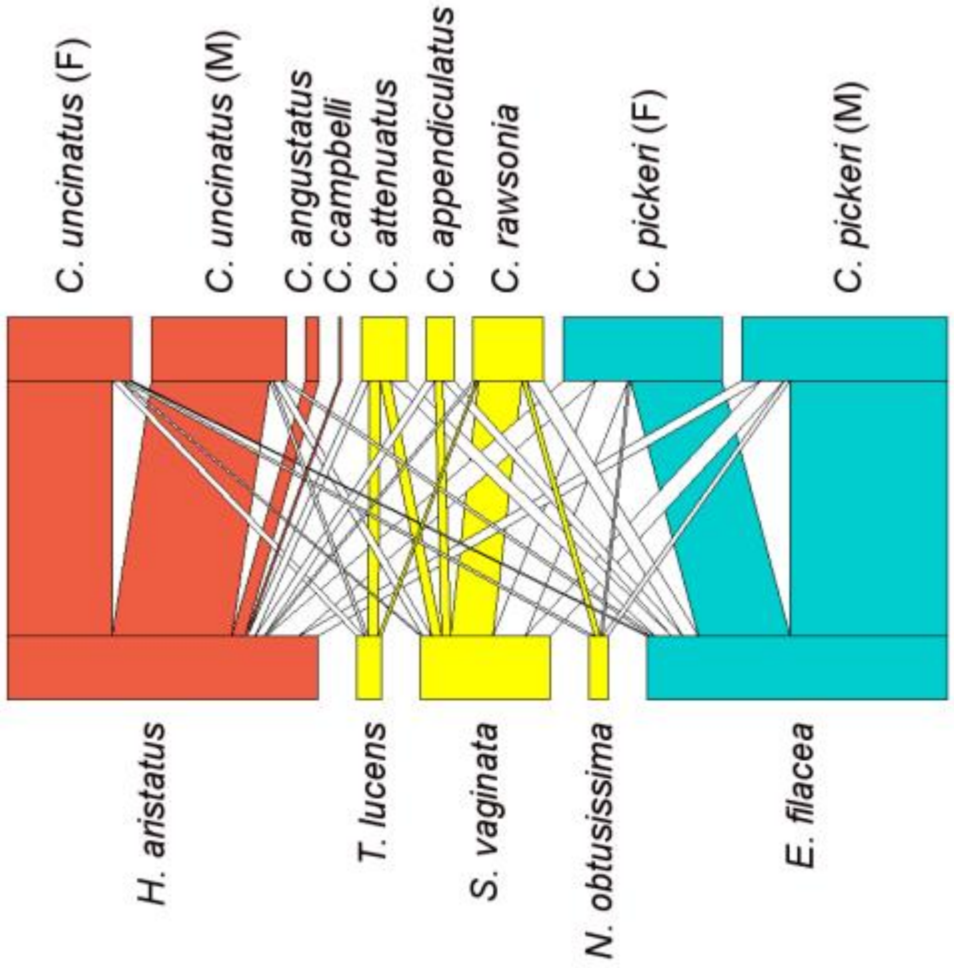


Figure 3.1: A weighted network of a *Cephalelus* - restio community sampled for two years at Pringle bay. The upper level shows the six *Cephalelus* species at the site, with males (M) and females (F) of *C. uncinatus* and *C. pickeri*. The widths of their blocks show their relative densities on all of the host plants at the site. The lower level shows the five restio species at the site, and the relative densities of all *Cephalelus* species collected from them. The number of individuals of each *Cephalelus* species caught from each restio species generates the interaction strength between the two. I identified three different modules indicated by three colours. Sexes of the same species grouped together in the same modules. Coloured interactions are within module interactions, and unfilled interactions are between module interactions. Notice the strong within module interactions between *C. uncinatus* and *H. aristatus*, *C. rawsonia* and *S. vaginata* and, *C. pickeri* and *E. filacea*.

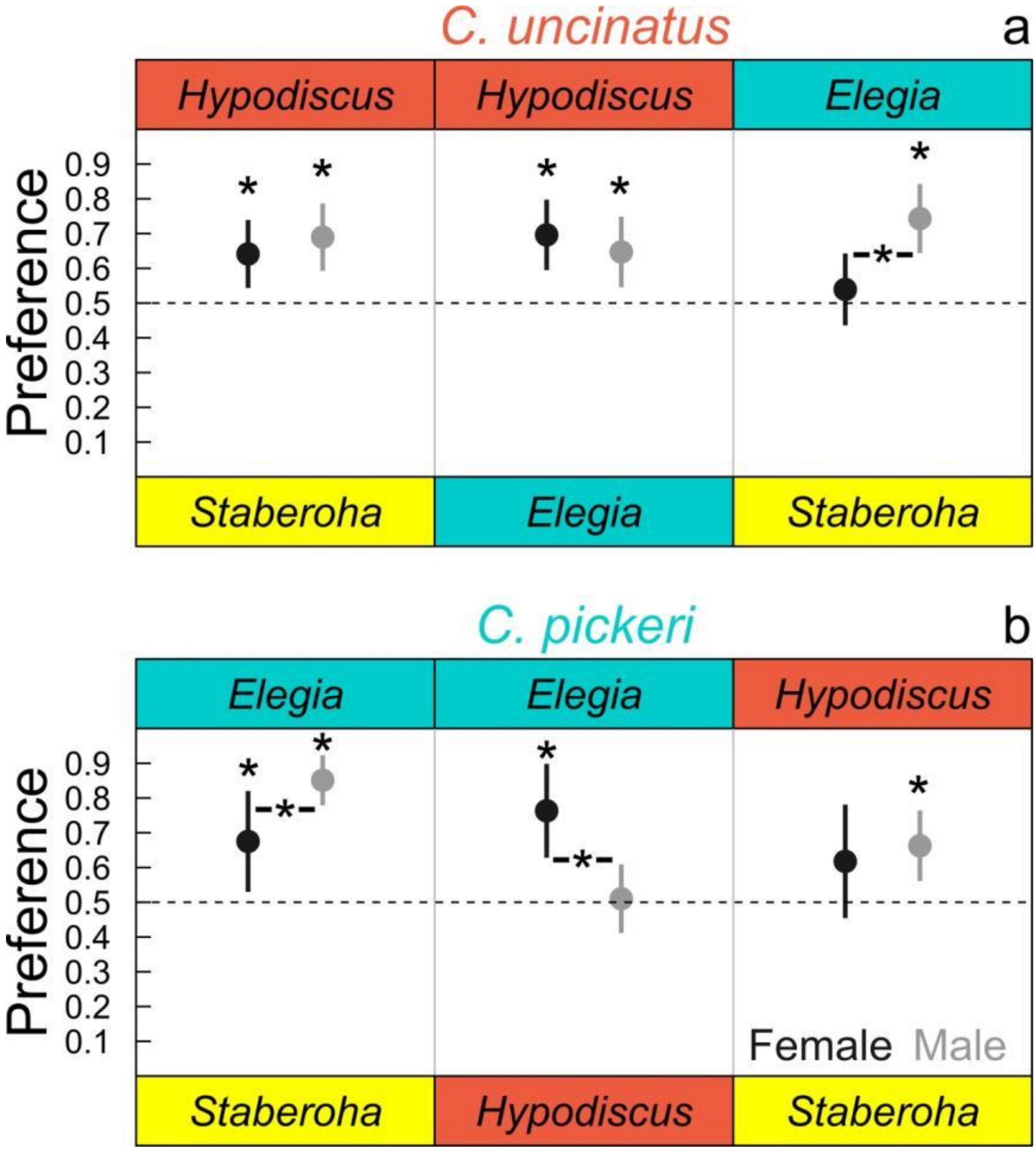


Figure 3.2: Preferences of *C. uncinatus* adults (panel a) and *C. pickeri* adults (panel b). Female preference is represented by black points and bars (GLM estimated means and 95% confidence intervals) and male preference by grey points and bars. The restio species names above and below plot areas indicate the choices offered, and their colours represent their module membership. When 95% CIs do not overlap with the dotted line there is a significant preference (indicated with *). When the bar is above the dotted line the preference is towards the species indicated above the plot area, when it is below the preference is towards species indicated below the plot area. Significant differences in preference between females and males are indicated with (-*-).

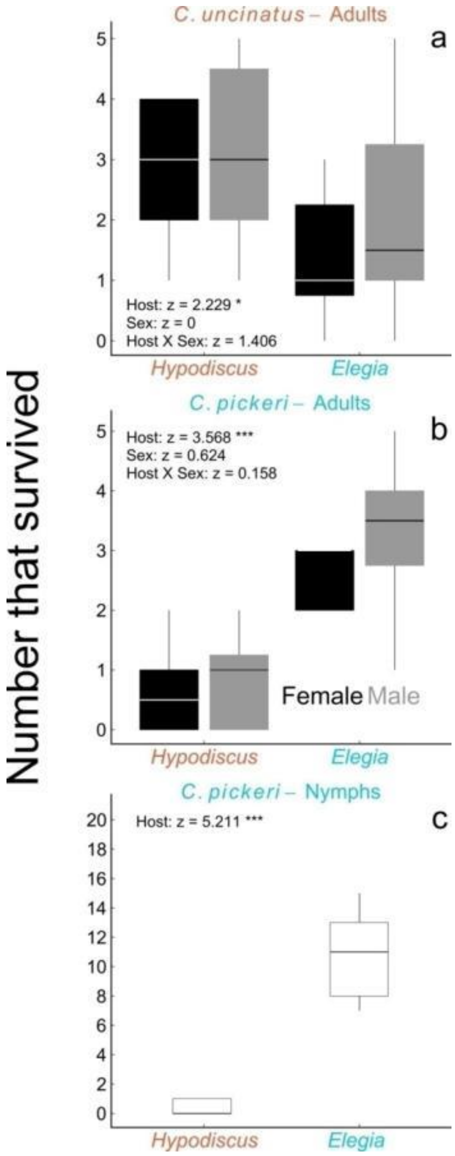


Figure 3.3: Cage survival experiments. Adults of *Cephalelus uncinatus* from the red module (Fig 3.1) and *C. pickeri* from the blue module were introduced to cages containing either *H. aristatus* (from the red module) or *E. filacea* (from the blue module). The same was done for *C. pickeri* nymphs. Panel a. shows the survival of *C. uncinatus* adults after 20 days, and panel b. shows the same for *C. pickeri* adults. Black boxes represent female survival and grey boxes represent male survival. Adult survival was out of 5 individuals per sex per cage. Panel c. shows the survival of *C. pickeri* nymphs out of 20 individuals per cage after 12 days. The x axis shows the species identity of caged host plants that are colour coded to match the colour of their module shown in Figure 3.1. z values from GLMMs for the effects of host plant species identity, hopper sex and their interaction on survival are shown. For nymphs, only the effect of host plant is shown. Host species was the only strong and significant predictor for survival. Survival did not differ between females and males, and they responded similarly to host plants. Asterisks indicate significance at 0.05 (*) and 0.001 (***) levels. Box and whisker plots show medians, upper and lower quartiles.

Supplementary material

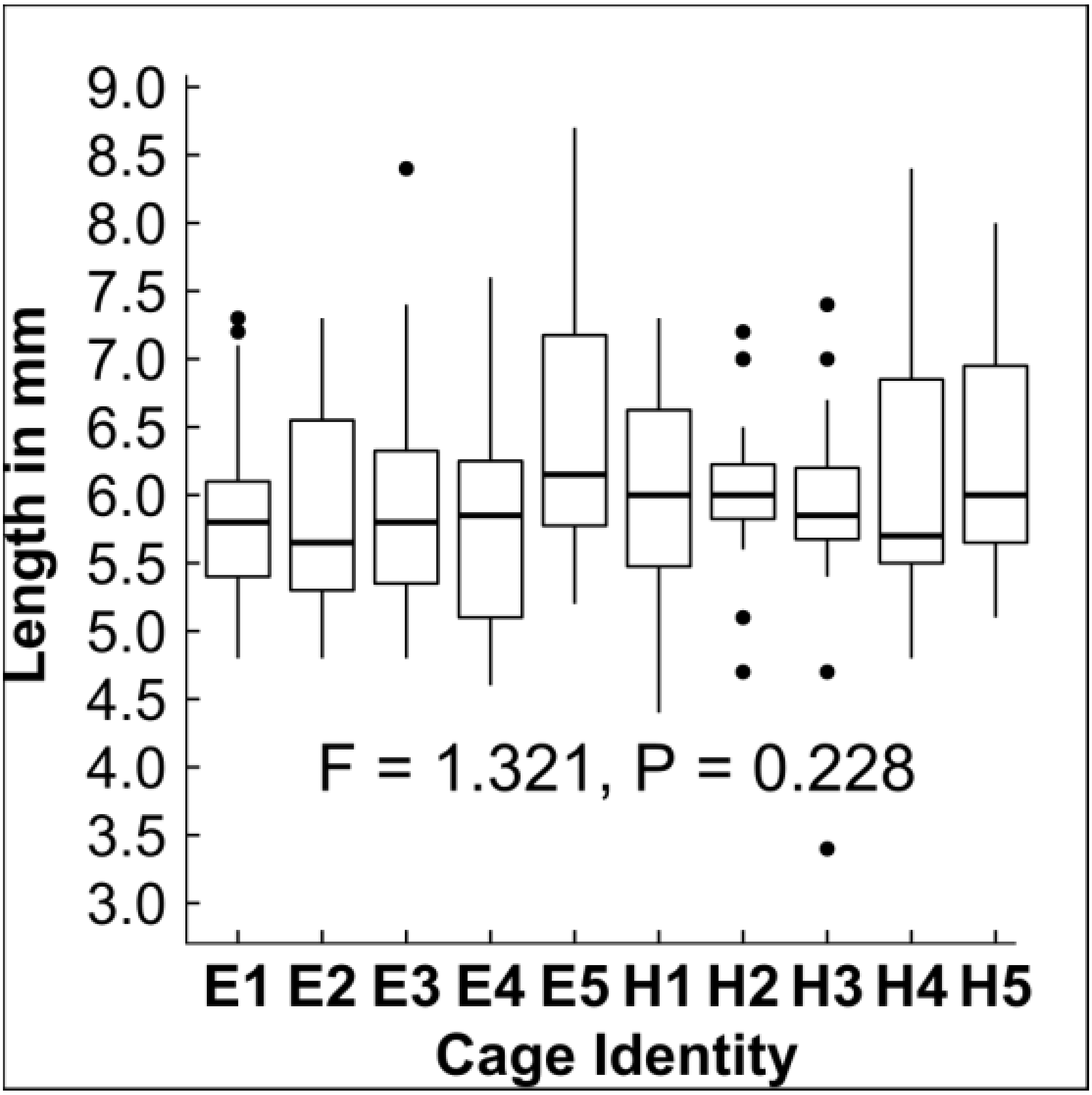


Figure S3.1: Lengths of *Cephalelus pickeri* nymphs introduced to different cages. The x axis shows the different cages. The y axis shows nymph total length. Cages starting with E contain *Elegia filacea*, and those starting with an H contain *Hypodiscus aristatus*. A one-way ANOVA was used to test if nymph sizes differed between cages. Average nymph size did not differ between cages (F = 1.321, df = 9, P = 0.228). Box and whisker plots show medians, upper and lower quartiles.

Chapter 4

Strongly niche partitioned leafhopper communities are not structured by current interspecific competition

Abstract

Niche partitioning in communities of functionally similar organisms is often interpreted as the consequence of interspecific competition, which could involve niche breadth reduction in response to current competition or niche evolution in response to selection imposed by competitors in the past (i.e. ECD). Previous studies in a single community suggest that restio leafhopper species (Cephalelini, Hemiptera: Cicadellidae) may exhibit host specificity to different species of plants from the Restionaceae family (restios). Here I sample broadly to investigate whether restio leafhoppers do in fact partition their potential food niches, and what the causes of niche partitioning may be. I first used a null-modelling approach to determine whether host plant partitioning is non-random and whether niche partitioning patterns could have resulted from strong evolved host specificity as opposed to host shifts in response to competition without increased specificity. I then used a co-occurrence analysis to test whether competitive exclusion (through limiting similarity) can explain host partitioning. I also performed an experiment to test whether restio leafhopper host use patterns result from behaviourally plastic responses to interspecific competition, or whether host preference is evolutionarily fixed. Null models suggest that host partitioning is non-random, and results from strong host specificity and not competition induced host shifts. I also find no evidence that competitive exclusion is driving patterns of host plant partitioning, and preference experiments indicate that host preference is not altered by potential competitors, suggesting that it is an evolutionarily fixed trait. Taken together, I conclude that patterns of host-use partitioning in this group of insect herbivores is the result of host specialisation, and does not result from current interspecific competition.

Introduction

Interspecific competition has been hypothesised to be a major factor shaping biological communities (Dayan and Simberloff 2005). One pattern that supports this hypothesis is that communities are often characterised by functionally similar species using different resources i.e. niche partitioning (Strong et al. 1979, Dayan and Simberloff 2005). Interspecific competition can give rise to this pattern in two different ways: through character displacement or competitive exclusion (Dayan and Simberloff 2005). In the case of ecological character displacement (ECD), co-occurring species reduce each other’s fitness when they use the same resources. Over time they may evolve trait differences which enable them to reduce competition by exploiting different resources (Brown and Wilson 1956). When this process occurs in a community context between several species, it is termed community-wide ECD (Strong et al. 1979). Unlike community-wide ECD, competitive exclusion through limiting similarity does not lead to evolved trait differences. Here interspecific competition causes such a significant reduction in fitness that species that share resources with other community members go locally extinct, or drive other community members to extinction (Hardin 1960). Thus, both competitive exclusion and/or community wide ECD would result in communities of organisms with non-overlapping resource use (Stuart and Losos 2013).

The community-wide ECD – competitive exclusion dichotomy might, however, be overly simplistic. Schluter (1984) points out that resource partitioning patterns can arise from a variety of processes. In a recent review Stuart and Losos (2013) suggest that patterns of ECD can arise when species diverge in resource use in allopatry, followed by the colonisation of the same geographic region. Thus, in the zone of sympatry, it may appear as if species evolved to use different resources in response to interspecific competition. This is especially relevant when investigating the community patterns of a group of closely related organisms undergoing adaptive radiation. It has, for example, been recognised that speciation often occurs (or is initiated) as a result of populations undergoing resource shifts in allopatry followed by secondary contact thereafter (Rundle and Nosil 2005).

An additional level of complexity is that both phenotypic plasticity and evolved character differences can drive patterns of niche partitioning (Pfennig and Murphy 2000). Contemporary interspecific competition within communities has been shown to decrease the fundamental niche to the realised niche, without leading to competitive exclusion (Connell 1961, Arakaki and Tokeshi 2011). Conversely, release from current interspecific competition has been shown to lead to realised niche expansion (Bolnick et al. 2010). Therefore, when testing whether community structure is determined by ECD or competitive exclusion, it is necessary to account for the possibility that plastic responses to contemporary interspecific competition could be driving resource use patterns (Schluter and McPhail 1992). The specific mechanisms driving niche breadth reduction differ between study systems. In spade foot toads, for example, phenotypic plasticity in response to exploitative competition is partly responsible for the field resource use patterns (Pfennig and Murphy 2000). Behavioural responses to interference competition may also cause resource-use shifts, and cause patterns of niche partitioning (Harrington et al. 2009). In the case of herbivorous insects, patterns of resource partitioning may also result from behavioural plasticity. Specifically, behaviour may be modified by chemical signals or aggressive behaviour between species (McLain 1981, Raupp et al. 1986, Flamm et al. 1987).

Despite good evidence that herbivorous insects often compete when using the same resource in ecological time (Denno et al. 1995), there is little evidence that interspecific competition structures herbivorous insect communities. Several studies have shown that even though species compete, they do not necessarily competitively exclude each other, and nor do they appear to have evolved to use different host plants or different parts of the same plants (McClure and Price 1975, 1976, Rathcke 1976, Tack et al. 2009, Hochkirch and Gröning 2012). The best support that interspecific competition structures herbivorous insects communities arguably comes from studies comparing host specificity between herbivorous insects in the tropics to those in temperate areas (Novotny et al. 2006, Dyer et al. 2007, Condon et al. 2008). These studies test the prediction that niches should be narrower in the tropics because higher species richness should result in increased levels of interspecific competition. This logic assumes that niches should be more saturated towards the tropics, requiring more specialisation (i.e. decreased trait variation (Bolnick et al. 2011)) to reduce niche overlap and interspecific competition. Ricklefs and Marquis (2012) provide some evidence for this by showing that despite more niche space towards the tropics in terms of plant traits, the more dramatic increase of insect species richness probably leads to more completely filled niches. In recent years, studies both supported (Dyer et al. 2007, Condon et al. 2008) and rejected (Novotny et al. 2006) the hypothesis that herbivorous insects are more host-specific in the tropics. Nonetheless, a recent global study by Forister et al. (2014) that included the dataset of Novotny et al.(2006), concluded species in most herbivorous insect feeding guilds exhibit higher host specificity towards the tropics.

Restio leafhoppers from the tribe Cephalelini (Hemiptera: Cicadellidae) are sap feeding insect herbivores consisting of two genera, *Cephalelus* (19 species) and *Duospina* (3 species). These genera are endemic to the Cape Floristic Region (CFR) of South Africa, and occur exclusively on plants in the Restionaceae family (Prendini 1995). The African Restionaceae (restios) are one of the most dominant and diverse families in this region comprising more than 360 species, with only 10 species occurring outside the CFR. Augustyn et al. (2013) showed that at a local community scale (2500 m^2 in this instance), up to seven restio leafhopper species can co-occur, mostly using different restio species. This pattern of niche partitioning suggests that local restio leafhopper communities might be structured by current interspecific competition at a local scale. However, the generality of this pattern across multiple local communities has not been confirmed. Also, if the pattern holds across multiple local communities it can be driven by several different processes.

Here I investigate the potential causes behind putative host-plant partitioning in seven local restio leafhopper communities. While I recognise that patterns of host-plant partitioning can arise from a wide array of processes I attempt to reject two hypotheses. Namely, host-plant partitioning can result from current competition in the form of competitive exclusion or behavioural plasticity (Table 4.1). I use a null model approach to test whether host use in restio leafhopper communities is non-random in all communities (i.e. restio hoppers partition resources in all communities). I also construct null models with different level of constraint on niches to test if niche partitioning is the result of strong specialisation, or host shifts in response to contemporary competition without changes in host specificity. I then analyse the co-occurrence patterns of restio leafhopper species across sites to test whether species using the same host plants tend not to co-occur, a pattern that would suggest that competitive exclusion is structuring restio leafhoppers communities (Diamond 1975). I also experimentally test whether host preference is a behaviourally plastic response to interspecific competition or if it is evolutionarily fixed. If restio leafhopper host preferences remain unchanged in the presence or absence of interspecific competition, and if these preferences reflect field host use patterns, then host use patterns likely reflect evolutionarily fixed niche preferences and not current competition.

Materials and Methods

Sampling of restio leafhopper communities

To quantify restio leafhopper niche overlap and co-occurrence patterns, I surveyed 27 sites across the CFR of South Africa from May 2011 to May 2013 (see map in Fig. S4.1a). Sampling was restricted to late summer and autumn months to control for temporal niche partitioning which is not within the scope of this chapter. As plant composition in the CFR (of which restios are a dominant component) can change dramatically over short distances (Cowling 1990) I confined my sampling to 1 hectare plots of the same habitat, comprising relatively homogenous host plant composition. In 16 of the local communities I used a modified leaf blower (Stewart and Wright 1995) to sample restio leafhoppers from 40 individual plants of each restio species present in each community. At 11 sites, 80 plants were sampled per restio species using the leaf blower (see sites in Fig S4.1). The increase in sampling effort did not change the number of restio leafhopper species detected on restio species significantly (Fig. S4.2), suggesting that my sampling efforts were sufficient. Insects were identified by dissecting out male genitals and matching females with males using the most recent species descriptions by Davies (1988) and Prendini (1997). Restios were identified by means of an interactive key (Linder 2011). This sampling strategy allowed me to construct species lists and to determine the host-use (niches) of each restio leafhopper species present at each site.

Niche partitioning

Community-wide niche overlap was determined using Pianka's index (Pianka 1973) which ranges from 0 (no host overlap) to 1 (complete host overlap). I measured niche overlap in the seven communities listed above. These were the only local communities with enough species (of both restios and restio leafhoppers) to allow generation of sufficient numbers of independent randomized datasets in all null models. I used three null models which differ in the extent to which they account for evolutionarily determined aspects of species niches (following Lawlor 1980). One thousand random matrices were simulated under each null model. For each community, one tailed tests were used to determine whether the observed degree of niche overlap was lower than those generated by each null model.

RA1 – In the least constrained model host-use of restio leafhoppers was randomized across all available restio species in each community, with no constraint on preference hierarchies (i.e. relative frequency of host use) or on the restio species that could be used by each restio leafhopper species. The number of restio and leafhopper species in each community was kept constant.

RA2 (modified) – In the second model the fundamental niche of each restio leafhopper species was constrained – i.e. in randomisations restio leafhopper species could only use restio species on which they are known to occur(using records from my CFR wide host-use database of the Cephalelini). This model did not constrain relative frequencies of host-use.

RA3 – The third randomization model only constrained community-level host specificity (i.e. relative frequencies of host use). For each leafhopper species, observed relative host use frequencies were randomized across all restio species in the community. This model did not constrain fundamental niches.

Thus the three models represent different levels of constraint on the niches of restio leafhoppers. Lower observed niche overlap relative to expected values under RA1 indicates non-random host use and niche partitioning, but whether niche partitioning results from specialisation or host shifts (without increased specificity) cannot be inferred from this model. Departure of observed from expected niche overlap values under RA2 would indicate that niche partitioning results from narrow host preference hierarchies (i.e. specialisation). Departure of observed from expected values under RA3 would suggest that host shifts in response to contemporary competition are determining patterns of niche partitioning. All null model analyses were performed in the R package EcoSimR (Gotelli and Ellison 2013).

Spatial co-occurrence

I constructed a presence-absence co-occurrence matrix across the 27 sites to assess whether competitive exclusion can drive patterns of niche partitioning. To analyse co-occurrence patterns of species pairs I used the probabilistic model of species co-occurrence of Veech (2013). This algorithm calculates the expected co-occurrence of species pairs based on their total number of occurrences. If the observed level of co-occurrence of a species pair is significantly smaller than the expected, it is exhibiting segregation. If the opposite is found it is exhibiting aggregation. And if no pattern is found (i.e. $P > 0.05$), the species pair associate randomly. I followed Veech (2013) and removed all species pairs with an expected co-occurrence of less than 1. I then tested whether segregated pairs share host plants (> 0 overlap in number of host plants) and aggregated pairs use different host plants. If so, competitive exclusion through limiting similarity cannot be ruled out as a determinant of niche partitioning.

Host preference

To determine whether congeneric restio leafhopper species compete through interference competition and consequently become locally more host specific (i.e. narrow their preference hierarchies), I conducted host preference experiments in a laboratory environment. I also tested whether intraspecific competition drives niche broadening, i.e. whether restio leafhoppers become less host specific in the presence of a conspecific. I tested host preferences of males of two species, *Cephalelus angustatus* and *Cephalelus pickeri*, in the absence (no competition) and presence of one another (interspecific competition) and in the presence of conspecifics (intraspecific competition). *Cephalelus angustatus* often associates with *Hypodiscus aristatus* in the field, and *C. pickeri* has been found to associate with both *H. aristatus* and *E. filacea*, albeit more strongly with *E. filacea* (Augustyn et al. 2013, Chapter 2). Both species often co-occur (See Fig. S4.1b). Each species was collected from locations where they were abundant. *C. pickeri* was caught from *E. filacea* at Pringle Bay and *C. angustatus* from *H. aristatus* at Franschhoek. All insects were collected on 30 April 2013. Experiments were initiated on 1 May 2013.

Individuals of each species (*C. pickeri* and *C. angustatus*) were marked with non-toxic acrylic paint and divided into four groups of 20. These groups were then, in different orders, cycled through an experiment consisting of three different treatments, each lasting 12 hours. These were: a no competition treatment, an intraspecific competition treatment, and an interspecific competition treatment. During the no competition treatment, insects were placed in a 750 mm jar and simultaneously offered 135 mm cuttings of *E. filacea* and *H. aristatus* culms (culms of both species are unbranched and approximately half a meter long at Pringle bay where they were collected). To keep culms fresh I attached Eppendorf vials containing distilled water to the bottom of each culm. Insects could easily move over the glass surface of the jars and find culms to perch on. After 12 hours I recorded the identity of the culm the insect had settled on as the chosen host plant. If the insect made no choice (i.e. was not perched on a culm), the experiment was not included in any analysis. For the intraspecific treatment I used the same approach as above except that a single conspecific individual was added to each jar. For the interspecific treatment the same approach as described above was used except that one individual each of *C. pickeri* and *C. angustatus* were placed together in a jar. Insects could easily come in contact with each other, thus allowing the possibility of aggressive behaviour such as vibrational signals (known to be able to travel further than 135 mm in other leafhopper species (Claridge 1985)). Only trials where both individuals made a choice were included in the analyses.

I ran two separate binomial Generalised Linear Mixed Models (GLMMs) with logit link functions for *C. pickeri* and *C. angustatus* to test whether the different competition treatments had an effect on host choice. Competition treatment (alone, intraspecific and interspecific) was included as a fixed factor in the analysis. I included two random factors: test group (of 20, see above) and individual nested within group. Group was included to control for a possible effect of the order of treatments received. The effects of group and competition treatment on host use were tested using likelihood ratio tests to compare models with and without these factors. To test for preference of *H. aristatus* over *E. filacea*, means and 95% CI were estimated from the GLMMs and back transformed to the scale of the response variable. Non random preference for one restio species was established when 95% CI did not overlap with a 50% preference.

Using a single GLMM with a logit link function, I compared host preference for *H. aristatus* over *E. filacea* between *C. angustatus* and *C. pickeri*. Here failed competition trials were included in the analysis (i.e. I included trials where only one insect made a choice), because the role of competition was not being assessed. Species identity was included in the model as a fixed factor. Individual was included as a random factor, thus treating each treatment as a repeated measure for each individual restio leafhopper. Significance of species identity was assessed using the same approach as described above. Preference for *H. aristatus* over *E. filacea* was also assessed using the same 95% CI estimation process as described above. All GLMMs were conducted using the R package lme4, and back transformations were done using the R package effects.

Results

Niche partitioning

The majority of restio leafhopper species are strikingly host specific within local communities i.e. they predominantly utilize a single restio species (Fig 4.1). Similarly, in most cases each restio species is only utilised by a single leafhopper species within a community. However, several restio species are not exploited at all (or very rarely) and occasionally restio species are exploited by multiple leafhopper species. Observed levels of niche overlap in all local communities were significantly lower than niche overlap generated by RA1 (Fig 4.2), which put no constraints on host specificity when generating random communities. Observed levels of niche overlap in all local communities were also lower than niche overlap generated by RA2 (modified) (Fig 4.2), which constrained which host plants could be used (i.e. the fundamental niche). Observed levels of niche partitioning did, however, not differ from niche overlap generated by RA3 (Fig 4.2) which only constrained preference hierarchies (i.e. relative frequencies of host use).

Spatial co-occurrence

I found no evidence that segregated species pairs shared any host plants (Fig 4.3). For the pair wise analysis of co-occurrence, 23 pairs out of 153 could be analysed (i.e. had expected co-occurrences of more than 1). Of these 23 pairs, 16 co-occurred randomly, 6 pairs had aggregated co-occurrence patterns and one pair showed segregated patterns of co-occurrence. This segregated pair consisted of *C. angustatus* and *C. bicoloratus*, two species that share no restio hosts. The six aggregated species pairs were: *C. attenuatus* with *C. campbelli*, *C. rawsonia* and *Duospina capensis*; *C. campbelli* with *C. rawsonia*; and *C. rawsonia* with *D. capensis*. All of the species pairs that aggregated have at least one host plant in common (Fig 4.3). Of the species pairs that associated randomly, 10 shared host plants and 6 did not share any restio species. Results from the pairwise test are summarised in Table S4.2.

Host preference

We found no evidence that either interspecific or intraspecific competition influence host preference behaviour of *C. angustatus* and *C. pickeri*. The presence of congeners or conspecifics had no significant effect on host preference for *H. aristatus* over *E. filacea* for both *C. angustatus* (LR = 0.684, *P* = 0.710, Fig 4.4a) and *C. pickeri* (LR = 0.645, *P* = 0.724, Fig 4.4b). GLMMs including the test group factor did not differ significantly from those excluding it (LR = 0, P = 1: *C. angustatus*; LR = 2.733, P = 0.098: *C. pickeri*) and in all cases the simpler models had the lowest AICs. Thus results reported are for models that exclude the test group. *C. angustatus* and *C. pickeri* differed in their preferences for *H. aristatus* over *E. filacea* (LR = 45.126, $P < 0.001$, Fig 4.4c). Both species preferred their host plants over non-hosts: *C. angustatus* preferred *H. aristatus* (mean preference higher than 0.5, and 95% CI does not overlap with 0.5), and *C. pickeri* preferred *E. filacea* (mean preference lower than 0.5, and 95% CI does not overlap with 0.5) (Fig 4.4c).

Discussion

Niche partitioning

All local restio leafhopper communities in this study exhibit strong niche partitioning, a pattern rarely reported for herbivorous insects (Lawton and Strong 1981). The levels of niche overlap found in local communities were far lower than that of unconstrained null models (RA1). Observed levels of niche overlap within local communities were also much lower than that of null models that constrained which host plants could be used i.e. the fundamental niche (RA2 modified). However, observed levels of niche overlap within local communities were not lower than niche overlap generated by null models that constrained preference hierarchies (but not which plants can be used) (RA3). This suggests that low niche overlap in restio leafhopper species is because they have narrow host preferences, and not because they shift host plants to avoid interspecific competition.

Spatial co-occurrence patterns

I found no evidence for competitive exclusion. The only species pair (out of 23 tested pairs) with segregated co-occurrence patterns (a diagnostic signature of competitive exclusion Diamond 1975) did not even use the same host plants. Consequently the segregated patterns of occurrence exhibited by this species pair are unlikely to be driven by competitive exclusion. This suggests that niche partitioning could not have resulted from species using the same host plants excluding each other from local communities.

Host preference

Host preference experiments also did not support the idea that interference competition structures restio leafhopper communities. *C. pickeri* and *C. angustatus* males chose the restio species that they used in the field and did not have an effect on each other's preferences. Neither did I find any evidence that restio leafhoppers of the same species influenced each other's preferences. Both conspecifics and congeners often perched closely together without showing any visible interactions. Although I only examined one species pair, this suggests that interspecific aggression might not occur in restio leafhoppers. This conforms to the findings of the most recent quantitative review on interspecific competition in herbivorous insects by Kaplan and Denno (2007). They found that, contrary to previous emphasis on interference competition, few studies find support for it. Instead, most evidence for interspecific competition is for indirect host plant mediated interactions (Kaplan and Denno 2007). For example, herbivory by one insect species can induce heightened levels of plant defensive chemicals negatively affecting other insect species feeding on the same plant (Agrawal 1998). It is therefore a plausible pathway through which interspecific competition may occur in restio leafhoppers that we did not assess. However, our null models suggest that niche partitioning results from specialisation, which can be caused by a variety of processes. Nonetheless, it is possible that interspecific competition may have driven specialisation over evolutionary time (Armbruster and Muchhala 2009, Forister et al. 2012).

Evidence for community-wide ECD

Considering that niche partitioning appears to result from strong evolutionary specialisation and that I find no evidence for competitive exclusion, restio leafhopper communities possibly exhibit community-wide ECD. Findings from the current study and Chapter 2 provide some support for 5 out of the 6 criteria needed to demonstrate community wide ECD (summarised in table S4.3). However, it has not been demonstrated that restio leafhoppers compete through resource exploitation (in the consumptive sense) when they use the same resource. This is an important, yet infrequently demonstrated criterion that needs to be tested in order to demonstrate ECD (Stuart and Losos 2013).

Even if interspecific competition can be demonstrated, it does not mean that the community wide ECD pattern arose from interspecific competition. Stuart and Losos (2013) suggest that allopatric resource shifts followed by the colonisation of a region of sympatry can give rise to patterns which are identical to ECD. In the case of restio leafhoppers this may occur if a population shifts to a novel host plant in allopatry and becomes reproductively isolated from its ancestral population (Ehrlich and Raven 1964). Once enough reproductive isolation has evolved (e.g. when the host shift is complete (Feder et al. 1994)) and dispersal occurs back into the ancestral range, an ECD-like pattern of host-use would be generated in the absence of interspecific competition. Allopatric resource shifts could, however, also promote ECD. Rice and Pfennig (2005) argue that initial release from interspecific competition in allopatry can allow intraspecific competition to increase trait variation related to resource use. Thus, once secondary contact is made, selection can act on the new existing variation and specialisation through interspecific competition might occur (Rice and Pfennig 2005). Either way, considering that restio leafhoppers are a group of closely related insects, investigating their patterns of speciation could be more fruitful in explaining their ECD-like community host use pattern as investigations of interspecific competition.

Conclusions

Quantitative reviews by Schluter (2000) and Stuart and Losos (2013) did not find any peer reviewed literature on herbivorous insects that claim to have detected ECD. However studies on herbivorous insects do report findings consistent with community wide-character displacement, for example, the recent finding by Forister et al. (2014) that herbivorous insects exhibit more host-specificity towards the tropics (where there might be more competition (Ricklefs and Marquis 2012)). Nonetheless, pattern-based studies can seldom rule out the possibility that patterns may be caused by other factors. For example, more temporal stability in species rich regions may favour the evolution of specialisation (Kassen 2002). While pattern based approaches may often be a good starting point to identify potential cases of ECD, it is clear that it is often difficult to dismiss other potential drivers of these patterns. For example, mustelids (weasels etc.) were previously shown to exhibit community-wide ECD (Dayan et al. 1989). However, more recently it was demonstrated that they exhibit more trait overlap in sympatry than in allopatry (Meiri et al. 2011). It was concluded that local adaptation across environmental clines is more important than character displacement in what was once considered a model example of ECD (Meiri et al. 2011). I suggest that future research should also investigate whether allopatric host shifts can explain ECD-like host use patterns in restio leafhoppers and other systems displaying the signatures of ECD.

Figures and Tables

Table 4.1: Four hypotheses that can explain niche partitioning via interspecific competition. The first three rows relate to findings expected from null models. I expect observed levels of niche overlap to be lower than that generated by null models with no constraints on models if restio leafhopper communities exhibit niche partitioning. Constraining host specificity allows us to detect whether niche partitioning is the result of host specificity or host-shifts (without changes in specificity). Note, however, that niche overlap null models cannot entirely distinguish between evolved and ecological processes. Nonetheless, if the fundamental niches in models are constrained, and observed values are similar to models, it indicates that host-use is somewhat evolved (i.e. not the result of plasticity). Competitive exclusion and ECD can only be distinguished by means of the co-occurrence patterns that they generate. Plastic responses to competition can be distinguished from evolutionary processes if restio leafhoppers are only host specific in the presence of interspecific competition.

	Evolved response to competition		**Current competition**	
Attribute	**ECD (specialisation)**	**ECD (shifts without specialisation)**	**Competitive exclusion**	**Plastic response to competition**
Niche overlap vs. models with no constraints (RA1)	*Less overlap than null model*	*Less overlap than null model*	*Less overlap than null model*	*Less overlap than null model*
Niche overlap vs. models with constraints on which plants can be used (RA2 modified)	*Less overlap than null model*	*Less overlap than null model*	*Less or the same overlap as null model*	*Less or the same overlap as null model*
Niche overlap vs. models with constraints on specificity (RA3)	*Same overlap as null model*	*Less overlap than null model*	*Less or the same overlap as null model*	*Less or the same overlap as null model*
Co-occurrence	*Random*	*Random*	*Segregated*	*Random*
Preference	*Unaffected, but chooses field host*	*Unaffected, but chooses field host*	*Unaffected, but chooses field host*	*Weak/no preference in absence of interspecific competitor*

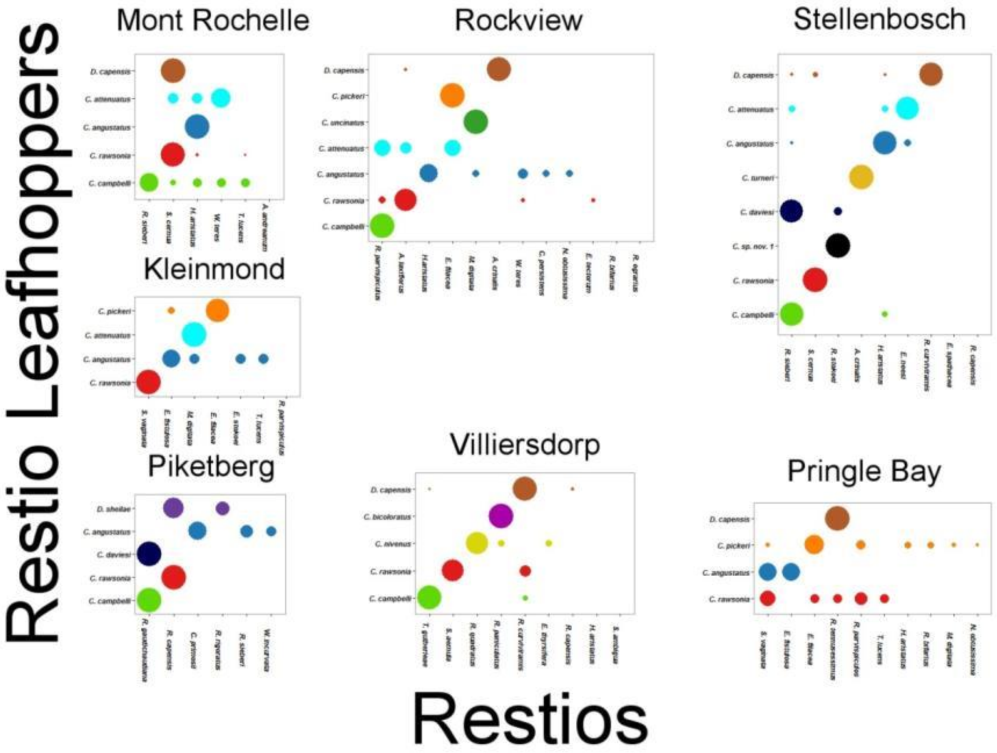


Figure 4.1: Restio leafhopper communities included in the community structure null model analyses: Mont Rochelle, Rockview, Stellenbosch, Kleinmond, Piketberg, Villiersdorp and Pringle bay. Circle size represents the proportional use of restios in columns by restio leafhoppers in rows. Restio leafhoppers species are colour coded.

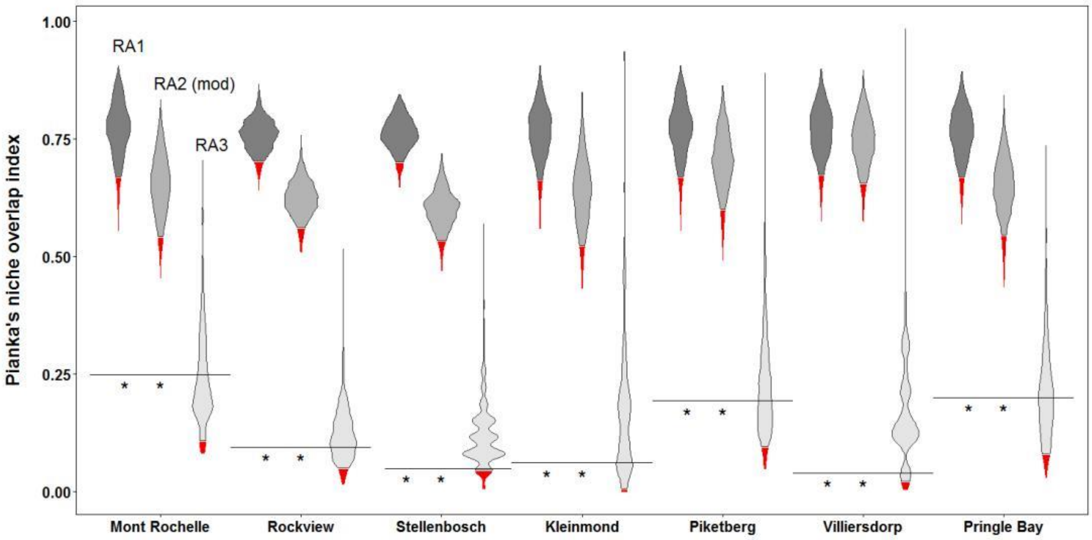


Figure 4.2: Observed niche overlap in seven restio leafhopper communities (horizontal lines) versus expected niche overlap generated by three different null models. Communities with a Pianka's niche overlap value of 0 have no overlap in host plant use, whereas communities with a Pianka's index of 1 have complete overlap in resource use. Null model results (starting from left for each community): RA1, RA2 (modified) and RA3 are shown as kernel density distribution plots (with bandwidths of 0.7). Observed values falling within or below the red filled areas (below one tailed 95% CIs) are non-random at the $p < 0.05$ level. RA1 generates random communities with no constraints on community member host-use. RA2 (modified) generates random communities, but constrains which hosts can be used based on range-wide host use (but not the relative preferences for these hosts). Non-random patterns would arise if restio leafhoppers specialise to avoid niche overlap. Lastly, RA3 generates random communities which account for local host specificity and relative frequencies of host use. Thus, non-random patterns would indicate that restio leafhoppers shift host plants, without increased specialisation, to avoid niche overlap. Note that the observed level of niche overlap was never different from the expected degree of niche overlap generated by the latter model (indicated by the lack of an *).

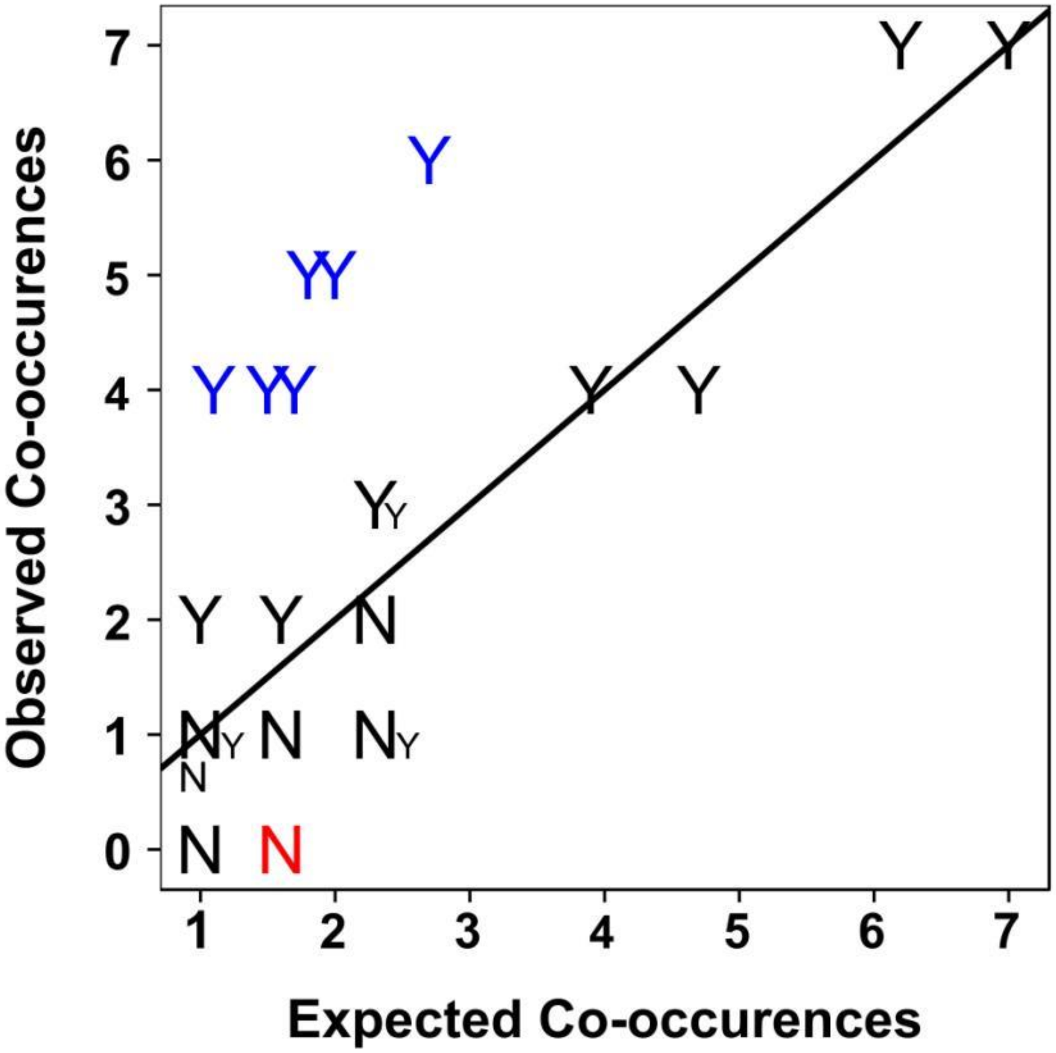


Figure 4.3: Observed versus expected pair wise species co-occurrences. Expected co-occurrences were generated from frequencies of occurrence of each species across the dataset. Each symbol represents a species pair, a Y symbol (yes) indicates that at least one host plant is shared and N (no) indicates that no host plants are shared. Competitive exclusion through limiting similarity can only happen if host plants are shared. Therefore, if host plants are shared and co-occurrence patterns are indicative of segregation, competitive exclusion cannot be dismissed. Segregation, without shared host plants likely reflects allopatry. Significantly segregated pairs are shown in red, significantly aggregated pairs in blue and randomly associated pairs in black. The diagonal line indicates where observed and expected co-occurrence patterns are equal. Pairs above the line tend to be aggregated and pairs below the line tend to be segregated. Small symbols are points that overlap completely with the closest large symbol.

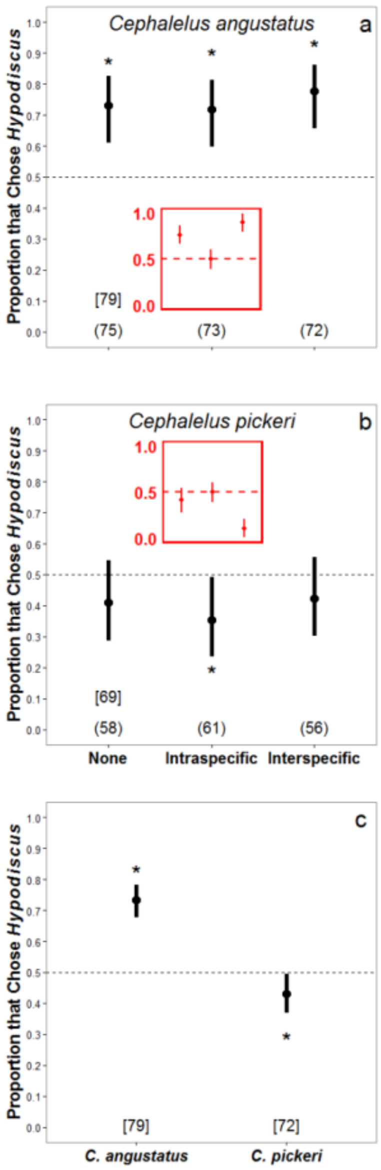
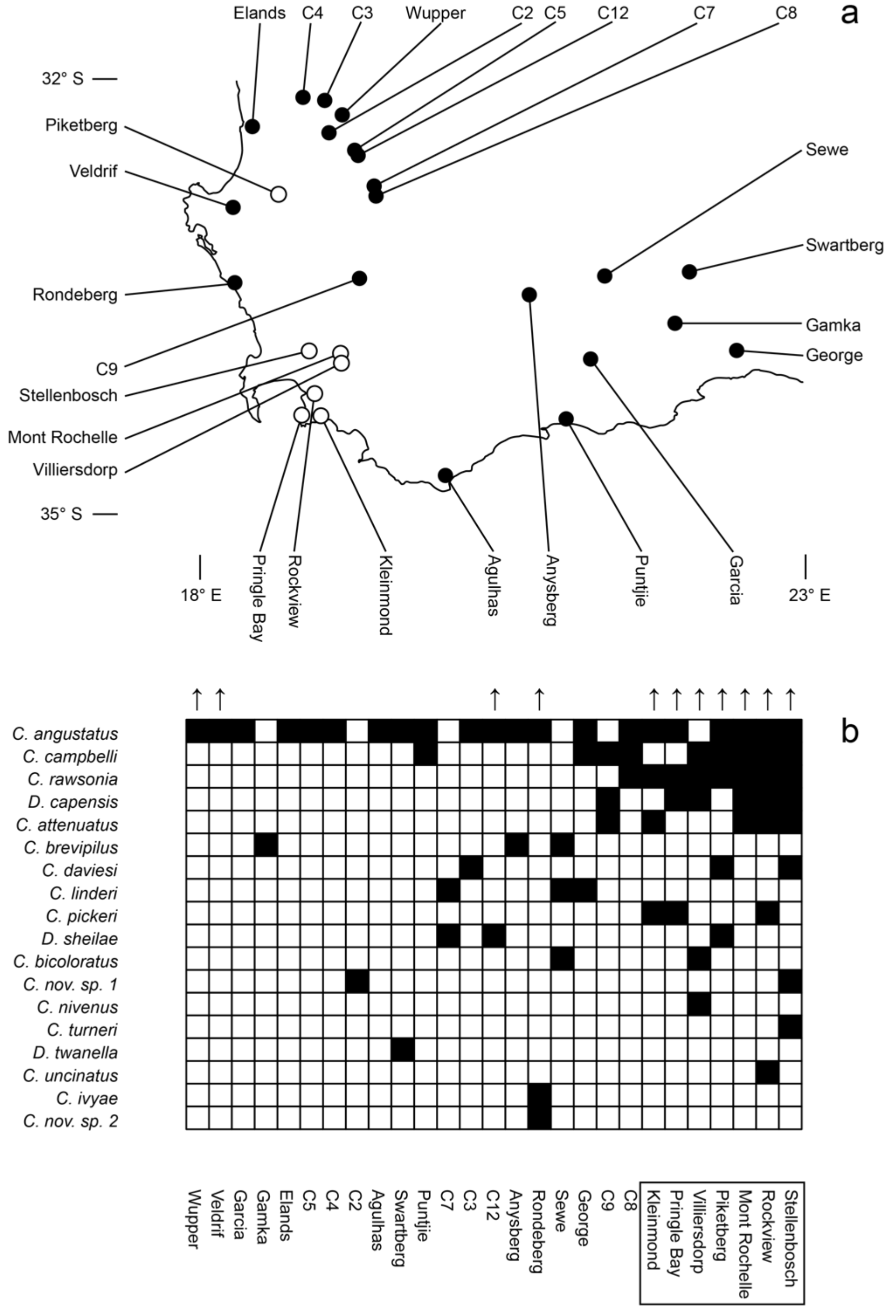


Figure 4.4: Preferences for *H. aristatus* over *E. filacea* for *C. angustatus* (a) and *C .pickeri* (b) under a control treatment with no competition (None) and two competition treatments; with a conspecific restio leafhopper (Intraspecific) and with a congener (with *C. pickeri* in the case of *C. angustatus* and vice versa) (Interspecific). Overall preference is compared between *C. angustatus* and *C. pickeri* (c). If the 95% CI bars are higher than 0.5 there is a significant preference for *H. aristatus* and if below 0.5 there is a significant preference for *E. filacea*. Asterisks (*) indicate significant preferences. Preferences did not differ between controls and treatments for both *C. angustatus* ($\chi^2 = 0.684$, $P = 0.710$, a) and for *C. pickeri* ($\chi^2 = 0.654$, $P = 0.724$, b). Overall preference differed between *C. angustatus* and *C. pickeri* ($\chi^2 = 45.126$, $P<0.001$, c). Considering that intraspecific competition is generally stronger than interspecific competition, good evidence for competition would have been if preferences in the intraspecific competition treatment were close to 0.5 for both species (i.e. niche expansion where each individual uses a different host). Good evidence for interspecific competition would then be preferences close to 1 for *C. angustatus* and close to 0 for *C. pickeri* (i.e. niche reduction). These hypothetical expectations if competition was important are shown in red. Sample sizes per species are shown in square parenthesis and per treatment sample sizes are shown in rounded parenthesis.

Supplementary figures and tables



	Wupper	Veldrif	Garcia	Gamka	Elands	C5	C4	C2	Agulhas	Swartberg	Puntjie	C7	C3	C12	Anysberg	Rondeberg	Sewe	George	C9	C8	Kleinmond	Pringle Bay	Villiersdorp	Piketberg	Mont Rochelle	Rockview	Stellenbosch
	↑	↑												↑		↑					↑	↑	↑	↑	↑	↑	↑
C. angustatus	■	■	■	□	■	■	■	□	■	■	■	□	■	■	■	■	□	■	□	■	■	■	□	■	■	■	■
C. campbelli	□	□	□	□	□	□	□	□	□	□	■	□	□	□	□	□	□	■	■	■	□	□	■	■	■	■	■
C. rawsonia	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	■	■	■	■	■	■	■	■
D. capensis	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	■	□	□	■	■	□	■	■	■
C. attenuatus	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	■	□	■	□	□	□	■	■	■
C. brevipilus	□	□	□	■	□	□	□	□	□	□	□	□	□	□	■	□	■	□	□	□	□	□	□	□	□	□	□
C. daviesi	□	□	□	□	□	□	□	□	□	□	□	□	■	□	□	□	□	□	□	□	□	□	□	■	□	□	■
C. linderi	□	□	□	□	□	□	□	□	□	□	□	■	□	□	□	□	■	■	□	□	□	□	□	□	□	□	□
C. pickeri	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	■	■	□	□	□	■	□
D. sheilae	□	□	□	□	□	□	□	□	□	□	□	■	□	■	□	□	□	□	□	□	□	□	□	■	□	□	□
C. bicoloratus	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	■	□	□	□	□	□	■	□	□	□	□
C. nov. sp. 1	□	□	□	□	□	□	□	■	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	■
C. nivenus	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	■	□	□	□	□
C. turneri	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	■
D. twanella	□	□	□	□	□	□	□	□	□	■	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
C. uncinatus	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	■	□
C. ivyae	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	■	□	□	□	□	□	□	□	□	□	□	□
C. nov. sp. 2	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	■	□	□	□	□	□	□	□	□	□	□	□

Figure S4.1: Twenty seven sites sampled across the CFR (a) and their associated restio leafhopper fauna (b). Unfilled points in a) indicate sites used for niche partitioning null model analyses. Filled points indicate sites included in a co-occurrence analysis. Filled squares in b) indicate presence, and unfilled squares indicate absence from a site. Up arrows above columns indicate sites where 80 individual plants (instead of 40) were sampled per plant species. Sites used for niche partitioning null models are boxed.

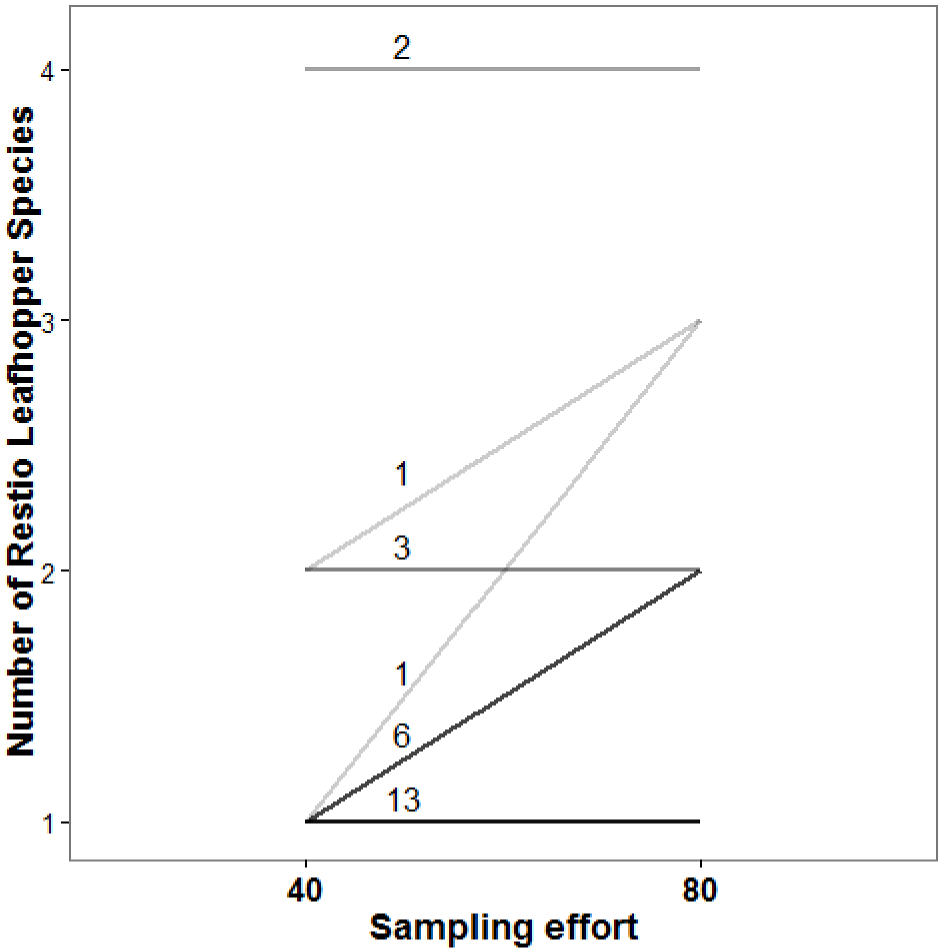


Figure S4.2: A verification that sampling 40 individual restios per species is sufficient for detecting the number of restio leafhoppers using that species. It should be taken into account that more sampling will always increase the number of restio leafhoppers detected, as single encounters are inevitable. Each line represents an experiment where a restio species was first sampled 40 times, and then another 40 times. After the first 40 restios sampled, all restio leafhopper species were recorded, and then again after 80 plants were sampled. To test whether the species richness estimates significantly increase after additional sampling a GLM with a Poisson distribution was used. Sampling effort was nested in each experiment (there were 26restio species sampled across 7 sites). The doubling of sampling effort did not significantly increase the number of restio leafhoppers detected (Sampling Effort: $\chi^2 = 1$, $P = 0.32$, Sampling Effort/experiment: $\chi^2 = 0.04$, $P = 0.98$) suggesting that sampling 40 individual plants is adequate.

Table S4.1: Summary of standardised effect sizes of niche partitioning in relation to three different null models at seven different sites. One tailed tests, test whether the observed degree of niche overlap differs significantly from the expected level of niche overlap generated by null models. P values are shown in superscripts.

Site	RA1	RA2 (modified)	RA3
Mont Rochelle	-9.034 $^{P < 0.001}$	-6.226 $^{P < 0.001}$	0.057 $^{P = 0.626}$
Rockview	-19.637 $^{P < 0.001}$	-13.886 $^{P < 0.001}$	-0.498 $^{P = 0.341}$
Stellenbosch	-20.332 $^{P < 0.001}$	-13.738 $^{P < 0.001}$	-1.115 $^{P = 0.13}$
Kleinmond	-12.364 $^{P < 0.001}$	-8.172 $^{P < 0.001}$	-0.782 $^{P = 0.244}$
Piketberg	-10.003 $^{P < 0.001}$	-8.143 $^{P < 0.001}$	-0.292 $^{P = 0.193}$
Villiersdorp	-13.478 $^{P < 0.001}$	-12.92 $^{P < 0.001}$	-1.183 $^{P = 0.094}$
Pringle Bay	-10.378 $^{P < 0.001}$	-7.140 $^{P < 0.001}$	-0.107 $^{P = 0.516}$

Table S4.2: Results of a probabilistic pairwise co-occurrence analysis for 23 species pairs. The first column shows the species pair that was compared and the second column shows whether the compared species pair shares any restio host plants. *P* values for segregation or aggregation are shown. Significance was taken at the P < 0.05 level, the finding of each pair is shown in the last column.

		Co-occurrence				
Pair	Any restio sp. shared?	Observed	Expected	P (segregate)	P (aggregate)	Association
C. attenuatus- C. campbelli	Yes	4	1.7	0.99844	0.02965	Aggregate
C. attenuatus - D. capensis	Yes	4	1.1	0.99993	0.00398	Aggregate
C. attenuatus - C. rawsonia	Yes	4	1.5	0.99931	0.01717	Aggregate
C. campbelli - D. capensis	Yes	5	2	0.99972	0.00795	Aggregate
C. campbelli - C. rawsonia	Yes	6	2.7	0.9997	0.00608	Aggregate
D. capensis - C. rawsonia	Yes	5	1.8	0.99991	0.00369	Aggregate
C. angustatus - C. attenuatus	Yes	4	3.9	0.74794	0.69688	Random
C. angustatus - C. brevipilus	No	1	2.3	0.11453	0.99316	Random
C. angustatus - C. campbelli	Yes	7	7	0.67678	0.69537	Random
C. angustatus - D. capensis	Yes	4	4.7	0.40422	0.89907	Random
C. angustatus - C. daviesi	Yes	3	2.3	1	0.4547	Random
C. angustatus - C. linderi	Yes	1	2.3	0.11453	0.99316	Random

C. angustatus - C. pickeri	Yes	3	2.3	1	0.4547	Random
C. angustatus - C. rawsonia	Yes	7	6.2	0.90834	0.40592	Random
C. angustatus - D. sheilae	No	2	2.3	0.5453	0.88547	Random
C. angustatus - C. uncinatus	Yes	2	1.6	1	0.59829	Random
C. angustatus - C. nov. sp. 1	No	1	1.6	0.40171	0.95726	Random
C. brevipilus - C. campbelli	No	0	1	0.27897	1	Random
C. campbelli - C. daviesi	Yes	2	1	0.97128	0.25026	Random
C. campbelli - C. linderi	No	1	1	0.74974	0.72103	Random
C. campbelli - C. pickeri	Yes	1	1	0.74974	0.72103	Random
C. campbelli - D. sheilae	No	1	1	0.74974	0.72103	Random
C. angustatus - C. bicoloratus	No	0	1.6	0.04274	1	Segregate

Table S4.3: Support for ECD provided by the restio leafhopper study system, according to the criteria of Schluter and McPhail (1992).

Criteria	Satisfied by the restio leafhopper system
1. Pattern could not have arisen by chance.	By means of null models I demonstrated that niche partitioning is non-random.
2. Differences between allopatric and sympatric populations should have a genetic basis.	Not demonstrated. Nonetheless, both (Schluter 2000a)'s and the most recent review by Stuart and Losos (2013) assume that when demonstrating community-wide ECD, differences in sympatry should by default have a genetic basis.
3. Competitive exclusion (species sorting) should be ruled out as a driver of the pattern.	Restio leafhoppers do not exhibit co-occurrence patterns indicative of competitive exclusion.
4. Phenotype should reflect differences in resource-use.	We showed that restio leafhoppers actively choose restio species that they perform best on and use in the field (here and in Chapter 2). Thus I have identified behaviour as a phenotypic link related to resource exploitation.
5. Relevant differences between sympatric and allopatric populations should be controlled for.	When community-wide ECD is demonstrated all species are sympatric, thus it is deemed unnecessary to demonstrate the allopatry-sympatry pattern, but see Meiri et al. (2011).
6. Species using the same resources should reduce each other's fitness.	Not demonstrated.

Chapter 5

Negative relationship between species richness and host specificity in a clade of specialised herbivorous insects

Abstract

A positive relationship between species richness and specificity is a common pattern in nature and interspecific competition has been hypothesised to drive it. The pattern is often thought to arise because escalations in species richness increase interspecific competition, which in turn selects for specialization. Here I investigated whether increased species richness drives increased host specificity in populations of restio leafhoppers (Cicadellidae: Cephalelini) within the Cape Floristic Region of South Africa, thus controlling for the influence of biogeographic history. I studied the effects of both local (species richness within a 1 ha site) and regional (the number of species distribution ranges overlapping a 1 ha site) restio leafhopper species richness on the host specificity of restio leafhopper populations (quantified by d’ estimated from community interaction networks). I reasoned that if local species richness is a surrogate for current competition, increased richness would drive reductions in niche size, producing host use patterns akin to specialisation. In contrast, specialisation (i.e. evolved niche reduction in response to competition) is more likely to be related to regional species richness because this is a better proxy for the number of species a population could have competed with over evolutionary time. I investigated whether niche partitioning, estimated with a network metric (H2’), changes with local and regional species richness. If niche partitioning remains constant despite increased species richness, it would indicate that apparent specialisation or specialisation prevents niche overlap. I found no correlation between local species richness and host specificity. Moreover, increased regional species richness correlated negatively with host specificity. Consequently, niche partitioning in restio leafhopper communities decreased with increasing local and regional species richness. My findings suggest that while interspecific competition is more likely in species rich communities because of increased niche overlap, it does not drive host specialisation in restio leafhopper populations. My results suggest that studies finding a positive relationship between specificity and decreasing latitude need to consider mechanisms other than increased competition in species rich communities, such as variable biogeographic history, as explanations for this pattern.

Introduction

Specialisation, the process whereby organisms evolve to use an increasingly narrower range of resources, has important implications for species diversification and persistence (Poisot et al. 2011). For this reason the link between species richness and specificity has been studied in various taxa and contexts. In a phylogenetic context, species within species rich clades are thought to be more specialised than those in species poor clades because they have responded to higher niche overlap and interspecific competition with clade members (reviewed in Ricklefs 2012). However, a clade may also be species rich because its members have a tendency to be specialised. The high diversity of orchids, for example, may reflect a tendency towards strong specialisation on multiple niche axes (Gravendeel et al. 2004, Cozzolino and Widmer 2005). In contrast, Armbruster and Muchhala (2009) suggest that species richness drives floral specialisation in pollination systems through character displacement (an evolutionary response to interspecific competition). Similarly, the most recent review on the evolution of specialisation in herbivorous insects suggests that host specialisation is potentially a consequence of interspecific competition (amongst other interactions), because there is limited evidence for the alternative possibility that genetic trade-offs (i.e. the ability to exploit one host makes an insect worse at exploiting another) favour specialisation in herbivorous insects (Forister et al. 2012).

Herbivorous insects have been focal organisms in the study of specialisation (Futuyma and Moreno 1988, Nosil and Mooers 2005, Forister et al. 2012), species richness (Erwin 1982, Novotny et al. 2007), and the link between specificity and species richness (Novotny et al. 2006, Dyer et al. 2007, Winkler and Mitter 2008, Ricklefs and Marquis 2012, Forister et al. 2014). A commonly reported pattern is the relationship between latitude and host specificity (e.g. Novotny et al. 2006, Dyer et al. 2007, Condon et al. 2008, Forister et al. 2014). Latitude is a good surrogate for species richness because of the almost ubiquitous increase of species richness towards the tropics for most groups of organisms (Hillebrand 2004). In recent years, studies have both supported (Dyer et al. 2007, Condon et al. 2008) and rejected (Novotny et al. 2006) the hypothesis that herbivorous insects are more host-specific towards the tropics. Nonetheless, a recent global study by Forister et al. (2014), that included the dataset of Novotny et al. (2006), concluded that most herbivorous insect feeding guilds exhibit higher host specificity towards the tropics. However, as for other organisms, the positive relationship between latitude and host specificity can be explained by a variety of processes. One hypothesis is that niches are more saturated in the tropics than in temperate regions. Ricklefs and Marquis (2012) argue that although there are more niches (measured in terms of leaf traits) in the tropics, insect species richness increases disproportionately, therefore filling niche space more completely in the tropics than in temperate regions. This suggests that insects in the tropics have specialised more in response to interspecific competition than those in temperate regions (Ricklefs and Marquis 2012).

One problem with investigating the relationship between specificity and species richness across large geographic regions is that biogeographic history may confound the relationship. For example, tropical lineages are older than those in post glacial temperate regions (reviewed in, Wiens et al. 2006, Mittelbach et al. 2007). Thus, species richness in the tropics may simply be the result of groups having had more time to speciate regardless of whether tropical lineages tend to be more specialised (see time-for-speciation effect by Stephens and Wiens (2003)). Also, specialisation increases with evolutionary time (Nosil 2002), and is favoured by temporal stability (Kassen 2002). Thus, tropical rain forests may simply hold more host specific insect species than temperate regions because of relatively infrequent disturbance cycles (Chambers et al. 1998, Scott 2000, Bond et al. 2005). Therefore, to understand the link between host specificity and species richness, it is beneficial to study it within smaller biogeographic regions and biomes. Another common shortcoming of studies that investigate the link between species richness and specificity is that specificity is measured at the species level. That is, they test whether species are more host specific in the tropics than in temporal regions (e.g. Novotny et al. 2006, Dyer et al. 2007, Condon et al. 2008).However, specificity is often a local population level phenomenon (Fox and Morrow 1981), shaped by local selection pressures (Anderson et al. 2014). Thus, if species richness is thought to drive host specificity through interspecific competition, it should be investigated at the population level.

The Cape Floristic Region (CFR) in South Africa is an anomaly in the latitudinal species richness trend, with plant species richness unparalleled by similar ecosystems of the same latitude (Cowling and Rundel 1996). Within this biome plant species richness peaks in the south-west and tails off towards the north and the east (Linder 2003). Plants in the restio family (Restionaceae) shows a geographic species richness trend similar to that of other CFR plant groups (Linder 2003). The restio family is also one of the oldest (Verboom et al. 2009) and most species rich (Linder 2003) plant families in the CFR. Because of its dominance, it is one of the definitive components of Fynbos, the main vegetation type in the CFR. The Cephalelini (Cicadellidae), hereafter called restio leafhoppers, is one of the best described clades of herbivorous insects in the CFR. Currently there are 21 described species, with several species occurring widely throughout the CFR (Prendini 1997). They occur exclusively on restios (Prendini 1995) and at the species level they tend to be restricted to restios of either the Restioneae or Willdenowieae sub tribe (Wiese 2014). They also exhibit niche conservatism; closely related restio leafhopper species tend to use restio species from the same sub tribe (Wiese 2014). At local scales, however, restio leafhoppers tend to be highly host specific, often strongly interacting with single host plant species (Chapters 2, 3 and 4). This chapter, and others further examines factors driving host specifity at the local scale. Data from other chapters suggest that neither current competition nor competitive exclusion is driving host plant partitioning (referred to as niche partitioning henceforth) (Chapters 3 and 4), but that niche partitioning is the result of restio leafhoppers having narrow host preferences (Chapter 4). This suggests that character displacement may have happened via host specialisation in restio leafhoppers (Chapter 4).

Here I explore the link between species richness and population level host specificity in restio leafhoppers within the CFR; a small biogeographic region. I first map species richness of restio leafhoppers within the CFR. I then study species richness at two spatial scales: local species richness refers to all restio leafhopper species occurring within a 1 ha site and regional species richness refers to the number of restio leafhopper species distribution ranges overlapping a 1 ha site. The 1 ha site in which a restio leafhopper population is located may have relatively low local species richness, but it may occur within a zone of high regional species richness, depending on its geographic location. I predict that if current interspecific competition is important, local species richness should drive increased host specificity (as quantified from networks). Host specificity at this spatial scale may, however, not be the result of evolutionary specialisation; it might simply be a result of the reduction of the fundamental niche to the realised niche (Connell 1961, Arakaki and Tokeshi 2011). I will refer to this process as apparent specialisation. I do, however, expect a positive relationship between regional species richness and host specificity to reflect evolved host specialisation (hereafter referred to simply as host specialisation). This is because regional species richness should be a better reflection of the number of species a population has competed with over evolutionary time (implied by Cornell and Lawton

(1992)). I also expect these processes to be reflected by community structure. If current competition increases apparent specialisation, I expect local community niche partitioning to remain constant despite increases in local species richness. Similarly, if interspecific competition drives true host specialisation, I expect niche partitioning to remain constant despite increases in regional species richness.

Materials and Methods

Sampling of restio leafhopper communities

To determine whether local and regional species richness drives population level host specialisation in restio leafhoppers, I sampled 32 communities from 27 sites across the CFR, encompassing a gradient of leafhopper species richness (see Fig 5.1 and Table S5.1). Twenty four of these communities (i.e. those with two or more leafhopper species) were also used to determine whether local community niche partitioning remains constant across species richness gradients (because of apparent or true specialisation). All sites were sampled in late-summer or autumn (Feb-May) when most restio leafhopper species peak in abundance. In addition, the Kleinmond, Kogelberg, Pringlebay, Rockview and Veldrift sites were sampled again in spring (Aug-Sept). For these sites spring and autumn samples were analysed as separate communities as they comprised different suites of restio leafhopper species (i.e. those with spring or autumn abundance peaks, (see Augustyn et al. 2013).

Sites were approximately 1 ha in extent. For most sites I sampled restio leafhoppers from 40 individuals of each restio species present at the site. For seven sites (Villiersdorp, Piketberg, Rockview, Mont Rochelle, Pringle bay, C12 and Rondeberg), 80 plants were sampled per restio species. I standardised sampling by sucking insects from each restio plant for 5 seconds using a modified leaf blower (Wilson et al. 1993). After collecting, restio leafhoppers were preserved in alcohol, and identified in the laboratory by dissecting out the genitalia of male restio leafhoppers and comparing them with the most recent species descriptions (Davies 1988, Prendini 1997). Females were matched with males through external morphology. Restios were also collected and identified in the laboratory using an interactive key (Linder 2011), and my own collection. Restio leafhopper and restio voucher specimens are held at the Department of Botany and Zoology, Stellenbosch University.

Mapping restio leafhopper species distribution ranges and determining species richness

Local species richness was quantified as the number of restio leafhopper species detected within each sampled community, while a mapping procedure was used to determine regional species richness. A species richness map for the CFR was constructed using locality data compiled from taxonomic treatments of restio leafhoppers (Davies 1988, Prendini 1997), collections for phylogeny reconstruction (Wiese 2014), broader restio herbivore surveys (Kemp 2014), and my own restio leafhopper survey dataset. Regional species richness associated with each surveyed site was quantified as the number of restio leafhopper species distribution ranges overlapping each site. All restio leafhopper species from these records were identified either by Davies (1988), Prendini (1997) or myself. To estimate species ranges whilst avoiding bias due to uneven or low sampling effort, I drew convex hulls around the outermost collection localities of each restio leafhopper species. I then added a 20 km buffer around each convex hull so that species distribution records represented by one or two points were not underrepresented; these were *C. gonubiensis* and *C. sp. nov. 2*. Only three sites were less than 20 km away from each other, these were: C5 and C12, C7 and C8 and Kleinmond and Kogelberg. Ranges were then rasterised, and clipped (masked) with outlines of the Fynbos, Renosterveld, Strandveld and Thicket biomes as stencils. These vegetation types were used for two reasons; they all contain restios, and restio leafhopper species localities were reported from all four vegetation types. Regional species richness was then mapped by overlaying the ranges of all restio leafhopper species. ArcMap11 was used for all mapping procedures.

Estimating population level specificity and niche partitioning from bipartite networks

Weighted bipartite networks were constructed separately for each community. As the sampling effort on each restio species occurring at a site was equal, the relative abundances of restio leafhopper species on each plant species are representative of their degree of host specificity. Thus connections between restio leafhoppers and restios represent utilisation rates. For 10 locally rare restio species (4.93% of all sampled species) fewer than 40 (or 80) individuals were present at a site. In these cases the weight of interactions was adjusted to account for reduced sampling effort.

For each community two quantitative network metrics, d’ and H2’ (Blüthgen et al. 2006) were used to quantify population level host specificity and niche partitioning respectively. Both d’ and H2’ are robust to differences in sampling intensity and network size (Blüthgen et al. 2006, Morris et al. 2014).

d’ is a population level measure of specificity that ranges from 1 (complete specificity) to 0 (complete generalism). Commonly d’ is measured in two different ways. It can measure the lack of niche overlap with other species within a network. For example, a restio leafhopper species within a network connecting strongly with many restios can have a d’ value close to 1 as long as few other restio leafhopper species are connected to the restio species it uses. The rationale is that host specific species within a community are less likely to overlap in host use with other species than generalist species (Bolnick et al. 2011). Alternatively, d’ can measure specificity directly whilst taking into account the relative abundance of the lower trophic level (e.g. restios). Thus, a restio leafhopper species that is strongly interacting with several restio species with very low abundances would have a high d’ value. However, a restio leafhopper species connecting strongly to one restio species that is very abundant might have a low d’ value. Measuring d’ this way accounts for the possibility that generalists might be exhibiting high host specificity because of current ecological processes (through for example search behaviour optimisation, (see Bernays and Wcislo (1994)) and not evolutionary processes. However, experimental evidence has shown that restio leafhoppers using locally abundant host plants survive better on them (e.g. *C. pickeri* on *E. filacea*, Chapter 2). Thus, accounting for restio relative abundance underestimates (or overestimates) the specificity of restio leafhoppers. For this reason, I measured d’ directly whilst assigning all restio species equal abundances. By this approach relative connection strength alone determines host specificity. I calculated d’ values this way for 78 populations of 20 species from 32 local community networks.

H2’ is a weighted measure of network wide specificity (Blüthgen et al. 2006). It measures different community properties depending on the sampling approach. As I sampled host plants equally H2’ represents the degree of niche overlap in a restio leafhopper community network (see Figure S5.1 for a detailed explanation). Thus, an H2’ value of 1 (maximum) represents a community exhibiting no niche overlap, and a H2’ value of 0 (minimum) represents a local community exhibiting complete niche overlap. H2’ can be influenced by two factors; the trait variance of community members (i.e. the degree of host specialisation) and the trait means (i.e. which hosts are used) (Blüthgen et al. 2006, Bolnick et al. 2011) (Fig 5.2). For this reason, H2’ can be increased through the specialisation of community members and/or through host shifts (Fig 5.2b and c). I determined H2’ for a total of 24 communities throughout the CFR.

Phylogenetic grouping of restio leafhopper species

Knowledge of phylogenetic relationships of restio leafhoppers was used to restrict correlations between species richness and population level host specificity to closely related species. As restio leafhoppers exhibit phylogenetic niche conservatism in host-use (Wiese 2014), species richness of closely related species may be the main driver of apparent or true specialisation. Restio leafhopper species were grouped into four clades identified by Wiese (2014) on a phylogeny derived from Co1 and H3 gene sequences. However, three restio leafhopper species sampled in my study were not included in this phylogeny. These species were placed by first determining their closest relatives from an earlier phylogeny based on genital morphology (Prendini and Linder 1998), and then assigning them to the clade of their closest relative on the Wiese (2014) phylogeny.

Species richness driving local host specificity (d’)

Generalized Linear Models (GLMs) with gamma distributions and log link functions were used to test whether host specificity of restio leafhopper populations increase (i.e. d’ increases) with increased restio leafhopper species richness. As host specificity data were left skewed (i.e. most d’ values were close to 1) I converted d’ values (1 - d’) to fit a gamma distribution which is right skewed. The results were then back transformed for plotting. In all models restio leafhopper species identity was included as a random factor to control for differences in specificity between species. Four models were used to test for the influence of restio leafhopper species richness on the specificity of populations. These tested for the role of: 1) local species richness, 2) local species richness of species from the same clade, 3) regional species richness, and 4) regional species richness of species from the same clade. As the effect of species richness was tested on the specificity of each restio leafhopper population, a population's own contribution to local and regional species richness was not taken into account (i.e. total species richness - 1). All models were also conducted with and without local restio (host) species richness as factor to control for the possibility that restio leafhopper richness and specificity might be correlated through their potential shared dependence on available niche space in a community. Models including and excluding restio species richness were then compared to test whether restio species richness has a significant effect on models.

Species richness and niche partitioning (H2’)

Two GLMs were used to test whether increased local (Local 1) and regional species richness (Regional 1) has no effect on niche partitioning (H2’), or whether increased species richness leads to decreased niche partitioning (in the case of no apparent or true specialisation). The same GLM type and data transformation procedure was used as for the species richness and specificity (d’) models. Models Local 1 and Regional 1 included restio leafhopper species richness and sampling season (summer-autumn or spring) as predictors of H2’. As increased restio (host plant) species richness may lead to more available niche space, and therefore lead to increased H2’ I conducted another two models including restio species richness. Models Local 2 and Regional 2 included restio species richness as a factor, but were otherwise the same as Local 1 and Regional 1. Models including and excluding restio species richness were tested against each other by means of F tests (i.e. Local 1 against Local 2 and Regional 1 against Regional 2). If models differ significantly it would suggest that restio species richness has an important effect on models estimating niche partitioning. All of the abovementioned models were conducted without two populations that were statistical outliers (thus n = 24 - 2). They were excluded on the basis that their H2’ values of 0 (see Figure 5.4) were lower than 0.658 (determined as the first quartile – 1.5 times the interquartile range) (Tukey 1977). These were two sites that consisted of two restio leafhopper species *C. appendiculatus* and *C. uncinatus* both mostly using *Mastersiella digitata* (at Kleinmond) and *M. spathulata* (at De Hoop). Considering that these species might be the only pair that consistently use the same restio species, they may distort the more general pattern. Nonetheless, I also ran the models Local 3 (same factors as Local 1) and Regional 3 (same factors as Regional 1) with outliers to assess how they affect models.

Results

Restio leafhopper species richness is highest in the south western region of the CFR, with species richness declining towards the north and the east (Fig 5.1). In the region of highest species richness the distribution ranges of 18 (of 22 total) restio leafhopper species overlapped, while only one restio leafhopper species was recorded on the west coast of the greater CFR at the site with the lowest species richness.

Local specificity and species richness

Local specificity of populations (d’) did not increase with local species richness in any models. Instead, host specificity near significantly decreased with an increase in local species richness (t = -1.941, P = 0.057, Fig 5.3a). In this model species identity, as in all of the models below, had a significant effect on the model (F = 2.975, P < 0.001, S5.2). Including restio species richness, did not change the model (F = 2.014, P = 0.161), and was therefore excluded from the model. Thus, contrary to expectation, I found no evidence that apparent specialisation is occurring in restio leafhoppers.

Local specificity of populations showed no significant increase with an increase in the number of closely related species within a community (t = - 0.270, P = 0.788, Fig 5.3b). Species identity had a strong effect on the overall model (F = 2.879, P = 0.001, Table S5.2). Including restio species richness, however, did not change the model (F = 3.587, P = 0.063), and was therefore excluded from the model. Thus, the lack of a positive correlation between local species richness and host specificity is not a result of apparent specialisation in response to closely related species only.

Contrary to my expectation if specialisation occurs through character displacement, local specificity of populations significantly decreased in regions with high species richness (t = -3.163, P = 0.002, Fig 5.3c). Species identity had a strong overall effect on the model (F = 3.01, P < 0.001, Table S5.2), while I verified that restio species richness had no significant effect on the model (F = 1.011, P = 0.31).

Local specificity of populations also decreased significantly in regions with a high number of closely related species (t = - 3.750, P < 0.001, Fig 5.3d). Species identity had an important effect on this model (F = 3.374, P < 0.001, Table S5.2). Also, as for all of the abovementioned models, I verified that restio species richness had no significant effect on the model (F = 1.145, P = 0.289).

Niche overlap

Contrary to what is expected if increased species richness leads to increased apparent or true specialisation, niche partitioning (H2’) decreased (rather than increased) with increased local (t = -3.484, P = 0.002, Figure 5.4, Local 1 in Table 5.1) and regional species richness (t = -2.825, P = 0.011, Figure 5.4, Region 1 in Table 5.1). However, local species richness had a stronger negative relationship with niche partitioning (β = -0.681, Table 5.1) than regional species richness (β = -0.217, Table 5.1).

Inclusion of restio species richness as a factor improved the model for local species richness (Local 2 in Table 5.1, F = 5.723, P = 0.027, AIC smaller), but niche partitioning was not significantly higher in communities with more restio species (t = -1.784, P = 0.091, Table 5.1). Importantly, the negative relationship between local leafhopper species richness and niche partitioning was not affected when accounting for restio richness (i.e. niche space) (t = -4.464, P < 0.001, Table 5.1). In contrast, inclusion of restio richness in the regional model did not improve the model (Regional 2 in Table 5.1, F = 1.674, P = 0.21), and rendered the negative relationship between regional richness and niche partitioning non-significant (t = -1.381, P = 0.184, Table 5.1). When outliers were included in models Local 1 and Regional 1 neither local (t = 0.058, P = 0.954) nor regional species richness (t = 0.256, P = 0.8) had significant relationships with niche partitioning (Table S5.3).

Discussion

Species richness and host specificity

Restio leafhopper species richness is highest in the south western CFR and declines towards the north and the east. This is the same pattern of species richness exhibited by their restio host plants and that of plant diversity in the CFR (Linder 2003). However, host specificity in restio leafhopper populations sampled across this gradient of species richness does not follow the same trend. Neither local community species richness nor regional species richness was positively related to population level host specificity. In fact, in contrast to my expectation, regional species richness correlated negatively with population level host specificity. As the measure of specificity that I used, d', is robust against the number of resource categories within networks (Blüthgen et al. 2006), increased host species richness should not lead to decreased d'. I verified this by showing that restio species richness had no significant effect on models estimating host specificity. I am therefore confident that restio leafhopper species richness is not driving apparent or true specialisation through current competition or character displacement. In contrast, my results suggest that an unidentified process is hindering host specialisation in the region of highest regional restio leafhopper and restio species richness. I speculate that, in species rich regions, selection might not favour adaptation to a single (or few) restio species that may not always be encountered between colonisation events. I base this speculation on the fact that exceptionally high beta diversity (i.e. small spatial scale turnover in plant communities) underlie plant species richness in the CFR (Cowling and Rundel 1996).

Niche overlap

The lack of a positive correlation between restio leafhopper species richness and host specificity means that I should expect a decrease in niche partitioning with an increase in species richness. This is because less host specific restio leafhopper populations should be more likely to share restio species with other community members (Devictor et al. 2010, Bolnick et al. 2011). Also, as host specificity of restio leafhoppers decreases towards species rich regions, increased regional species richness should lead to a stronger decline in niche partitioning than increased local species richness. I found that both local community species richness and regional species richness were associated with a decrease in niche partitioning when not controlling for restio species richness. However, counter to expectation local species richness was associated with a stronger decrease in niche partitioning ($\beta = -0.681$) than regional species richness ($\beta = -0.217$) and its effects were not altered when accounting for restio species richness (Table 5.1). Increasing restio species richness did not lead to increased niche partitioning (i.e. did not alleviate niche overlap). Therefore increased total niche space does not explain why local communities in species rich regions exhibit relatively high niche partitioning despite that community members are relatively more generalised.

Niche overlap can, however, be reduced without specialisation if host shifts (changes in trait means) occur without specialisation (changes in trait variance) (Bolnick et al. 2011). Therefore it is possible that restio leafhoppers have avoided niche overlap in species rich regions by host shifting (see Fig. 5.2c). I therefore cannot rule out the possibility that increases in regional restio leafhopper species richness have led to increased interspecific competition that has led to host shifts, but not specialisation. Host shifts may also occur in the absence of competition as a result of allopatric changes in the availability of host plants (Bush 1975, Wasserman et al. 1981, Nosil et al. 2006, Futuyma 2008). Spatial heterogeneity in selection pressures is thought to have been an important driver of speciation, and community patterns, in plants in the CFR (Ellis et al. 2014). Therefore allopatric host shifts followed by secondary contact may explain niche partitioning in local restio leafhopper communities(see other examples reviewed in Stuart and Losos 2013).

Other influences on restio leafhopper species richness

Although my study focuses on species richness as a driver of host specificity, the causality can be reversed. That is, the lack of competition between restio leafhopper species might mean that they are less likely to be host specific, and therefore less likely to speciate (following arguments of Armbruster and Muchhala 2009). As both restio leafhopper and restio species richness peaks in the south western CFR, it appears that restio species richness alone may explain restio leafhopper species richness (as was initially suggested by Novotny et al.(2006) for tropical insect species richness). A more detailed quantitative approach is, however, needed to assess the geographic correspondence between restio and restio leafhopper species richness directly. Restio leafhopper specialisation and diversification is likely also influenced by interspecific interactions other than interspecific competition. Predation has been argued to be more important than interspecific competition in herbivorous insect community structure and diversification (e.g. Lawton and Strong 1981, Nosil and Crespi 2006). Restio leafhoppers are likely no exception; they appear to mimic the leaf sheaths of restios in order to avoid predators (see Figure 1 in Augustyn et al. (2013)). As not all restio species have the same colour sheaths (and differ in other traits, see Linder (2011)), predation might be an important selective pressure favouring the evolution of leaf sheath matching to a restricted set of restios. However, I have previously demonstrated that survival declines in the absence of predation when restio leafhoppers are transferred to restio species that they avoid (Chapter 3). Thus, like in other cryptic insects, multiple selective pressures likely explain the evolution of specialisation in restio leafhoppers (see *Timema*, Nosil and Sandoval 2008).

Conclusion

My study has three strengths that are often lacking in studies investigating the link between species richness and specialisation. As it was conducted within a single biogeographic region and largely within a single vegetation type I excluded the influence of large differences in biogeographic history (Stephens and Wiens 2003). In addition I studied the role of species richness on host specificity at the local population level which is often the appropriate spatial scale for the study of selection pressures (Anderson et al. 2014) and specialisation (Fox and Morrow 1981). Although, tropical studies use only local estimates of specificity (Novotny and Basset 2005, Forister et al. 2014), they do not compare specificity in populations across species richness gradients (Robinson and Schluter (2000) used an approach similar to mine for stickleback fish). My standardised sampling approach also allowed me to express host specificity as realistic weighted interactions instead of the number of host plant species used as in most large scale diversity studies (e.g. Novotny et al. 2006, Dyer et al. 2007, Condon et al. 2008, Forister et al. 2014). These studies do, however, make use of rearing experiments that account for incidental host use records (Dyer et al. 2007). Nonetheless my analyses are unlikely to be affected by incidental encounters (Blüthgen et al. 2006). Despite the advantages my approach offers, I detected no causal link between species richness and host specificity in restio leafhoppers. This may be because restio leafhoppers do not exhibit interspecific competition, or that specificity at the population level does not translate into higher speciation rates in a straightforward way. Nonetheless, I suggest that more studies such as mine are required to validate whether the now established correlation between latitude and host specificity in herbivorous insects (Forister et al. 2014) reflects a causal link between species richness and host specificity.

Figures and Tables

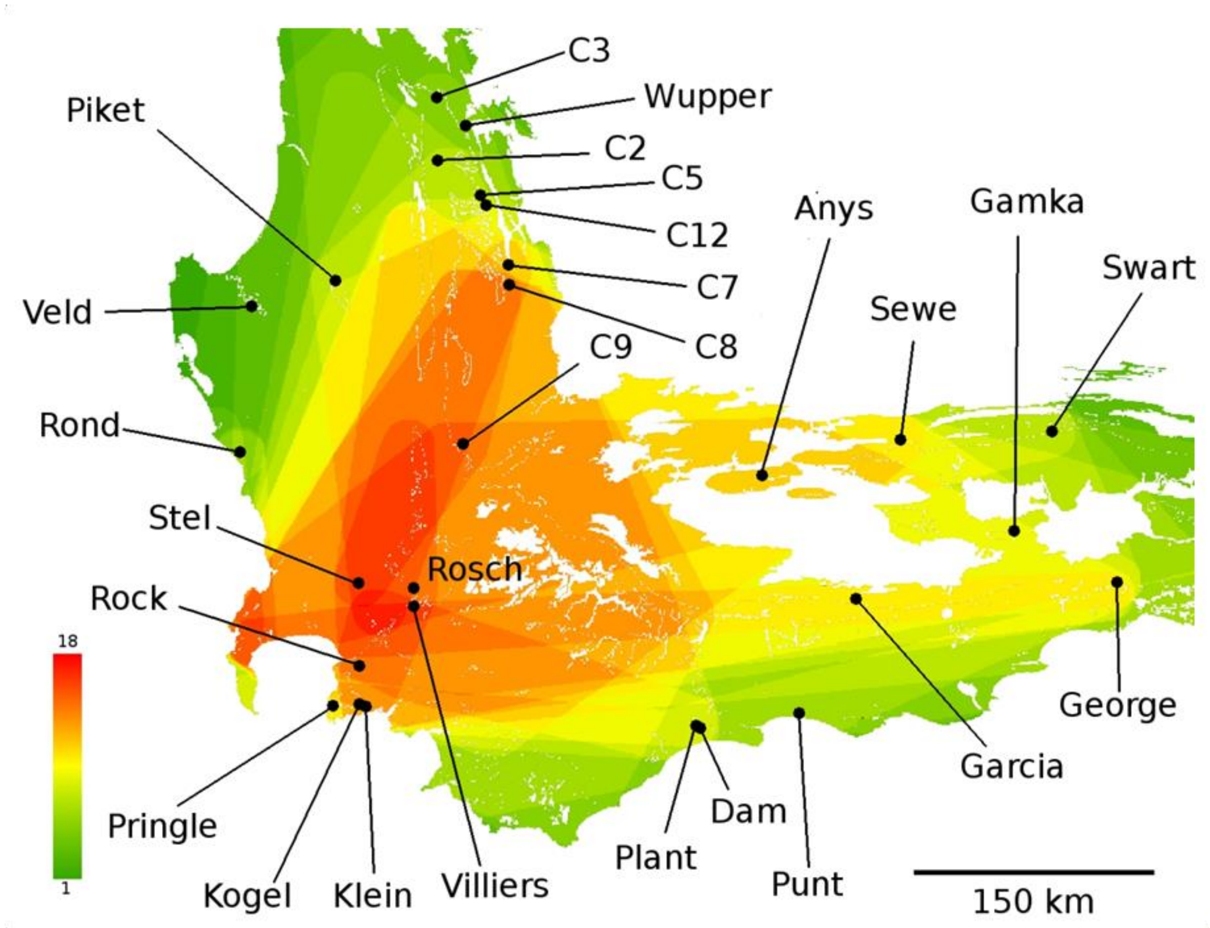


Figure 5.1: Map showing 27 sampled sites across a restio leafhopper species richness gradient. The colour gradient indicates the number of overlapping restio leafhopper distribution ranges across the CFR (which I refer to as regional richness) – the highest number of overlapping ranges was 18 and the lowest 1.

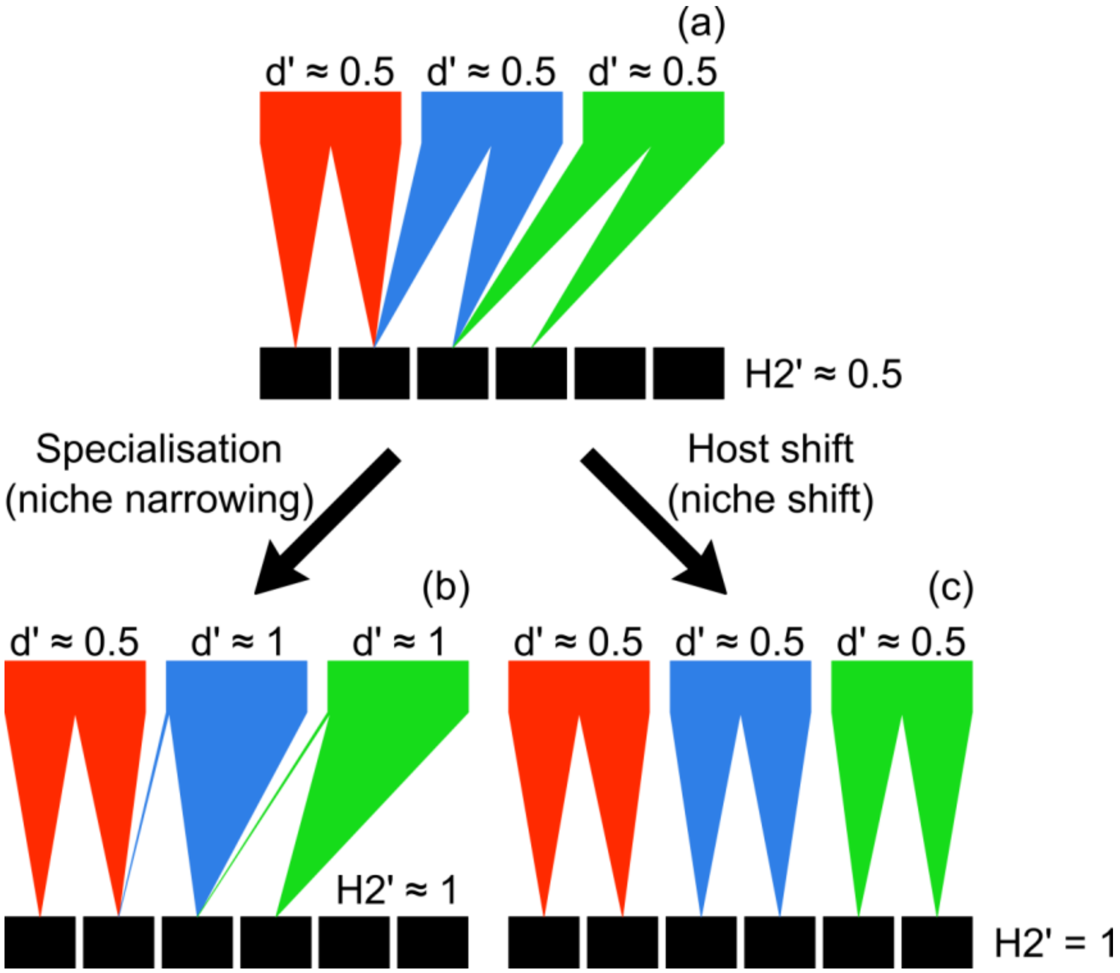


Figure 5.2: Niche overlap reduced by specialisation or host shifting without narrowing host use. A hypothetical community consists of a red, blue and green restio leafhopper species and there are six different restio species within the community (in black). In the first example (a) three different species are equally host specific; d’≈ 0.5 for all species. In addition, they overlap in which restio species they use. Thus, niche partitioning within the community is relatively low, H2’ = 0.5. However, niche overlap can be reduced if species specialise. In example (b) the red species is not more host specific than in example (a); d’ ≈ 0.5. However, the blue and the green species now each mostly use a single restio species; their d’ values are now close to 1. The community also exhibits almost complete niche partitioning; H2’ ≈ 1. However, niche partitioning can occur without specialisation. There is no difference between the specificity of restio leafhopper species in example (a) and (c), however species have shifted hosts in a way that reduced niche overlap, thus H2’ = 1.

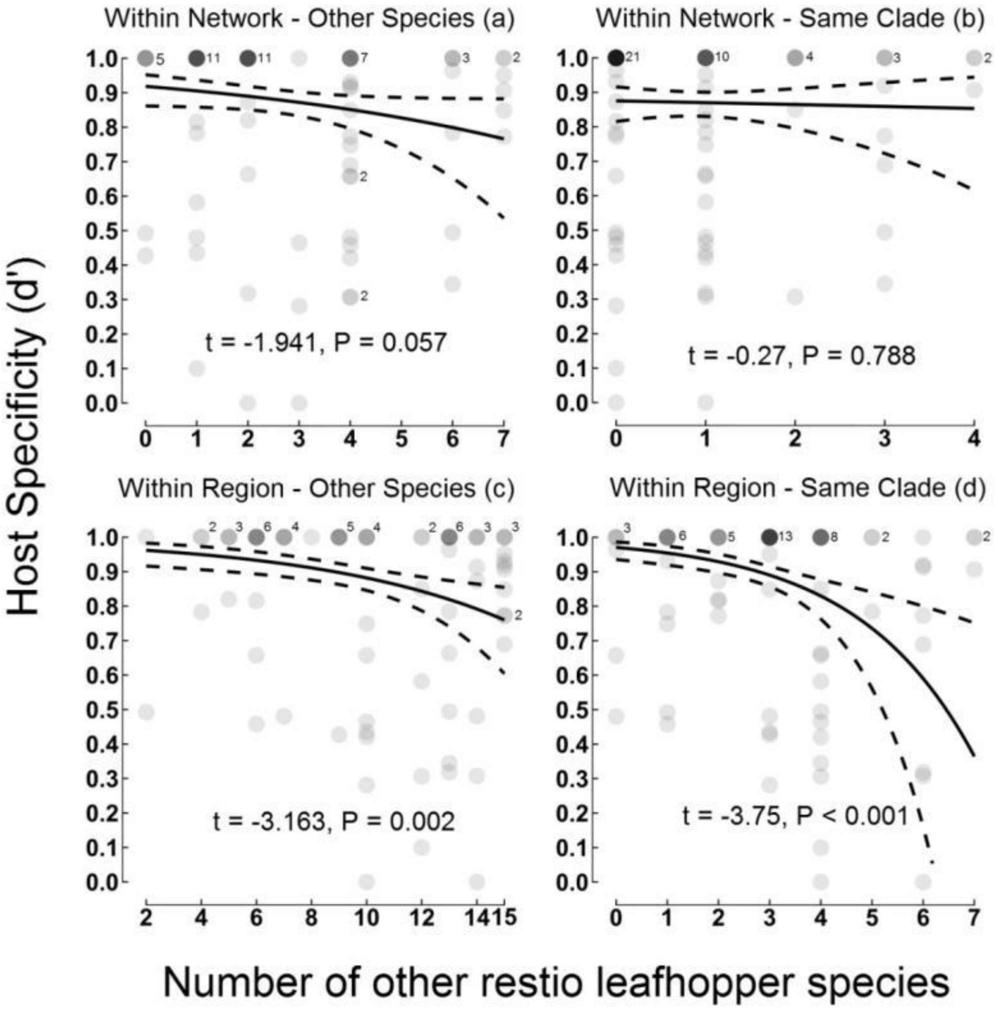


Figure 5.3: The effect of species richness on restio leafhopper host specificity. Each dot represents a host specificity estimate (d’) of a restio leafhopper population. Dots are semi-transparent, dark dots represent multiple overlapping points. Small numbers next to dark dots indicate the number of overlapping points. X axes represent the number of species other than the population for which d’ was estimated (i.e. total species richness - 1). The effect of local community species richness (within network, a and b) and regional species richness (within region, c and d) was assessed. Also, the richness of all restio leafhopper species (other species, a and c) and only closely related species (same clade, b and d) was assessed separately. Populations ranged from complete generalists (0) to complete specialists (1). Solid lines represented GLM estimated d’ value means and dashed lines represent 95% confidence intervals. Their associated GLM estimated t and P values are indicated. Contrary to expectations from interspecific competition, increased species richness did not lead to increased host specificity. Host specificity showed a near significant decrease with increasing local community species richness (a). Increasing within-clade local community species richness had no significant effect (b). Increased regional species richness of all (c) and only closely related (d) restio leafhopper species was associated with decreased host specificity.

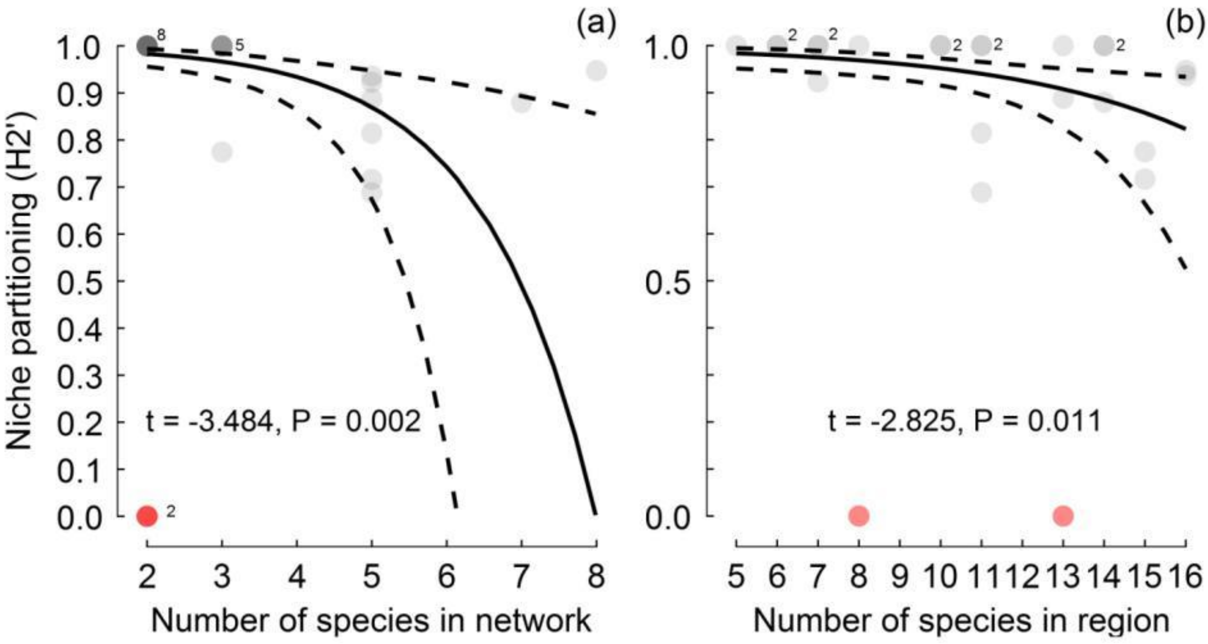


Figure 5.4: The influence of increasing within local community species richness (a) and regional species richness (b) on niche partitioning. Each point represents a niche partitioning (H2') estimate of a local community network. If host specificity increases H2' should remain high despite increased species richness. Conversely, if specificity does not increase with species richness, H2' is expected to decrease (i.e. niche overlap increases). However, increased niche partitioning can occur without increased specificity see Fig 5.2c. H2' ranged from 0 (complete niche overlap) to 1 (complete niche partitioning). Dots are semi-transparent, dark dots represent multiple overlapping points. Small numbers next to dark dots indicate the number of overlapping points. The two populations showing complete overlap (indicated in red) both consisted of *C. uncinatus* and *C. appendiculatus* specialised on *M. digitata*. GLMs reported here were performed excluding these outliers (estimated t and P values in black) (thus only Local 1 and Region 1 models from Table 5.1 are shown here). Results of models including outliers are shown in Table S5.3. Solid lines are GLM estimated means and dashed lines are 95% confidence intervals. Since increased species richness did not lead to increased host specificity, niche partitioning declined with increased local community (a) and regional species richness (b).

Table 5.2: GLMs exploring the relationship between increased local and regional species and niche partitioning (H2'). At both scales, models Local 1 and Region 1 included restio leafhopper species richness and sampling season. Models Local 2 and Regional 2 included the additional variable, restio species richness, to account for increased niche partitioning with increased restio species richness (i.e. niche availability). Note that increasing local community species richness was always associated with decreased niche partitioning. However, this was not true for regional species richness which had no significant effect on H2' in Regional 2. In addition, for both local and regional richness, Local 2 and Regional 2 had lower AICs than models Local 1 and Regional 1. Nonetheless, increased restio species richness did not lead to increased niche partitioning (i.e. did not alleviate niche overlap).

Model	AIC	Coefficient	β	SE	t	P
Local 1	-79.883	Species richness	-0.681	0.196	-3.484	0.002
		Season	-0.1357	0.982	-0.138	0.892
Local 2	-84.943	Species richness	-0.627	0.14	-4.464	< 0.001
		Season	0.074	0.671	0.11	0.913
		Restio richness	-0.148	0.083	-1.784	0.091
Regional 1	-72.711	Species richness	-0.217	0.077	-2.825	0.011
		Season	-0.719	0.773	0.929	0.364
Regional 2	-73.066	Species richness	-0.154	0.094	-1.644	0.118
		Season	-0.352	0.814	0.433	0.67
		Restio richness	-0.15	0.109	-1.381	0.184

Supplementary tables and figures

Table S 5.1: Summary of all networks used in the study. The name of a network corresponds to a site, suffixes indicate which season a network was sampled in (A = autumn, S = Spring).Note that not all sites were sampled in both seasons. Niche partitioning (H2') is shown for all networks with more than one species with the exception of Kogel1; here one hopper species consisted of a single observation. The number of restio leafhopper species within each network is shown followed by the number in the region of each network. The number of restio species in each network is shown followed by the number of individual plants sampled per restio species.

Network	H2'	Local species richness	Regional species richness	Restio species richness	Sampling effort per plant species
Anysberg A	1	2	11	8	40
C12 A	1	2	8	5	80
C2 A	1	2	7	4	40
C3 A	1	2	5	7	40
C4 A	NA	1	4	1	40
C5 A	NA	1	4	4	40

C7 A	1	2	11	2	40
C8 A	1	3	14	5	40
C9 A	0.77	3	15	13	40
DeHoopDam A	NA	1	7	2	40
DeHoopPlant A	0	2	8	2	40
Gamkaberg A	NA	1	9	4	40
Garcia A	NA	1	10	6	40
George A	1	3	10	7	40
Kleinmond A	0.89	5	13	7	80 (two 40)
Kleinmond S	0	2	13	7	80 (two 40)
Kogelberg A	NA	2	13	7	80
Kogelberg S	1	2	13	7	80 (two 40)
Piketberg A	0.92	5	7	6	80 (two 40)
Pringle A	0.81	5	11	10	100
Pringle S	0.69	5	11	10	100
Puntjie A	1	2	6	2	80
Rockview A	0.88	7	14	12	80

Rockview S	1	3	13	12	80
Rondeberg A	1	3	6	3	80
Rosch A	0.72	5	15	6	80 (One 40)
Sewe A	1	3	10	10	40 (One 30)
Stelberg A	0.95	8	16	9	80
Swartberg A	1	2	7	7	40
Veldrift A	NA	1	3	2	80
Villiers A	0.94	5	16	10	80
Wuppertal A	NA	1	5	6	80

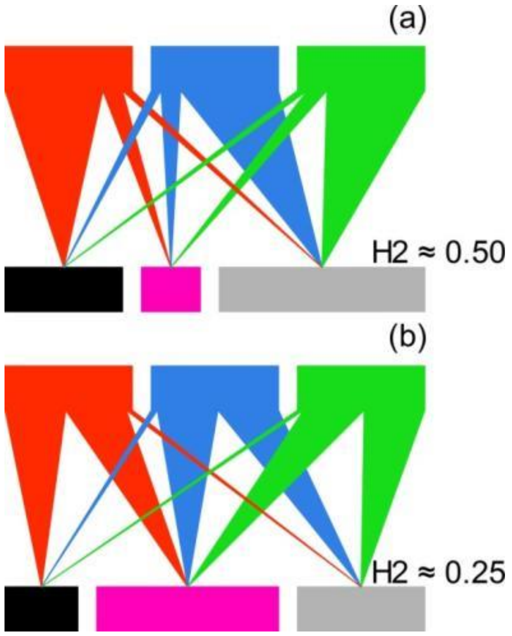


Figure S5.1: A hypothetical community sampled using two different techniques that have different outcomes forH2’. The community consists of a red, blue and green restio leafhopper species (arrows) and a black, pink and grey restio species (rectangles). The thicknesses of the arrows represent the strength of utilisation of restios by restio leafhopper species, and the thicknesses of rectangles below show the relative extent to which restios are used by all restio leafhopper species. In (a) all restio species were sampled equally for restio leafhoppers, and in (b) plants were sampled according to their relative abundances. The pink restio species was five times more abundant than the grey and the black restio species. Note that in (a) all restio leafhopper species interact weakly with the pink restio species, while in (b) all restio leafhopper species connect strongly with the pink restio species as it was sampled frequently because of its high abundance. In (a) H2’ is a traditional measure of niche partitioning while (b) is a measure of the outcome of niche partitioning on interspecific interactions. That is, restio leafhopper species have evolved to avoid the pink restio species to avoid interspecific competition resulting in a relatively low H2’ value in (a). However, as the pink restio species is common, restio leafhopper species often interaction it resulting in a low H2’ value (b). In this study I were interested in niche partitioning and therefore sampled all restio species equally as in (a).

Table S5.2: GLMs testing for the effects of increased species richness on host specificity. Letters in the model column correspond to models shown in Figure 5.3a-d. Species identity is reported as the difference between a model including and excluding it.

Model	Coefficient	F	β	SE	t	P
a	Local species richness		-0.151	0.078	-1.941	0.057
	Species identity	2.975				< 0.001
b	Local species richness (same clade)		-0.041	0.151	-0.27	0.788
	Species identity	2.879				0.001
c	Regional species richness		-0.142	0.045	-3.163	0.002
	Species identity	3.01				< 0.001
d	Regional species richness (same clade)		-0.439	0.117	-3.75	< 0.001
	Species identity	3.374				< 0.001

Table S5.3: Results of Models Local 3 (same factors as Local 1) and Regional 3 (same factors as Regional 1) conducted with outliers shown in red in Figure 5.4 (main text).

Model	AIC	Coefficient	β	SE	t	P
Local 3	-47.635	Local species richness	0.0133	0.229	0.058	0.954
		Season	-1.058	1.049	-1.008	0.325
Regional 3	-47.76	Regional species richness	-0.033	0.129	0.256	0.8
		Season	-1.009	1.173	0.86	0.34

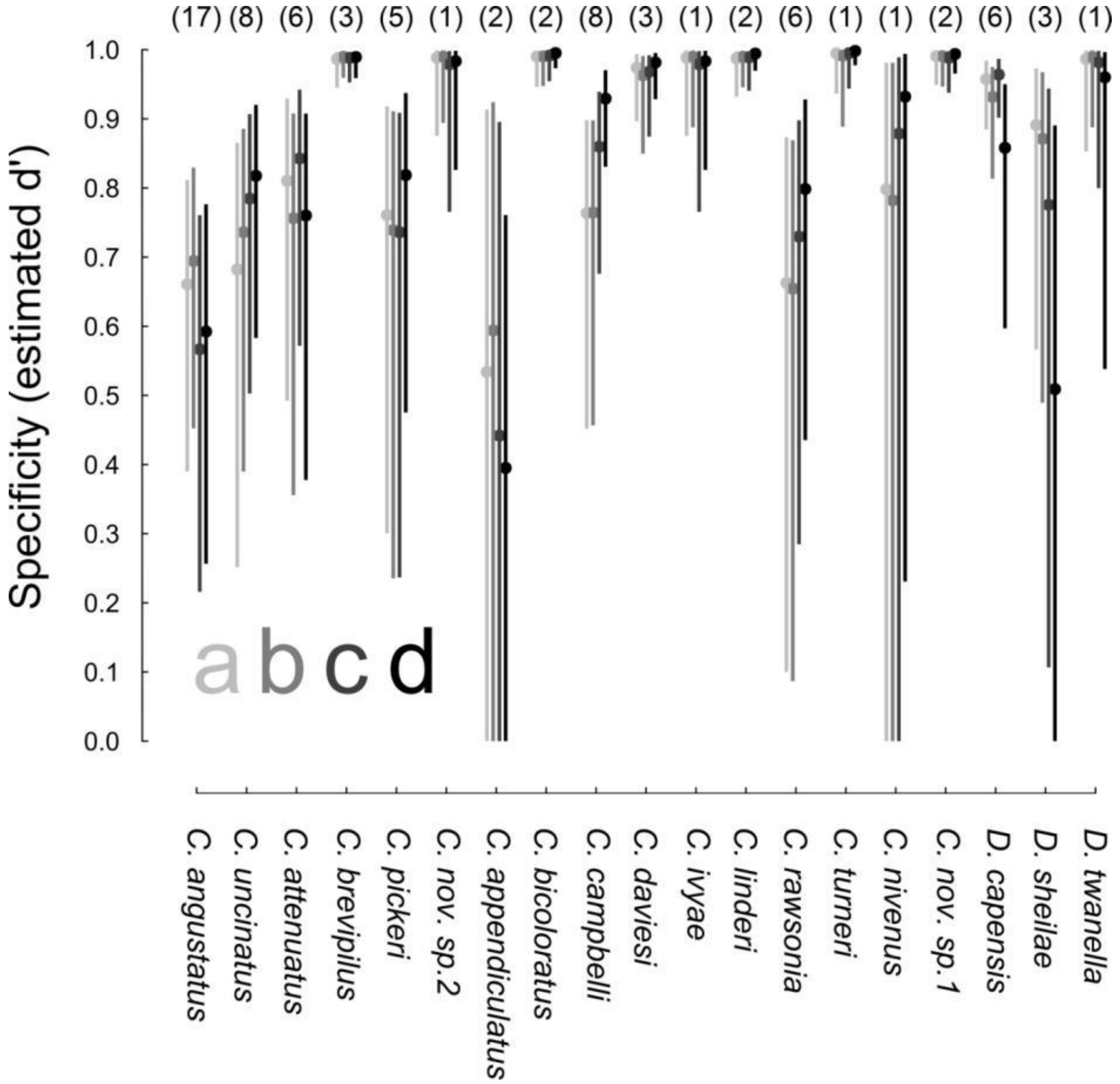


Figure S5.2: Local specificity estimates for different species of hoppers under four different models, GLM estimated specificity means (d’) and 95% CI are shown in the same order as shown in Figure 5.3 (main text): a) local species richness, b) local species richness of species from the same clade, c) regional species richness, and d) regional species richness of species from the same clade. A mean d’ value of 1 suggests complete host specificity and a mean close to 0 suggests complete generalism. Numbers in parenthesis above means and 95% CIs shows the sample size (i.e. number of networks d’ was estimated from).

Chapter 6

Spatial heterogeneity in plant distributions drives host-shifts in *Cephalelus uncinatus*, a broadly distributed restio leafhopper species

Abstract

Ecological speciation is the evolution of reproductive isolation resulting from divergent selection. Herbivorous insects are often used to study this model of speciation because speciation of insect herbivores is frequently initiated by shifts to novel host plants which act as divergent selective environments. Host shifts can occur when the ancestral host plant of an herbivorous insect population is not available, forcing initially inferior host plants to be incorporated into the diet of a founder population of insects. The plant-diverse Cape Floristic Region of South Africa is characterised by plant species being patchily distributed across the landscape which may facilitate allopatric host-shifts in herbivorous insects. I used a restio leafhopper species, *Cephalelus uncinatus*, and its host plants from the Restionaceae family (restios) to determine whether restio leafhoppers are locally adapted to their host plants. I first show that *C. uncinatus* is forced to use different restio species in different regions because its host plants are not homogeneously distributed across the landscape. I then show, by means of reciprocal host presentation experiments, that *C. uncinatus* in both allopatric and parapatric populations have shifted their preferences to different host plants, but that this is not the case in sympatry. Morphological divergence in traits potentially related to predation (body size and colour) largely mirrors this pattern, suggesting that divergence of *C. uncinatus* has occurred through host-shifts in response to large-scale geographic heterogeneity in host plant distributions. My findings emphasise the importance of geographic heterogeneity in selective environments as an initiator of ecological divergence.

Introduction

Ecological speciation is the evolution of reproductive isolation as a consequence of divergent selection (Rundle and Nosil 2005). Under this model of speciation, reproductive isolation between populations is most likely to emerge when strong or multiple divergent selection pressures (multifarious selection) are experienced (Nosil and Sandoval 2008, Nosil et al. 2009) and when gene flow is limited (Via 2001). As both these conditions are most likely to be met in spatially isolated (allopatric) populations, classifying speciation on a geographic continuum (from total allopatry to total sympatry) is useful (Rundle and Nosil 2005). Under one particular scenario of ecological speciation, reproductive isolation initially evolves in allopatry with low gene flow, followed by secondary contact (Albert and Schluter 2004, Rundle and Nosil 2005, Jordal et al. 2006). Once secondary contact occurs, other processes such as reinforcement (i.e. selection against unfit hybrids) result in further increases in sexual isolation and eventual speciation (Nosil et al. 2003, Albert and Schluter 2004, Rundle and Nosil 2005). However, for reproductive isolation to be maintained, gene flow should not be too strong (Nosil et al. 2003).

In the case of herbivorous insects, reproductive isolation is primarily thought to be initiated when populations shift to novel host plants (Ehrlich and Raven 1964). The primary reason for this is that a host shift potentially translates into a dramatic change in the selective regime that an insect population experiences. This is because different hosts used by different insect populations can differ markedly in both their chemistry (Ehrlich and Raven 1964) and backgrounds against which insects are concealed against predators (Brower 1958, Nosil 2004). Thus, in herbivorous insects using different host plants divergent selection can be multifarious, and the probability of reproductive isolation evolving as a result is potentially high (Nosil and Sandoval 2008). If host shifts are accompanied by life history factors such as host fidelity (Feder et al. 1994), and if insects mate on their host plants (Bush 1969a, 1969b), reproductive isolation can evolve as a direct consequence of host shifting. In other words, assortative mating can occur as a direct result of host preference differences between ancestral and novel host-associated populations (called ecotypes henceforth) (Feder et al. 1994). Because divergent preferences act as such a strong sexual isolating barrier, they have often been viewed as a key factor allowing sympatric speciation (Bush 1969a, 1969b, Feder et al. 1994), albeit not conclusive evidence for it (Berlocher and Feder 2002).

Host shifts can occur across the sympatry-allopatry continuum (Futuyma 2008). When host shifts occur in sympatry, host-related selection gradients need to be steep

(Berlocher and Feder 2002). For this reason ecological factors such as competition are thought to be important in the initial host shift (Feder et al. 1995, Dieckmann and Doebeli 1999). In contrast, host shifts in allopatry can simply occur as a result of not encountering the ancestral host plant (Nosil et al. 2006). This can even occur in the absence of a choice between host plants (Wasserman et al. 1981, Nosil et al. 2006). Once enough divergence has occurred in the absence of strong gene flow, secondary contact can occur without leading to the breakdown of reproductive isolation. Providing that gene flow is not too strong, reproductive isolation between host related ecotypes can be maintained during secondary contact (Nosil et al. 2003). As previously isolated host associated ecotypes can now occasionally interact sexually, reinforcement can directly drive the evolution of reproductive isolation (Nosil et al. 2003).

If ecotype formation, and evolution of divergent preferences, results from host shifts occurring in allopatry (followed by secondary contact or not), spatial heterogeneity in host plants will promote the evolution of reproductive isolation. However, this is only likely if the spatial distributions of host plants are temporally homogenous (Kassen 2002). If plant distributions change too frequently selection will favour generalism and local adaptation is likely to be impeded (Kawecki and Ebert 2004). Spatial environmental heterogeneity (in, for example, soils, topography, rainfall) is thought to underlie plant diversification in the extremely diverse Cape Floristic Region (CFR) in South Africa (Ellis et al. 2014). Also, climatic stability in the region is thought to have facilitated diversification by providing ample time for divergence to take place (Ellis et al. 2014). Consequently divergent selection in allopatry is thought to have played an important part in the radiations of Cape plants. The role of environmental heterogeneity in generating plant speciation is also reflected by the components of plant diversity in this region. Plant local (alpha) diversity is not exceptionally high, but spatial turnover of plant species between different abiotic environments (beta diversity) is the highest of any Mediterranean-climate region in the world (Cowling and Rundel 1996). This suggests that, like plants, herbivorous insects in this biome might also respond to spatially disjunct selective environments. Founder populations of herbivorous insects might shift hosts in allopatry in response to the decline in availability of previously preferred host plants.

The African Restionaceae (hereafter called restios) is a highly diverse monophyletic plant clade which comprises about 350 species, with only 10 of these occurring outside the CFR (Linder 2003). It is one of the diagnostic components of fynbos vegetation and is one of the oldest clades in the CFR, with its origin estimated at about 65 million years ago (Linder et al. 2003). Restios are generally reed-like in appearance. Their photosynthetic stems have regular nodes with persistent dried out leaf sheaths in most species, but these drop off in some species. The leaf sheaths of restios appear to be mimicked by the morphology of cryptic herbivores called restio leafhoppers (Cicadellidae: Cephalelini), the dominant insects on restios (Kemp 2014). They are characterized by a small, slender body and have a diagnostic elongated crown (with the exception of *Duospina capensis*) that resembles the bracts and dried out leaf sheaths of restios. Currently there are 21 described species from two genera namely *Cephalelus* and *Duospina* (Prendini 1997). Recent findings suggest that restio leafhoppers did not co-diversify with restios; instead restio leafhoppers diversified much more recently (1-6 MYA) than restios (Wiese 2014). This is reflected by the fact that there are 15 times more described restio species than described restio leafhopper species. As a result, many restios are not exploited by restio leafhoppers (niches are unfilled). This might suggest that restio leafhoppers are in an early stage of an adaptive radiation during which host shifts can occur without interspecific competition (or homogenising hybridisation, e.g. Taylor et al. (2006)) impeding diversification (Mahler et al. 2010).

My study focuses on *Cephalelus uncinatus*, a broadly distributed restio leafhopper species which consumes the sap from several restio species. It mainly uses several genera of restios from the Willdenowieae tribe, as well as several species in the genus *Elegia* which belongs to the Restioneae tribe (Wiese 2014). I have previously shown experimentally that *C. uncinatus* from a single site actively chooses its predominant field host and also survives better on it than on unused restio species (Chapter 3). As these experiments were performed in the absence of predators, they suggest that preference is linked to performance through plant chemistry. However, *C. uncinatus* may gain additional protection from predators by choosing restios that serve as good backgrounds for camouflage. Across its distribution range, *C. uncinatus* uses different host plants, and it has been suggested that it consists of several ecotypes. Prendini (1997) noticed consistent differences in male genetalia between populations using *Willdenowia incurvata*, *Mastersiella digitata* and *Elegia nuda* as host plants.

Here I use *C. uncinatus* to determine whether restio leafhoppers have undergone host related ecotypic divergence in preference and morphology. Specifically, I ask (1) are host plants heterogeneously distributed across the CFR? (2) If so, are pairs of geographically isolated populations using different host plants more divergent in their host preferences than geographically nearby populations? I test this by reciprocally presenting restio hosts to *C. uncinatus* population pairs in allopatry, parapatry, and sympatry. If divergent preferences decrease with increasing geographic contact, it would suggest that ecotypic divergence is promoted by a geographic distance (likely because of reduced gene flow). I then ask (3) whether aspects of morphology (body size and colour) have also diverged in response to the use of different restio species. Considering that different restio species differ in culm thickness, large *C. uncinatus* individuals using restios with thin culms might be easily spotted by predators and selected against. Additionally, restio leafhoppers are thought to mimic the leaf sheaths of restios for camouflage (Osborn 1903), I therefore expect *C. uncinatus* individuals to have diverged in colour if the leaf sheaths of their host restios are divergent in colour. I also expect host preference and phenotype to have diverged together. This would be an indication that divergent selection through predation may contribute to the evolution of reproductive isolation in *C. uncinatus*.

Methods

Restio distributions

I assessed the spatial heterogeneity of host plant availability by making use of plant and insect distribution records. I sourced distribution records of *C. uncinatus* from an MSc thesis by Davies (1986) and an honours thesis by Prendini (1995). All other distribution records were obtained from my own opportunistic collecting and standardised sampling (Chapter 4). All *C. uncinatus* individuals were either identified by me, Davies (1986) or Prendini (1995). Restio distribution records were sourced from the leading expert on the Restionaceae, H.P. Linder (unpublished data). This data set mostly includes records from the Bolus herbarium and fieldwork conducted by H.P. Linder.

Only restio species used by more than one *C. uncinatus* individual from my own collection records (Chapter 4) were included in the spatial analysis. This included *Willdenowia incurvata*, *Willdenowia teres*, *Mastersiella digitata*, *Mastersiella spathulata*, *Hypodiscus aristatus* and *Hypodiscus synchroolepis* from the Willdenowieae tribe and *Elegia nuda*, *Elegia stokoei*, *Elegia muirii*, *Elegia fistulosa*, and *Elegia filacea* from the Restioneae (Fig. 6.1).

For all restio species I measured the most northern, southern, western and eastern points of each restio distribution using ArcGis 11. I did the same for the distribution of *C.uncinatus*. I then measured, to the nearest kilometre, the differences in latitude between the most northern and southern points, and the differences in longitude between the most western and eastern points of each distribution. I performed two analyses, one for longitude and another for latitude, to test whether these restio species have narrower longitudinal and latitudinal distributions than *C. uncinatus*. This was done by calculating the 95% bootstrap estimated confidence intervals of restio longitudinal and latitudinal range extents. If the longitudinal or latitudinal extent of the distribution of *C. uncinatus* exceeded the95% confidence intervals of restio species distributions I concluded that *C. uncinatus* has a larger distribution range than most restio species. Bootstrapping was performed using the one.boot function in the R package simpleboot, 95% CIs were calculated from 2000 bootstrap replicates.

Local adaptation in preference

I tested three pairs of putative ecotypes differing in their degree of spatial isolation (Fig 6.2). An allopatric comparison was made between *C. uncinatus* using *M. digitata* at Pringle Bay and *W. incurvata* at Rondeberg (Fig 6.2a). These two sites are 113 km away from each other and gene flow should be minimal. The distributions of the host plants are also largely non-overlapping (Fig 6.2b). This suggests that, at a large geographic scale, there should be little gene flow between *C. uncinatus* using *M. digitata* and *W. incurvata*. At a finer spatial scale of co-occurrence, I compared preferences of *C. uncinatus* using *M. spathulata* and *E. nuda* in parapatry at De Hoop (Fig 6.2b). The host plants were located 800 meters away from each other, and gene flow is probably higher than for the allopatric population. The distributions of the two host plant species also overlap partly, but are largely non-overlapping (Fig 6.2b). This suggests that, at a large spatial scale, populations of *C. uncinatus* using *M. spathulata* and *E. nuda* can experience gene flow in parts of their distributions, accentuated at sites such as De Hoop. However, as *M. digitata* and *E. nuda* are from different restio tribes, they might represent strongly divergent selective environments countering the homogenising effect of gene flow. At the finest spatial scale of co-occurrence, I tested populations occurring on different hosts in sympatry, and thus potentially experiencing high levels of gene flow (Fig 6.2c). *C. uncinatus* uses both *M. digitata* and *H. aristatus* at Pringle bay, which may represent different ecotypes. At a large spatial scale *M. digitata* and *H. aristatus* overlap extensively (Fig 6.2c). Therefore, at a large geographic scale, gene flow should be highest between *C. uncinatus* associated with *M. digitata* and *H. aristatus* than between the allopatric and parapatric pairs above.

For the allopatric comparison, insects were first collected from *W. incurvata* at Rondeberg on the 28th of October 2013. On the following day *C. uncinatus* was collected from *M. digitata* in Pringle Bay. When the experiment was repeated later, insects were first collected from *M. digitata* on the 19th of November 2013 at Pringle bay and then from *W. incurvata* the following day. For the parapatric comparison at De Hoop collections were made on the 6th and the 8th of January 2014. For the sympatric comparison at Pringle Bay, insects were collected on the 6th and the 13th of November 2013. Insects in this comparison were collected 700 m away from each other. *H. aristatus* consisted of pure stands while individuals from *M. digitata* were caught in the presence of a 50/50 mix between *H. aristatus* and *M. digitata*. For all experiments, insects were collected by vacuuming insects off plants by means of a modified leaf blower/shredder. During collecting, insects were placed singly into clean Eppendorf vials which were then placed in a cooling box. After collecting, insects were kept in a fridge at 10 °C until experiments started the next day (approximately 12 hours later) or two days later for the allopatric comparisons (36 hours). Restio culms were collected at the sites of insect collecting and placed into distilled water to keep them fresh. Once in the laboratory, culms were cut to the length of 135 mm and kept fresh in a fridge at 10 °C.

In each of the three experimental comparisons a minimum of 76 individuals of each putative restio leafhopper ecotype were presented with a choice of both restio species. In each case both ecotypes were tested simultaneously. To avoid competitive or sexual interactions, individual insects were dropped alone into 740 ml preserve jars with one 135mm cutting of each restio species. Culms were placed in the jar so that they were touching. To keep restio cuttings fresh, I attached 0.6 ml vials with distilled water to the bottom of each cutting. I prevented fogging of jars by replacing lids with fine gauze and jars were kept at a constant 25°C. Insects could easily move over the glass surface of the jars, and from one culm to another. After 24 hours preference of each individual was recorded as the restio species it was perching on. More than 90% of restio leafhoppers had settled on one of the two restio species offered after 24 hours (see Figure S6.1). Insects that did not perch on a restio within 24 hours were excluded from analyses.

I analysed host preference data using separate binomial generalised linear models (GLM) with log link functions for each of the three experiments. The response variable was always a binary choice for either *Mastersiella digitata* (in the allopatric and sympatric experiments) or *Mastersiella spathulata* (in the parapatric experiment). Each model tested for the role of the independent variables, sex and putative ecotype, in determining host choice. For each GLM I back transformed and plotted GLM estimates of means and 95% CIs on a scale ranging from 0 to 1. If 95% CIs did not overlap with a 0.5 preference, a preference for one of the compared restio species was inferred. All GLMs were implemented in the base GLM (glm) function in R version 3.

Morphological divergence

Body size

Restios in my experiments differ in their culm thicknesses (see Figure S2; therefore selection from predation might drive divergence in body size between populations using different restios. I have observed that restio leafhoppers hide behind the culms when disturbed by the viewer. Thus, large bodied restio leafhoppers might not be able to effectively hide behind thin restio culms. Using the specimens from the presentation experiments, I tested whether divergence in body size has occurred. I did this separately for female and male *C. uncinatus*. Similar to the preference experiments, I did pair wise comparisons between the abovementioned allopatric, parapatric and sympatric putative ecotypes (Fig 6.2). To do this, I measured elytron lengths (sample sizes shown in Fig 6.3) as a standardised measure of body size. For each separate geographic comparison I then conducted a two-way ANOVA including putative ecotype and sex as independent variables and elytron length as the dependant variable.

Body colour

Another aspect of phenotype that could be under divergent selection is body colour of putative ecotypes. This may occur if selection from predation favours restio leafhoppers that colour-match the leaf sheaths of restios which they are mimicking. Using an Ocean Optics USB4000 spectrometer I recorded spectral colour data of *C. uncinatus* caught on the five different host plant species used in the reciprocal restio presentation experiments. Before taking colour measurements, the spectrometer was allowed to heat up for approximately 45 minutes. Thereafter, light and dark calibrations were performed every 10 minutes or less. All insects were measured once while two repeat measures of each restio sheath were taken and averaged. *C. uncinatus* sample sizes are shown in Fig. 6.4. Restio sheath sample sizes were as follows: 18 individuals of *W. incurvata*, 15 of *M. digitata*, 12 of *H. aristatus*, 10 of *M. spathulata* and 11 of *E. nuda*.

I modelled the spectral data in tetrahedral colour space of a potential predator of restio leafhoppers using the R package PAVO. Specifically, I modelled colour as perceived by the Cape dwarf chameleon (*Bradypodium pumilum*) using the experimentally determined cone sensitivities supplied by Stuart-Fox et al. (2007). This insectivorous reptile species occurs on various fynbos plants including restios (Tolley et al. 2010, Herrel et al. 2011). It is also the only visual predator that I have observed on restios during sampling. Although birds are often model species in insect predation, I observed no birds searching for insects on restios. Other species of dwarf chameleon in the genus also occur in Fynbos, and are broadly distributed throughout the CFR. Chameleon vision is evolutionary conserved; therefore *Bradypodium pumilum* is thought to be a good representative model for the entire *Bradypodium* genus (Stuart-Fox et al. 2007). Modelling was conducted assuming D65 standard daylight.

Using the colour space model, I then tested whether each putative host race colour matches the leaf sheaths of their host better than non-hosts. Specifically I made the same pair wise comparisons as above (allopatric, parapatric and sympatric). For each individual insect, I determined the Euclidian distance (in colour space) between itself and the central colour point (centroid) within the cluster of sheath measurements of each restio species. A shorter Euclidian distance indicates a better colour match between the insect and sheath. I used two-way ANOVAs including the variables, putative ecotype origin (i.e. which restio species it is from) and restio species identity. As all individual insects were represented by two measurements (i.e. distance to host and distance to non-host), I also included individual identity as a random variable. Female and male insects were analysed separately. A significant interaction between putative ecotype origin and plant species identity would be indicative of local adaptation to sheath colour (Kawecki and Ebert 2004).

Results

Restios are not homogenously distributed across the greater CFR (Fig 6.1). For example, *W. incurvata* (W.i in Fig. 6.1) is the only restio species used by *C. uncinatus* that occurs on the west coast of the greater CFR. This species also does not overlap with, for example, *M. digitata* (M. d in Fig. 6.1) that occurs further south east than *W. incurvata*. Restio distribution ranges also tended to be much more restricted than that of *C. uncinatus* (C. u, red dots in Fig. 6.1). For example, *M. digitata* (always used by *C. uncinatus* when encountered) is restricted to the south western part of the distribution of *C. uncinatus* which extends up both the west and east coast of the CFR.

This was the general pattern: *C. uncinatus* had a north-south distribution length of 517 km while restios had an upper 95% north-south distribution length of 328.4 km. *C. uncinatus* also had broad west-east distribution of 768 km while restios had an upper 95% west-east distribution of 733.9 km. Therefore, *C. uncinatus* encounters different restio species in different parts of its distribution range.

Divergent Preference

Putative ecotypes from *M. digitata* at Pringle Bay and *W. incurvata* at Rondeberg (allopatric comparison, Fig 6.2a) had significantly different host preferences (z = 2.025, *P* = 0.043, Fig 6.3a). However, there was still a general preference for *W. incurvata*. Females and males from *W. incurvata* showed a strong preference for this species (95% CI lower than 0.5). However, females from *M. digitata* showed a weaker preference for *W. incurvata* and males had no significant preference (95% CI overlapped with 0.5). Generally males had a stronger preference for *M. digitata* than females (z = 2.025, *P* = 0.043, Fig 6.3a).

Putative ecotypes from *M. spathulata* and *E. nuda* at De Hoop (parapatric comparison, Fig 6.2b) had strongly divergent host preferences (z = 4.382, *P* < 0.001, Fig. 6.3b). Females and males from *M. spathulata* also had significant preferences for *M. spathulata*, while only males from *E. nuda* had a significant preference for *E. nuda*. Preferences did not differ significantly between the sexes for this comparison (z = 1.115, *P* = 0.265, Fig. 6.3b).

Putative ecotypes from *M. digitata* and *H. aristatus* at Pringle Bay (sympatric comparison, Fig 1c) did not have significantly different host preferences (z = 1.057, *P* = 0.291, Fig 6.3c). Also, males and females of both putative ecotypes showed no preference for either *M. digitata* or *H. aristatus* (all 95% CIs overlap with 0.5). Preferences between females and males were the same (z = 0.277, *P* = 0.782, Fig 6.3c).

Phenotypic divergence

Body size

Putative ecotypes in all comparisons differed in body size. Insects caught from *M. digitata* at Pringle Bay had significantly shorter elytra than those caught from *W. incurvata* at Rondeberg (allopatric comparison), F = 25.39, df = 1, *P* < 0.001, Fig. 6.4a. Sexes did not differ in elytron length, F = 0.002, df = 1, *P*= 0.969, and the interaction between sex and identity of host plant was not significant, F = 1.685, df = 1, *P* = 0.212. The largest difference was between females of different ecotypes (by 0.549 mm, *P* = 0.002, see post hoc test results summarised in Table S6.1).

At De Hoop (parapatric comparison), insects caught from *M. spathulata* were significantly larger than those caught from *E. nuda*, F = 46.517, df = 1, *P* < 0.001, Fig 6.4b. Females also had shorter elytra than males, F = 6.107, df = 1, *P* = 0.017, but there was no significant interaction between sex and identity of host plant, F = 0.007, *P* = 0.933. *M. spathulata* males and *E. nuda* females differed the most (by 0.475 mm, *P* < 0.001, Table S6.1), followed by females of different putative ecotypes (by 0.347 mm, *P* = 0.001, Table S6.1).

At Pringle bay (sympatric comparison), the size difference between insects caught from different plants were less pronounced. Nonetheless, insects caught from *M. digitata* were significantly smaller than those caught from *H. aristatus*, F = 4.821, df = 1, *P* = 0.042, Fig 6.4c. Sexes did not differ in elytron length, F = 0.313, df = 1, *P*= 0.583, and the interaction between sex and identity of host plant was not significant, F = 0.687, df = 1, *P*= 0.419. Difference in body size was most pronounced in females, albeit only near significantly (by 2.44 mm, *P* = 0.075, Table S6.1).

Restio Sheath Colour Matching

I observed one clear case of local adaptation in colour matching of restio leaf sheaths between putative ecotype pairs. In the female allopatric comparison (Fig 6.5a), I detected no difference in the degree to which putative ecotype pairs matched leaf sheaths (F = 0.208, df = 1, *P* = 0.652). I also found no evidence that the leaf sheath of one restio species was matched better than the other (F = 1.723, df = 1, *P* = 0.198). I did, however, detect a near significant interaction effect between ecotype origin and plant species (F = 2.910, df = 1, *P* = 0.096).

For males (Fig 6.5d) in the allopatric comparison I also detected no difference in the degree to which putative ecotype pairs match leaf sheaths (F = 0.00, df = 1, *P* = 0.995). Both putative ecotypes matched *W. incurvata* better than *M. digitata* (F = 40.501, df = 1, *P* < 0.001), and I detected no significant interaction effect between putative ecotype origin and plant species (F = 0.037, df = 1, *P* = 0.849).

In the female parapatric comparison (Fig 6.5b) I found strong evidence for local adaptation in colour matching. Although females from putative ecotypes did not differ significantly in colour matching (F = 0.153, df = 1, *P* = 0.698), and tended to match *M. spathulata* better than *E. nuda* (F = 51.407, df = 1, *P* < 0.001), there was a strong interaction between putative ecotype origin and host plant (F = 26.092, df = 1, *P* < 0.001). In other words females from *M. digitata* matched *M. digitata* leaf sheaths best, and females from *E. nuda* matched *E. nuda* leaf sheaths best.

Males in the parapatric comparison (Fig 6.5e), however, exhibited weak evidence for local adaptation. Between putative ecotypes, males from *E. nuda* matched leafs heaths of both *M. spathulata* and *E. nuda* better than males from *M .spathulata* (F = 16.238, df = 1, *P* <0.001). Similar to females both putative ecotypes matched *M. spathulata* leaf sheaths better than *E. nuda* leaf sheaths (F = 716.233, df = 1, *P* < 0.001), but there was only a near significant interaction effect between putative ecotype origin and host plant species (F = 3.467, df = 1, *P* = 0.068).

The sympatrically occurring populations showed no evidence for local adaptation or divergence in leaf sheath matching. Females (Fig 6.5c) of different putative ecotypes did not differ in the degree to which they matched leaf sheaths (F = 0.028, df = 1, *P* = 0.868). Also, both populations matched *M. digitata* better than *H. aristatus* (F = 45.749, df = 1, *P* <0.001), and there was no significant interaction between putative ecotype origin and host plant species (F = 0.044, df = 1, *P* =0.835).

Males (Fig 6.5f) had a very similar leaf sheath matching pattern to females. There was no difference in the degree to which different putative ecotypes matched leaf sheaths (F = 1.108, df = 1, *P* = 0.298). Also, both putative ecotypes matched *M. digitata* better than *H. aristatus* (F = 168.236, df = 1, *P* <0.001), and there was no significant interaction effect between putative ecotype origin and host plant (F = 0.538, df = 1, *P* = 0.467).

Discussion

I examined the potential for spatial turnover in host plant availability as a driver of ecotype formation in an insect species endemic to the greater CFR of South Africa. I found that *Cephalelus uncinatus* has a distribution that is broader than most restio host species that it is able to use. Consequently *Cephalelus uncinatus* is obliged to use different host species in different parts of its distribution range. This suggests that host-shifts can occur as a result of simply not encountering ancestral restio hosts (Wasserman et al. 1981, Nosil et al. 2006). Results from preference experiments are in agreement with this hypothesis. I found significant differences in host preferences between allopatric and parapatric populations, but not sympatric populations. Morphological differences between putative ecotypes largely mirrored this finding:

Allopatric and parapatric pairs differed more in body size than what the sympatric putative ecotype pair did (although I did detect a significant difference in the sympatric pair). However, in analyses of colour matching of restio leaf sheaths, I only found strong evidence for local adaptation in the parapatric putative ecotype pair. These findings suggest that divergent selection in allopatry and parapatry, but not sympatry, favours ecotype formation in *C. uncinatus*.

Divergence in host preference

I predicted that if gene flow inhibits host use divergence, putative ecotypes of *C. uncinatus* should show strongest divergence in host preference in allopatry, decreasing towards sympatric situations. Host preference is potentially a good measure of reproductive isolation in restio leafhoppers, as they complete their lifecycle and mate on their food plants (W. Augustyn, personal observation). Thus assortative mating in *C. uncinatus*, like in other insect groups that mate on their host plants can occur as a direct result of divergent selection on preference (Feder et al. 1994). I do, however, recognise that I did not investigate the genetic grounds for preference (e.g. by performing reciprocal rearing experiments, see e.g. Nosil et al. (2006)). Nonetheless, I found mixed evidence for gene flow as an inhibiter of host preference divergence. While both the allopatric and parapatric putative ecotype pairs exhibited significant differences in host preference (in contrast to the sympatric pair), divergence was weaker in the allopatric comparison than in the parapatric comparison. Specifically, in the parapatric comparison, the putative ecotype pair had reciprocally divergent host preferences, while in the allopatric comparison both pairs tended to prefer *W. incurvata*. There can be several explanations for this pattern. One possibility is that host preference is strongest in parapatry because reinforcement can only occur on secondary contact (provided that gene flow is not too strong) (Nosil et al. 2003). Another possibility is that the selection gradient in the parapatric species pair is more pronounced because this was the only experimental pair comprising hosts from different Restionaceae tribes. In Chapter 2 I showed that *C. uncinatus* using *H. aristatus* (Willdenowieae) had reduced survival on *Elegia filacea* (Restioneae), suggesting that physiological factors such as restio chemistry acts as a selection pressure. Considering that restio chemistry of the genus *Elegia* is markedly different from other restios (Harborne 1979), *M. digitata* (Willdenowieae) and *E. nuda* (Restioneae) in the parapatric comparison are likely to differ physiologically. This might be in combination with more pronounced morphological differences (e.g. sheath colour) between *E. nuda* and other restio species used by *C. uncinatus*.

Morphological divergence

In this study I did not measure physiological traits of *C. uncinatus*; instead I measured traits that are likely to be under selection through predation. As for preference experiments I cannot rule out plasticity or that trait values resulted from non-adaptive processes. Nonetheless, consistent with the findings from preference experiments, I detected stronger morphological divergence in the allopatric and parapatric population pairs than in the sympatric pairs. Body size differed between all population pairs, but was least pronounced for the sympatric pair (see Fig 6.4). It also seems to correspond largely with culm thickness. For all comparisons the larger bodied ecotype was always associated with a thicker culmed restio (see Fig. S6.2). I suggest that this could result from predation selecting for smaller insects on thinner culms, allowing them to hide behind culms. Alternatively, large bodied restio leafhoppers may not be able to hold on to thin culms in strong winds and vice versa. Either way, my finding suggests that there is selection on body size, which is often regarded as a classic magic trait because of its direct involvement in assortative mating (Servedio et al. 2011). Western skinks, for example, tend to choose similar sized mates (Richmond and Jockusch 2007). Herbivorous insects have been shown to communicate their body size with substrate borne vibratory signals (de Luca and Morris 1998). Also, vibratory signals in auchenorrhynchans (leafhoppers and planthoppers etc.) have been shown to be important in mate selection and assortative mating (Nuhardiyati and Bailey 2005, Rodríguez et al. 2006). Thus body size in *C. uncinatus* (and possibly other restio leafhoppers) might be an important trait involved in assortative mating and promoter of rapid reproductive isolation.

Although reduced fitness associated with adaptation is not directly related to assortative mating, it can automatically increase reproductive isolation through immigrant inviability (Nosil et al. 2005). This process simply requires that maladapted immigrants have reduced survivorship prior to mating, which inadvertently results in assortative mating (Nosil et al. 2005). I previously showed that host preference is potentially linked to survival in *C. uncinatus* (Chapter 2). This suggests that immigrant inviability might result from physiological trade-offs in putative *C. uncinatus* ecotypes. However, here I indirectly investigated predation as a potential driver of immigrant inviability. In Cape dwarf chameleon vision, I only detected significant divergence in restio sheath colour in the parapatric comparison between females from *M. digitata* and *E. nuda*. This suggests that immigrant females that are maladapted in terms of colour will be selected against by predation leading to assortative mating in the parapatric comparison (Nosil et al. 2005).

Conclusion

My findings suggest that large scale spatial heterogeneity of restios might be important in initiating host shifts in *C. uncinatus*. For example, *C. uncinatus* on the west coast will continuously encounter *W. incurvata* (Fig 6.2a) while *C. uncinatus* on the south west coast will continuously encounter *M. digitata* and *H. aristatus* (Fig 6.2a and c). This might allow fitness trade-offs to evolve with little gene flow (Hereford 2009). Once secondary contact is made, such as in the case of parapatric population pair at de Hoop (Fig 6.2b), strong selection might maintain reproductive isolation between divergent populations. I examined traits considered important for the maintenance or evolution of reproductive isolation. All traits, host preference, body size and colour can be directly or indirectly related to assortative mating in *C. uncinatus*. Also, considering that physiological performance is possibly linked to preference in restio leafhoppers (Chapter 2), selection might be multifarious (Nosil et al. 2009). That is, both plant chemistry and differential predation might be important in driving host plant related divergence (Nosil and Sandoval 2008). My findings suggest that ecological speciation is likely an important model for speciation in restio leafhoppers that are possibly in an early and rapid phase of radiation (Wiese 2014).

Figures

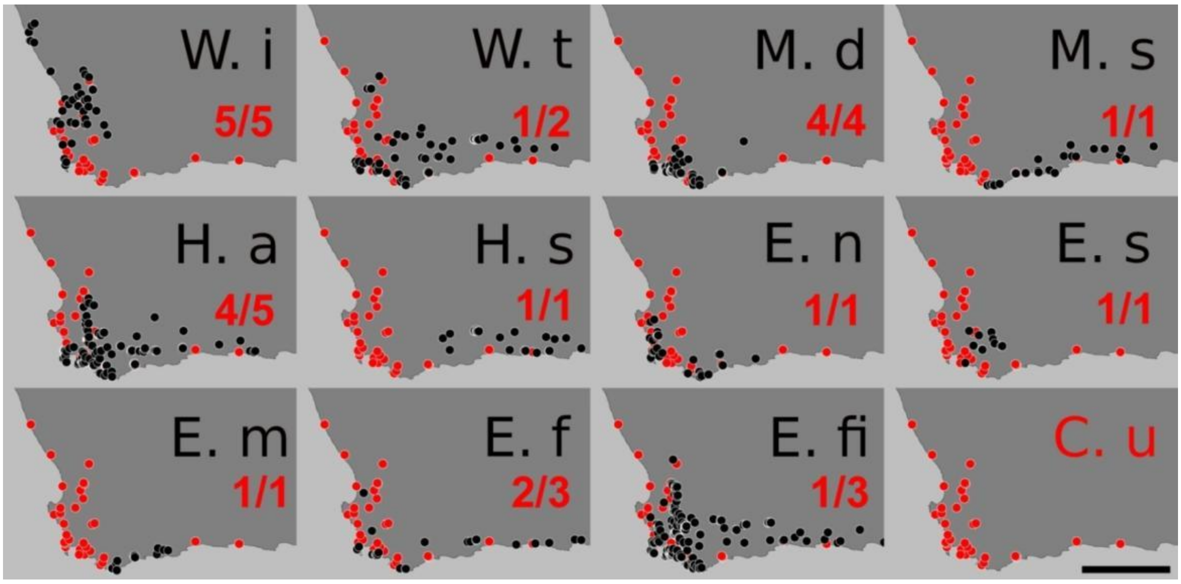


Figure 6.1: Known distribution ranges of *C. uncinatus* (C. u – red dots) and restios used by it. These are: *Willdenowia incurvata* (W. i), *Willdenowia teres* (W. t), *Mastersiella digitata* (M. d), *Mastersiella spathulata* (M. s), *Hypodiscus aristatus* (H. a), *Hypodiscus synchroolepis* (H. s), *Elegia nuda* (E. n), *Elegia stokoei* (E. s), *Elegia muirii* (E. m), *Elegia. fistulosa* (E. f) and *Elegia filacea* (E. fi). The fractions in red show how many times a restio species was used by *C. uncinatus* out of the number of times that the restio species co-occurred with *C. uncinatus* in my dataset. The scale bar in the bottom-right corner represents 300 km.

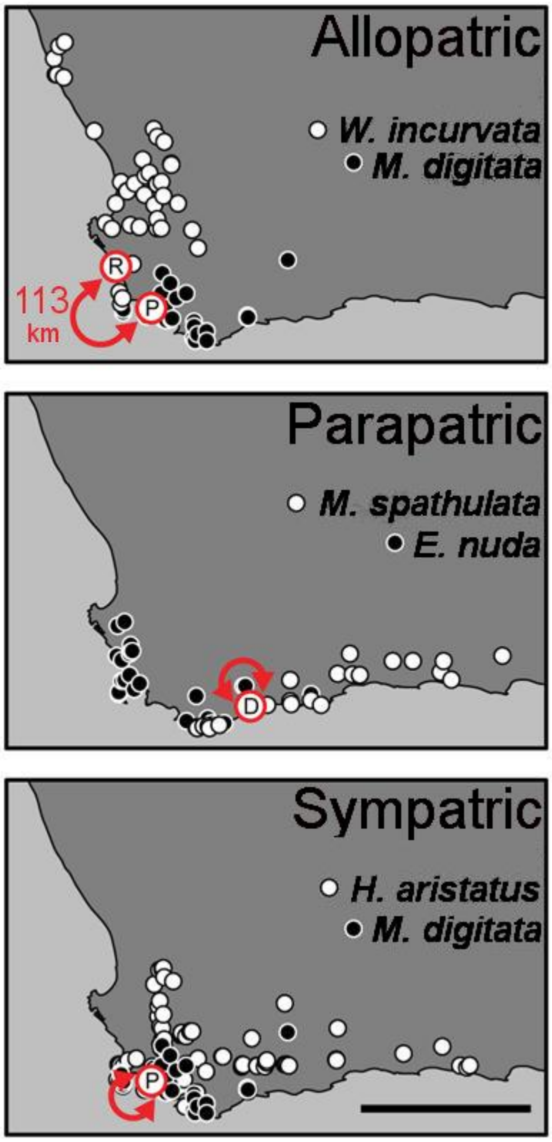


Figure 6.2 Local adaptation experiments were conducted at three sites: Rondeberg (R symbol), De Hoop (D symbol) and Pringle Bay (P symbol). Three experiments were conducted: an allopatric comparison (sites were 113 km away from each other) between *C. uncinatus* using *M. digitata* at Pringle Bay and *W. incurvata* at Rondeberg (top panel), a parapatric comparison at De Hoop between *C. uncinatus* using *M. spathulata* and *E. nuda* (middle panel), and a sympatric comparison at Pringle Bay between *C. uncinatus* using *M. digitata* and *H. aristatus* (bottom panel). The scale bar represents 300km.

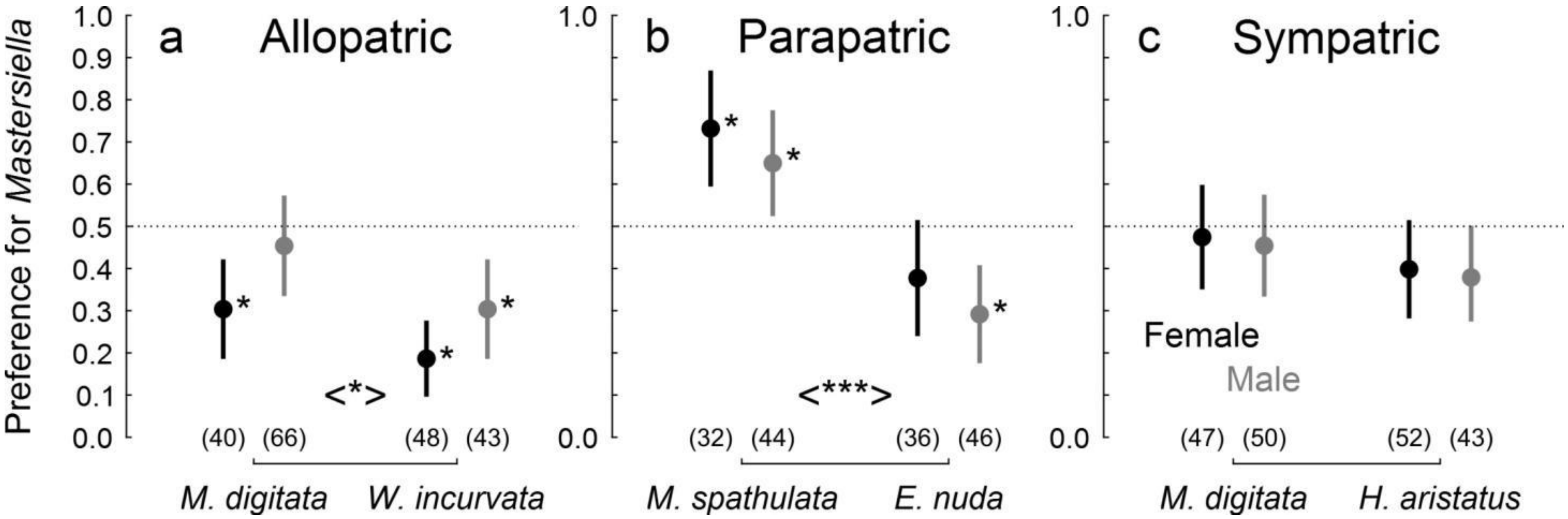


Figure 6.3: Reciprocal host presentation experiments. Panels correspond to comparisons in Figure 6.2.Panel a: The allopatric comparison between *C. uncinatus* caught from *M. digitata* at Pringle Bay and *W. incurvata* at Rondeberg, panel b: The parapatric comparison between *M. spathulata* and *E. nuda* at De Hoop, panel c: The sympatric comparison between *M. digitata* and *H. aristatus* at De Hoop. Names on the x axes correspond to the host plants insects were collected from. The y axes represent the probability that *Mastersiella* (*M. digitata* or *M. spathulata*) was chosen over the other species, estimated by means of binomial GLMs. GLM estimated means and 95% confidence intervals of females (black) and males (grey) are shown. When bars are above 0.5 it indicates a significant (indicated by *) preference for *Mastersiella*, when below it indicates a preference for the other species. There was a significant difference in host preference between individuals from *M. digitata* and *W. incurvata* (indicated by <*>) (a). There was a significant difference in host preference between individuals from *M. spathulata* and *E. nuda* (indicated by <***>) (b). There was no significant difference between individuals from *M. digitata* vs. *H. aristatus* (c). Sample sizes are shown in parenthesis.

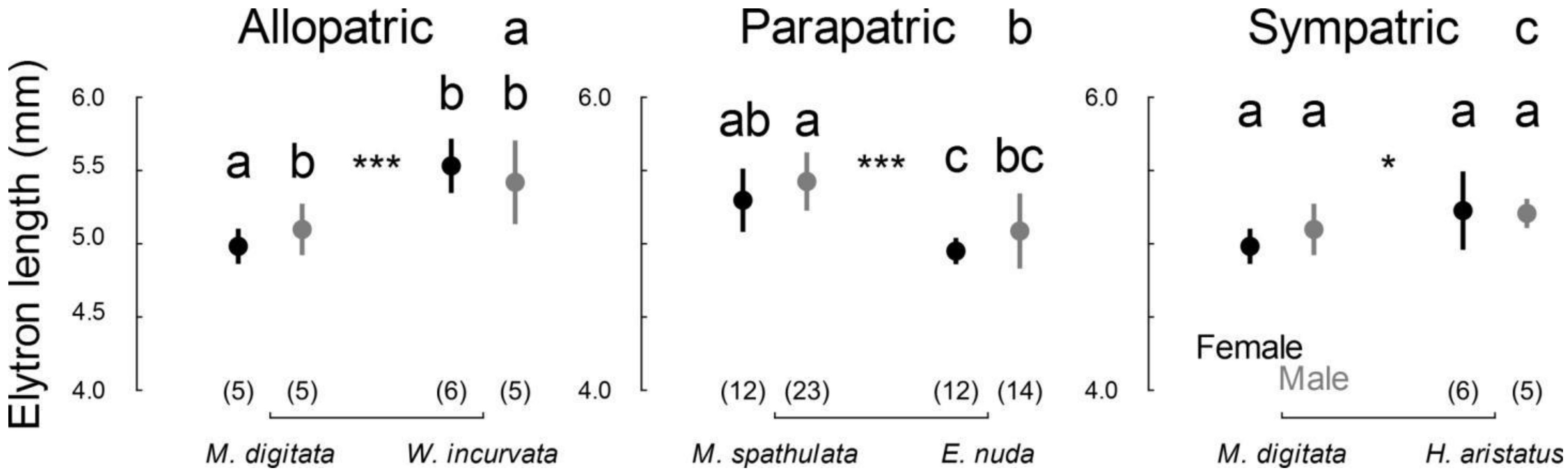


Figure 6.4: Differences in elytron length (a measure of body size) between allopatric, parapatric and sympatric population pairs. Panels correspond to comparisons in Figure 6.2.Panel a: The allopatric comparison between *C. uncinatus* caught from *M. digitata* at Pringle Bay and *W. incurvata* at Rondeberg, panel b: The parapatric comparison between *M. spathulata* and *E. nuda* at De Hoop, panel c: The sympatric comparison between *M. digitata* and *H. aristatus* at De Hoop. Names on the x axes correspond to the host plants that insects were collected from. Means are indicated by dots and standard deviations by bars. Black dots with bars correspond to females and males are indicated with grey dots and bars. Host effects from two way ANOVAs are shown, significance is indicated by *** (P< 0.001), and * (P< 0.05).Sexes and ecotypes not sharing letters are significantly different, as determined by post hoc tests. In all comparisons, insects caught from different plants differed in body size. Sample sizes are shown in parentheses.

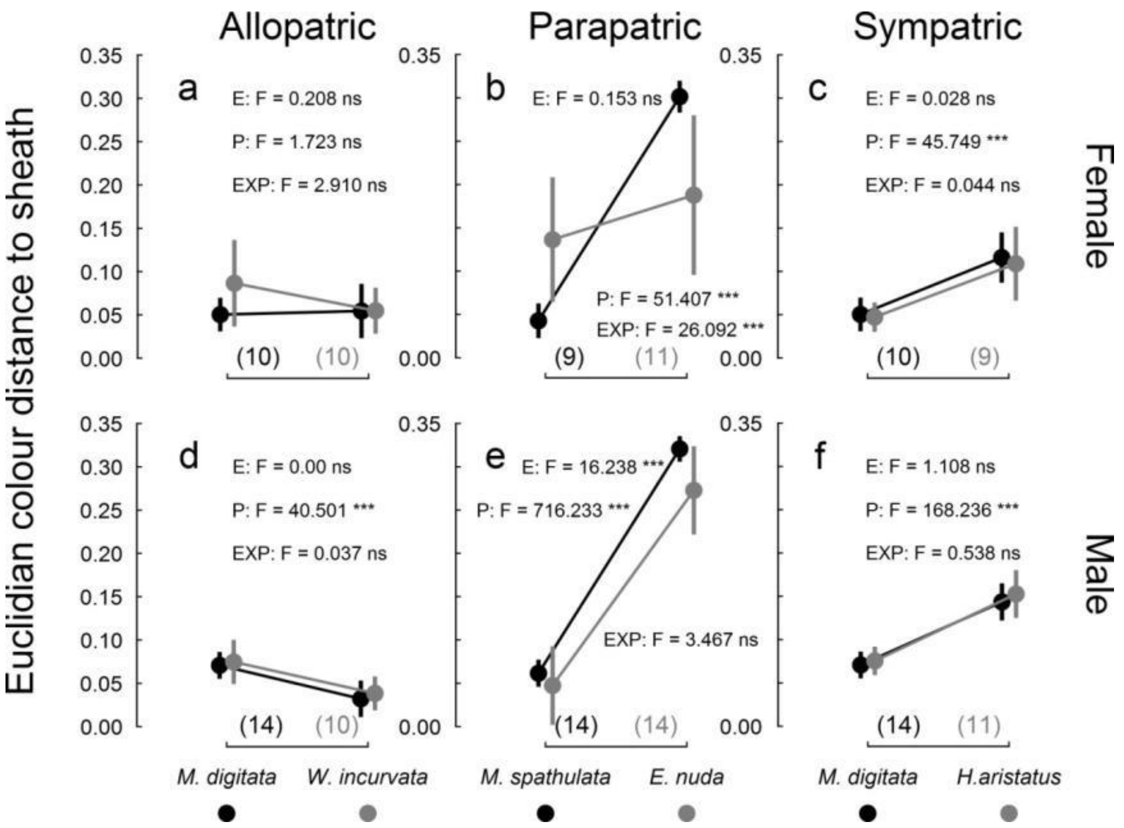


Figure 6.5: Sheath colour related local adaptation in Cape dwarf chameleon vision between allopatric (a and d), parapatric (b and e) and sympatric (c and f) populations. Females (a, b and c) were analysed separately from males (d, e, and f). Restio species are shown on x axes and Euclidian distances between restio sheaths and insects (in tetrahedral colour space) are shown on y axes. A short Euclidian distance is indicative of a close match between an insect and a leaf sheath (0 being a perfect match).Black points (means), bars (SE) and lines indicate populations associated with host plants on the left of each panel (i.e. always *Mastersiella* spp.) and those that are grey represent putative ecotypes associated with species on the right (i.e. *W. incurvata*, *E. nuda* or *H. aristatus*). Results from ANOVAs are shown on each graph: E represents putative ecotype origin, P represents plant species, and EXP represents the interaction between the two factors. Strong interaction effects are indicative of local adaptation (*** indicates < 0.001, ns indicate > 0.05). Sample sizes (number of individuals) are shown in parentheses. Note that only females in the parapatric comparison show a significant interaction effect.

Supplementary Tables and Figures

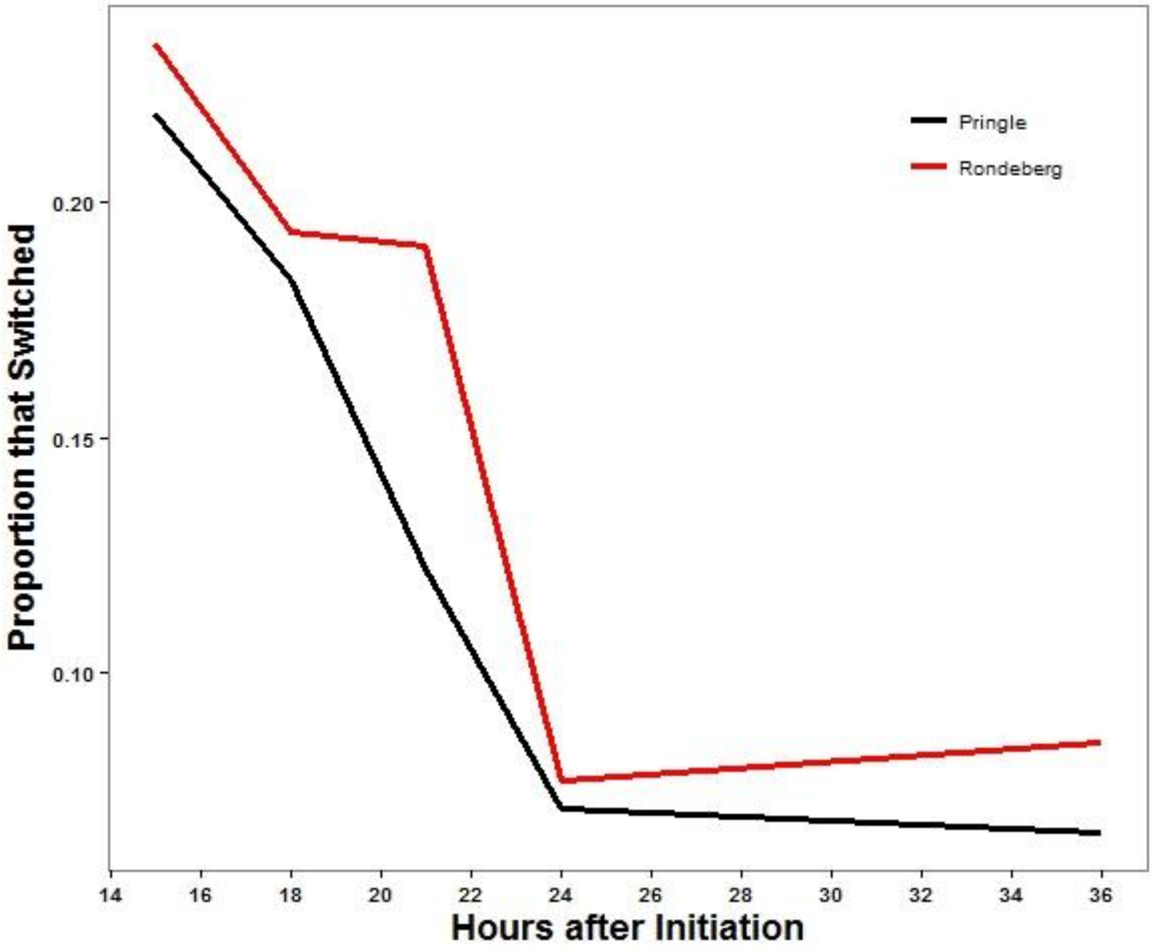


Figure S6.1: Settling of preference in local adaptation experiments. The x axis represents the proportion of individuals that changed their perching site (either between species or not on any species e.g. the side of the jar) from the previous observation. Observations were done at 12, 15, 18, 21, 24 and 36 hours after insects were introduced to preferences. For The Rondeberg experiments (red line) the choice was between (*W. incurvata* and *M. digitata*) and for the Pringle experiments it was between *M. digitata* and *H. aristatus*. Experiments at De Hoop are not shown because they were terminated after 24 hours, by which time insects had settled on a choice.

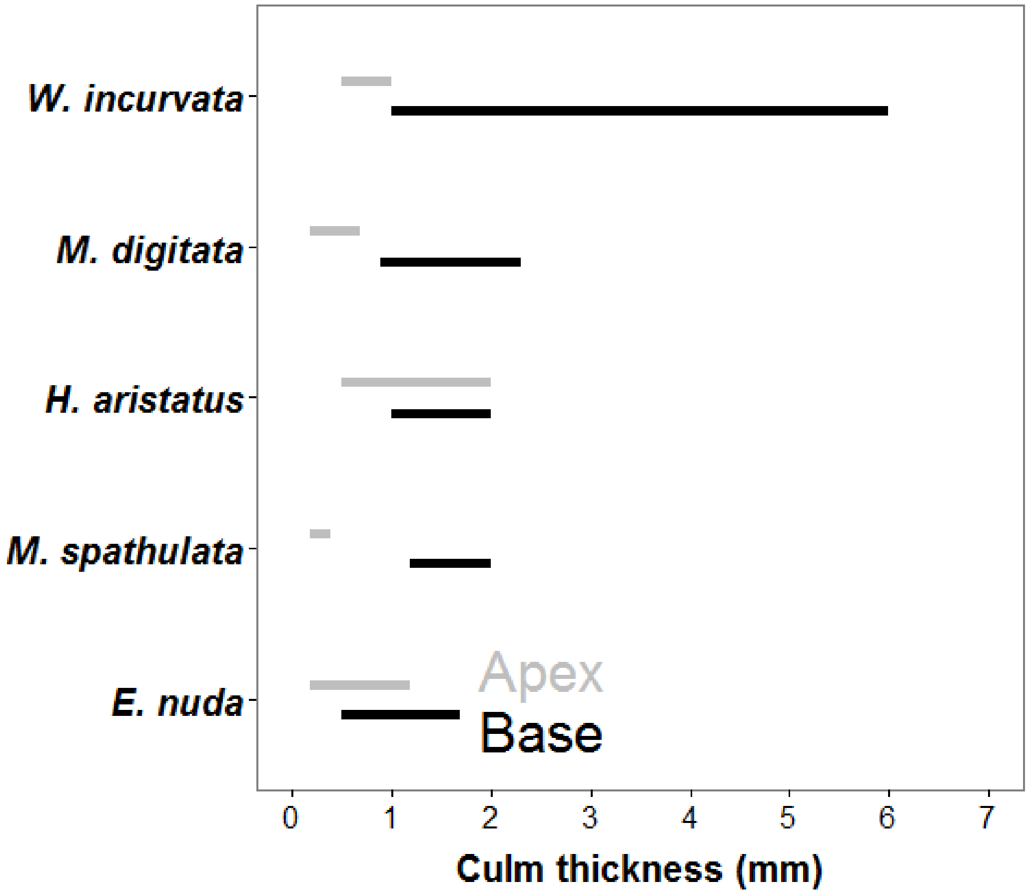


Figure S6.2: Range of culm thicknesses of the five restio species used in reciprocal restio presentation experiments. Grey bars represent the apex of restio culms just below the inflorescence and black bars represent the base of the culm just above rhizome. *W. incurvata*, *M. digitata* and *M. spathulata* culms are all branched; therefore there are large differences between the apex and the base culm thicknesses of these species. *H. aristatus* and *E. nuda* have straight unbranched culms. Data were extracted from an interactive identification key for restios (Linder 2011). Data on means are not available.

Table S6.1: Summary of Tukey HSD post hoc test on elytra lengths (a surrogate for body size) between ecotypes and sexes.

Comparison	Compared Pairs	Difference	Lower 95% CI	Upper 95% CI	*P*
Allopatric	*W. incurvata* Female - *M. digitata* Female	0.549	0.205	0.894	0.002
	W. incurvata Male - *M. digitata* Female	0.437	0.077	0.797	0.015
	M. digitata Male - *W. incurvata* Female	-0.434	-0.779	-0.090	0.011
	W. incurvata Male - *M. digitata* Male	0.322	-0.038	0.682	0.089
	M. digitata Male - *M. digitata* Female	0.115	-0.245	0.475	0.800
	W. incurvata Male - *W. incurvata* Female	-0.112	-0.457	0.232	0.791
Parapatric	*M. spathulata* Male - *E. nuda* Female	0.475	0.284	0.666	< 0.001
	M. spathulata Female - *E. nuda* Female	0.347	0.128	0.566	0.001
	M. spathulata Male - *E. nuda* Male	0.338	0.156	0.520	< 0.001
	E. nuda Male - *M. spathulata* Female	-0.210	-0.421	0.001	0.051
	E. nuda Male - *E. nuda* Female	0.137	-0.074	0.348	0.322
	M. spathulata Male - *M. spathulata* Female	0.128	-0.063	0.319	0.296
Sympatric	*M. digitata* Females - *H. aristatus* Females	-0.244	-0.562	0.075	0.170
	H. aristatus Males - *M. digitata* Females	0.225	-0.108	0.557	0.256
	M. digitata Males - *H. aristatus* Females	-0.128	-0.447	0.190	0.667
	M. digitata Males - *M. digitata* Females	0.115	-0.217	0.448	0.760
	M. digitata Males - *H. aristatus* Males	-0.109	-0.442	0.223	0.786
	H. aristatus Males - *H. aristatus* Females	-0.019	-0.337	0.299	0.998

Chapter 7

Conclusion

In the restio leafhopper system I found evidence that specialisation and divergence occur in response to both bottom-up (i.e. host chemistry/anatomy) and top-down mediated selection (i.e. predation) (Chapters 2, 3, and 6). I do not, however, find any evidence for the involvement of interspecific competition in specialisation, divergence, and community structuring (Chapters 3, 4, and 5). Nonetheless, restio leafhopper communities exhibit niche partitioning (i.e. host plant partitioning) (Chapters 2, 3, and 4), a pattern that is often interpreted as evidence for either current or past interspecific competition. In contrast, my results suggest that host plant partitioning is a consequence of the speciation process. That is, restio leafhopper populations expand their geographic ranges into regions where they are forced to use novel host plants in the absence of their ancestral host plants (Chapter 6). After expansion, populations using different host plants are subjected to divergent selection resulting in local adaptation, with reproductive isolation probably evolving as a by-product (Chapter 6). When multiple restio leafhopper species, which presumably diversified via host shifting (i.e. populations specialising on novel host plants), colonise the same local communities, a pattern of host plant partitioning is likely to emerge. Other studies on herbivorous insects often find that interspecific competition occurs but does not play a role in community structuring (Tack et al. 2009, Hochkirch and Gröning 2012). In contrast, I find evidence for niche partitioning, but conclude that it is an indirect consequence of the best studied selection pressures in ecological speciation in herbivorous insects (i.e. plant chemistry/morphology and predation) (Matsubayashi et al. 2010).

Agents of selection

Physiological trade-offs, resulting from specialising to overcome plant defences (Ehrlich and Raven 1964), do not fully explain divergent host plant adaptation in herbivorous insects (Futuyma and Moreno 1988, Singer and Wee 2005). Although plants often affect the physiological performance of herbivorous insects (Gripenberg et al. 2010), host plant linked predation and parasitism can be equally important sources of divergent selection (Singer and Stireman 2005, Matsubayashi et al. 2010). Interspecific competition is, however, often not a strong determinant of host-use patterns under natural conditions (Hochkirch et al. 2007, Tack et al. 2009). Nonetheless, in nature, multiple divergent selection pressures often occur simultaneously (Singer and Stireman 2005, Nosil et al. 2009). A bias of one selective pressure over another may, therefore, limit our understanding of host shift related divergence. Nosil and Sandoval (2008), for example, showed that divergent selection on morphological traits related to camouflage against predators in *Timema* stick insects lead to population level divergence, but not species level divergence. A combination of divergent selection on morphology and physiology, however, leads to species level divergence in *Timema* (Nosil and Sandoval 2008). Nosil and Sandoval (2008) interpreted these findings as support for the multifarious selection hypothesis which predicts that the completeness of speciation should be positively correlated with the number of genetically independent traits under divergent selection (Nosil et al. 2009).

Restio leafhopper populations and species use different restio species that likely vary in the number of ways that they differ from each other as selective environments. Some restio species used by populations of *Cephalelus uncinatus* may, for example, differ in culm thickness, but not dramatically in sheath colour. Other restio species may, however, differ in both culm thickness and sheath colour. Furthermore, body size and colour appear to be under divergent selection between populations of *C. uncinatus* using different restio species (Fig 7.1). Large bodied populations tend to be found on restios with thick culms, and populations tend to colour match the leaf sheaths of restios that they are using locally. Interestingly, between the population pairs that I investigated in Chapter 6, the population pair that showed the strongest divergent host preferences used restio species that differ in both culm thickness and sheath colour. Accordingly, this population pair differed in both body size and colour (Fig 7.1 e and f), whereas all the other population pairs that I investigated only differed in body size. It is also possible that physiological trade-offs evolved in this strongly divergent population pair. The restio species that this pair uses, *Mastersiella spathulata* or *Elegia nuda*, are distantly related restio species from different tribes within the restio family (Briggs and Linder 2009), and may differ chemically (Harborne 1979). The other population pairs that I investigated use restio species from the same tribe, and are possibly chemically more similar to one another (Ronsted et al. 2012). Furthermore, a sympatrically occurring restio leafhopper species pair (*C. uncinatus* and *Cephalelus pickeri*) that use restio species from different tribes exhibit evidence for physiological trade offs and strongly divergent host preferences (Chapter 3). Taken together, multiple selection pressures may be needed to complete, but not necessarily initiate, host shifts and diversification in restio leafhoppers. I therefore find indirect support for the multifarious selection hypothesis (Nosil et al. 2009), but more focused testing of this hypothesis is required.

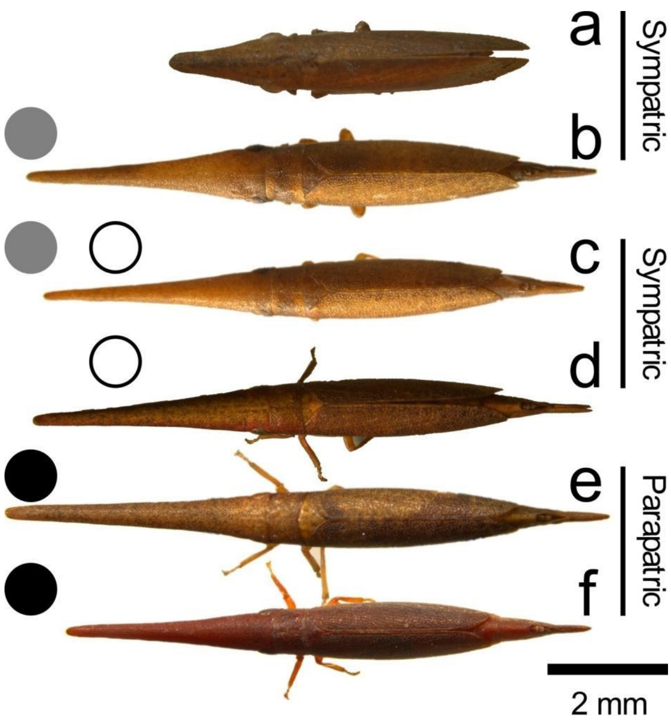


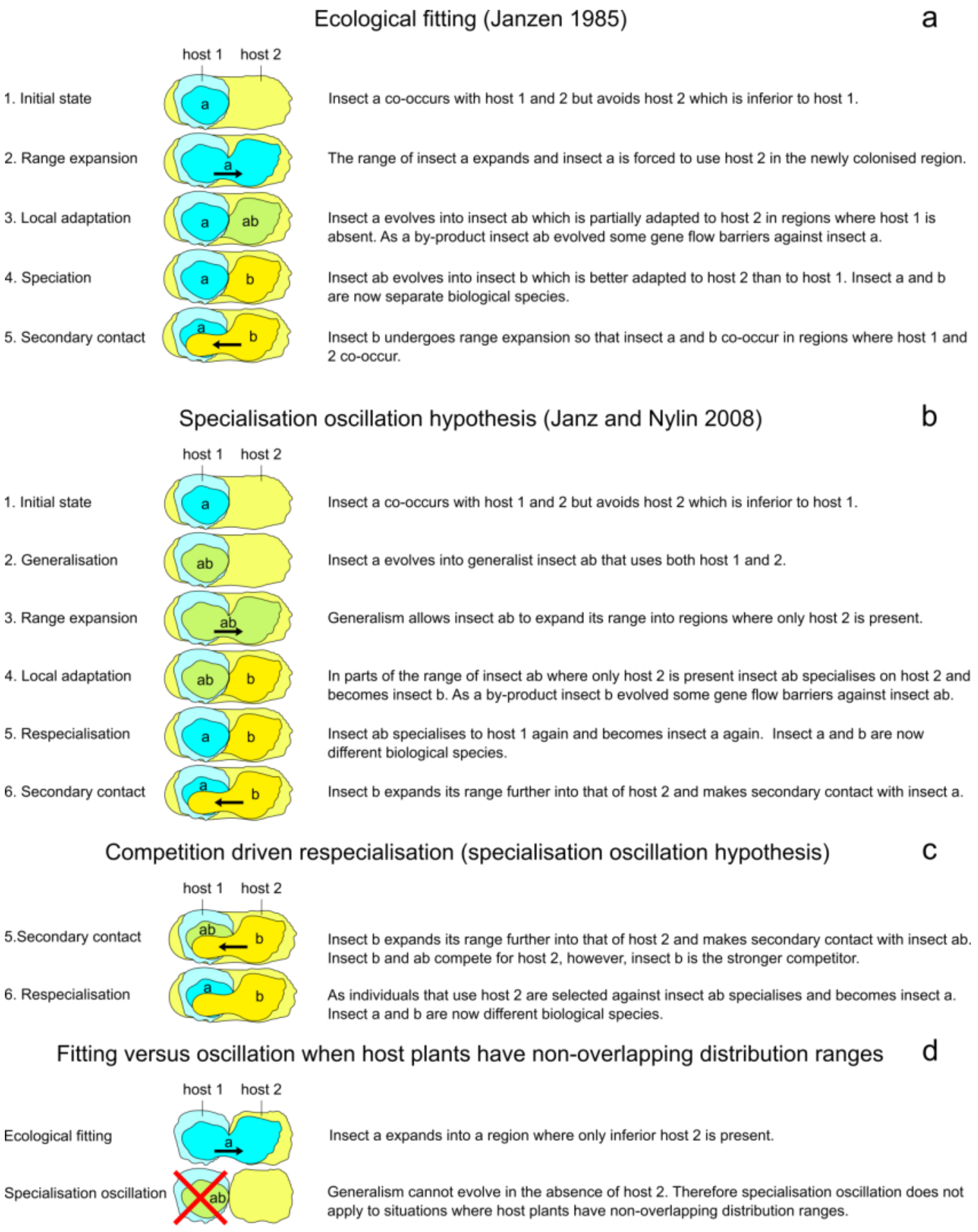
Figure 7.1: Females of putative ecotypes of *C. uncinatus* collected from: a) *Elegia elephantina*, b) *Willdenowia incurvata*, c) *Mastersiella digitata*, d) *Hypodiscus aristatus*, e) *Mastersiella spathulata*, f) *Elegia nuda*. The putative ecotype collected from *Elegia elephantina* (a) has been referred to as *Cephalelus. nov. sp. 2* throughout my thesis, but the male genetalia of this "species" resembles those of *C. uncinatus*. *Cephalelus. nov. sp. 2* may, therefore, be an ecotype of *C. uncinatus* (hence referred to as such in this caption). Putative ecotype pairs are allopatric unless indicated otherwise. Population pairs that were tested for divergent host preferences in Chapter 6 are indicated with matching dots. The black pair reciprocally avoided each other's host plants. The grey pair had divergent host preferences, but not reciprocal host avoidance. The white pair showed no divergence in host preference, and therefore probably represents a single generalist population.

The geography of speciation

Classifying speciation under different geographic modes (e.g. allopatric versus sympatric) has generally been replaced by classifying speciation under varying degrees of gene flow (Via 2001, Butlin et al. 2008, Mallet et al. 2009). Nonetheless, understanding how selection pressures differ geographically is still an important aspect of ecological speciation (Nosil 2012). The extensively overlapping distribution ranges of restio leafhopper species suggests that divergent selection, and not only genetic drift, may have played an important role in reproductive isolation (Wiese 2014). Nonetheless, speciation in restio leafhoppers is probably not initiated in the presence of strong gene-flow. In chapter 6 I showed that *C. uncinatus* is more broadly distributed than the restio species that it uses. This suggests that, like in possibly most host shifts in herbivorous insects (Futuyma 2008), populations of restio leafhoppers disperse into regions where their ancestral host plant species are absent, forcing populations to use novel host plants. Furthermore, I found evidence of allopatric and parapatric, but not sympatric, host plant related divergence (Chapter 6). This supports the speculation of Futuyma (2008) that non-sympatric speciation is likely the norm in herbivorous insects.

Non-sympatric speciation in herbivorous insects can occur under two geographic scenarios: ecological fitting (Janzen 1985) or specialisation oscillation (Janz and Nylin 2008) (SO). Janzen (1985) suggested that insect species may expand their geographic ranges into regions where they can, for example, use host plant species which they are not optimally adapted to. Janzen then argued that local adaptation (and eventually speciation) would occur so that two geographically isolated species are formed (Fig 7.2 a). Janz and Nylin, more recently, suggested that speciation can occur through specialisation (i.e. SO) as opposed to shifts to novel host plants. The main premise of SO is that insect species go through phases of generalism and re-specialisation, and that new species are formed during re-specialisation. Applied to non-sympatric speciation the SO hypothesis predicts that evolving generalism will favour geographic range expansion, and re-specialisation would lead to geographically isolated host races (Fig 7.2 b). Janz and Nylin are, however, not particular about how re-specialisation occurs. I suggest that, re-specialisation in populations that expanded their geographic range may occur from locally adapting, to for example, the only available host

(Fig 7.2 b, step 4). Explaining re-specialisation in the populations that remained in the same geographic area after evolving generalism is less intuitive. Janz and Nylin suggest that during a two step model of speciation (i.e. allopatric divergence followed by secondary sympatry) interactions between diverging populations may finish speciation, but they do not emphasise re-specialisation. I suggest that secondary contact between generalist and already re-specialised populations may drive re-specialisation in the generalist population (i.e. species now prefer different host plants, Fig 7.2 c). If the insects mate on their host plants there will now be prezygotic isolation between re-specialised populations.



Ecological fitting (Janzen 1985)

1. Initial state	Insect a co-occurs with host 1 and 2 but avoids host 2 which is inferior to host 1.
2. Range expansion	The range of insect a expands and insect a is forced to use host 2 in the newly colonised region.
3. Local adaptation	Insect a evolves into insect ab which is partially adapted to host 2 in regions where host 1 is absent. As a by-product insect ab evolved some gene flow barriers against insect a.
4. Speciation	Insect ab evolves into insect b which is better adapted to host 2 than to host 1. Insect a and b are now separate biological species.
5. Secondary contact	Insect b undergoes range expansion so that insect a and b co-occur in regions where host 1 and 2 co-occur.

Specialisation oscillation hypothesis (Janz and Nylin 2008)

1. Initial state	Insect a co-occurs with host 1 and 2 but avoids host 2 which is inferior to host 1.
2. Generalisation	Insect a evolves into generalist insect ab that uses both host 1 and 2.
3. Range expansion	Generalism allows insect ab to expand its range into regions where only host 2 is present.
4. Local adaptation	In parts of the range of insect ab where only host 2 is present insect ab specialises on host 2 and becomes insect b. As a by-product insect b evolved some gene flow barriers against insect ab.
5. Respecialisation	Insect ab specialises to host 1 again and becomes insect a. Insect a and b are now different biological species.
6. Secondary contact	Insect b expands its range further into that of host 2 and makes secondary contact with insect a.

Competition driven respecialisation (specialisation oscillation hypothesis)

5.Secondary contact	Insect b expands its range further into that of host 2 and makes secondary contact with insect ab. Insect b and ab compete for host 2, however, insect b is the stronger competitor.
6. Respecialisation	As individuals that use host 2 are selected against insect ab specialises and becomes insect a. Insect a and b are now different biological species.

Fitting versus oscillation when host plants have non-overlapping distribution ranges

Ecological fitting	Insect a expands into a region where only inferior host 2 is present.
Specialisation oscillation	Generalism cannot evolve in the absence of host 2. Therefore specialisation oscillation does not apply to situations where host plants have non-overlapping distribution ranges.

Figure 7.2: Ecological fitting (a) versus specialisation oscillation (b) as hypotheses to explain herbivorous insect speciation. Specialisation oscillation can be referred to as speciation by specialisation. Specialisation oscillation has some shortcomings: it does not explain why species in the same region become generalised and specialised again. I suggest that secondary contact during the speciation process can cause re-specialisation (c). Restio leafhoppers, however, do not specialise in response to competition (Chapter 5). Another shortcoming of specialisation oscillation is that in does not apply to scenarios where host plants have non-overlapping distribution ranges (otherwise it is just ecological fitting) (d).

Although SO cannot be ruled out in restio leafhopper diversification, I suggest that ecological fitting might occur more often than SO. First, I find little evidence for interspecific competition, which means that re-specialisation of populations that do not expand their geographic ranges, may not occur. Also, SO will not promote range expansion if host plant species are non-overlapping because there will be no host plants to generalise to (Fig 7.2 d). Knowing that the host plants of restio leafhoppers often have non-overlapping distributions, ecological fitting may occur more often than SO. My findings suggest that restio leafhopper populations expand their geographic ranges into regions where their ancestral host plants are absent; forcing them to use a new, initially inferior, host plant (Chapter 6). Selection then favours individuals that match the sheaths and culm thickness, as well as chemistry of the new host pant (i.e. local adaptation occurs) (Chapters 3 and 6). If local adaptation completes the evolution of reproductive isolation before the ancestral and the diverging population make secondary contact, then speciation is allopatric (Rundle and Nosil 2005). I have, however, found putative ecotypes of *C. uncinatus* in parapatry (and sympatry if *Cephalelus sp. nov. 2* is an ecotype of *C. uncinatus*, see Fig 7.1), suggesting the possibility of speciation in sympatry. An alternative to allopatric speciation, is a two-step process involving both allopatric and sympatric phases (Rundle and Nosil 2005). Once enough reproductive isolation has evolved through, for example, divergent host preferences (Feder et al. 1994), allopatrically diverging restio leafhopper populations may make secondary contact without homogenising into one population. Thus, considering that putative ecotypes of *C. uncinatus* may, in part, diverge in the face of gene-flow, diversification of restio leafhoppers may occur under the two-step model proposed by Rundle & Nosil (2005). This permits reinforcement (a form of reproductive character displacement) (Rundle and Nosil 2005, Nosil 2013) to operate during speciation in restio leafhoppers. Investigating accentuated differences in sexual traits in restio leafhoppers (e.g. mate calling behaviour (Claridge 1985), and genital morphology (Kameda et al. 2009)) may elucidate the importance of reinforcement in restio leafhopper diversification. Nonetheless, my work suggests that other forms of character displacement, like ECD, probably do not to play an important role in restio leafhopper diversification (Chapters 3, 4, and 5).

Community structure and specialisation

An interesting consequence of host-shifts followed by secondary sympatry is that it generates patterns of community-wide ECD (Chapter 4). Just like Antillean *Anolis* lizard communities (anole communities from now on), restio leafhopper communities comprise different species adapted to different microhabitats. While restio leafhopper species are adapted to different host plants, anole species are adapted to different vegetation structure types (Losos 2009a). A possible difference between anoles and restio leafhoppers is that anole species often use the same microhabitats, but then additional niche axes show partitioning (mostly thermal niches and prey size) (Losos 2009b). Nevertheless, niche partitioning in anoles is thought to be the result of interspecific competition (Losos 2009b). In particular, the anole system satisfies all six criteria provided by Schluter and McPhail (1992) to demonstrate ECD, making it one of the best studied examples of community-wide ECD (Stuart and Losos 2013). Perhaps the strongest pattern based evidence for ECD in the anole system is “species-for-species matching” (Schluter 1990). That is, each local anole community is a monophyletic clade and different local communities are different clades. Furthermore, a similar set of ecomorphs (e.g. a tree trunk specialist, a ground specialist etc.) has evolved in each local community (Losos 1998). Therefore, knowing that anole species often compete (Losos 2009b), it has been inferred that interspecific competition could have led to the “species-for-species-matching” pattern. Nevertheless, this is not irrefutable evidence that community structure in anole communities resulted from ECD. Multiple selective pressures (e.g. predation between anole species (Gerber and Echternacht 2000)) might have played a role in structuring anole lizard communities over evolutionary time.

The resemblance between restio leafhopper community structure and that of anoles, in which competition causes both microhabitat specialisation and shifts (Lister 1976), emphasises the importance of assessing ECD beyond the criteria provided by Schluter and McPhail (1992).Throughout my thesis I found support for five of Schluter and McPhail (1992)'s six criteria (summarised in Table 7.1). I did not, however, conduct experiments to test whether exploitative interspecific competition occurs between species that use the same host plants. Nonetheless, the lack of experimental evidence for aggression between species that overlap in host use (Chapter 4), suggest that agonistic character displacement (Grether et al. 2009) likely does not occur in restio leafhoppers. Importantly, however, if I conduct experiments and find evidence for exploitative competition it would be erroneous to infer community-wide ECD. This is because null models revealed that host plant partitioning in restio leafhopper communities is primarily the result of community members being highly host specific (Chapter 4). Although specialisation may be the result of interspecific competition (Lister 1976, Robinson and Schluter 2000, Rice and Pfennig 2005, Armbruster and Muchhala 2009, Ricklefs and Marquis 2012), this is clearly not the case for restio leafhoppers. Instead of showing a positive relationship between species richness and host specificity, populations of restio leafhoppers in restio leafhopper species rich regions are less host specific than those in species poor regions (Chapter 5). In addition, considering that restio leafhoppers possibly evolve host preferences for single restio species in the absence of a choice between plants

(Chapter 6), even the optimisation of host finding behaviour may explain host specificity (Bernays and Wcislo 1994).

Table 7.1: Schluter and McPhail (1992)'s Criteria for ECD met by restio leafhoppers.

Criteria	Satisfied by the restio leafhopper system
1. Pattern could not have arisen by chance.	In chapter 4 I demonstrated, by means of null models, that niche partitioning is non-random. Also, niche partitioning is likely the result of specialisation.
2. Differences between allopatric and sympatric populations should have a genetic basis.	Not demonstrated. Nonetheless, both (Schluter 2000a)'s and the most recent review by Stuart and Losos (2013) assume that when demonstrating community-wide ECD, differences in sympatry should by default have a genetic basis. Nonetheless, experimental findings in Chapters 3 and 4 suggest that current interspecific aggression is not driving patterns of niche partitioning.
3. Competitive exclusion (species sorting) should be ruled out as a driver of the pattern.	Restio leafhoppers do not exhibit co-occurrence patterns indicative of competitive exclusion (Chapter 4).
4. Phenotype should reflect differences in resource-use.	I showed that restio leafhoppers actively choose restio species that they perform best on and use in the field (Chapters 3, 4, and 6). Also body size appears to be linked to be linked to the culm thickness of the restio species used (Chapter 6). Thus I have identified behavioural and morphological traits linked to resource exploitation.
5. Relevant differences between sympatric and allopatric populations should be controlled for.	When community-wide ECD is demonstrated all species are sympatric, thus it is deemed unnecessary to demonstrate the allopatry-sympatry pattern (Chapter 4).
6. Species using the same resources should reduce each others' fitness.	Not tested for exploitative competition. Nonetheless, restio leafhoppers overlapping in host use show no aggressive behaviour towards each other (Chapter 4).

Although there is evidence that interspecific competition leads to specialisation, to my knowledge, no studies have tested whether it is the main contributor of patterns of community-wide ECD. This is partly because current statistical methods used to detect trait overdispersion (i.e. non-overlapping morphological traits related to resource exploitation) do not allow the exploration of trait variation. Trait overdispersion is usually determined by testing for unusually evenly spaced trait means within communities (See Fig. 7.3 a) (Schluter 2000b). This method has some shortcomings (Fig 7.3), but primarily it cannot be used to determine whether non-overlapping trait values result from narrow trait variation within species relative to across species or shifts in trait means (without decreased trait variation). This problem can be resolved by constructing different null models that test for causes of observed levels of overlap in trait variation by constraining different aspects of trait values (following a similar approach to what I used in Chapter 4). If findings from null models, for example, suggest that low trait overlap is the result of low trait variation, the cause of trait variation can be investigated to test whether community-wide ECD results from interspecific competition. This will allow for more rigorous assessment of community-wide ECD, and also provide new insight into the role of ECD. If, for example, interspecific competition only reduces trait variation, interspecific competition is likely less important in speciation than when it causes shifts in trait means (Rice and Pfennig 2005). This is because populations that underwent mean trait shifts without a reduction in variation would have acquired new phenotypes possibly making them reproductively isolated from populations that did not experiencing interspecific competition (Rice and Pfennig 2005). Populations that lost trait variation would, however, still be reproductively compatible with those that did not (Rice and Pfennig 2005).

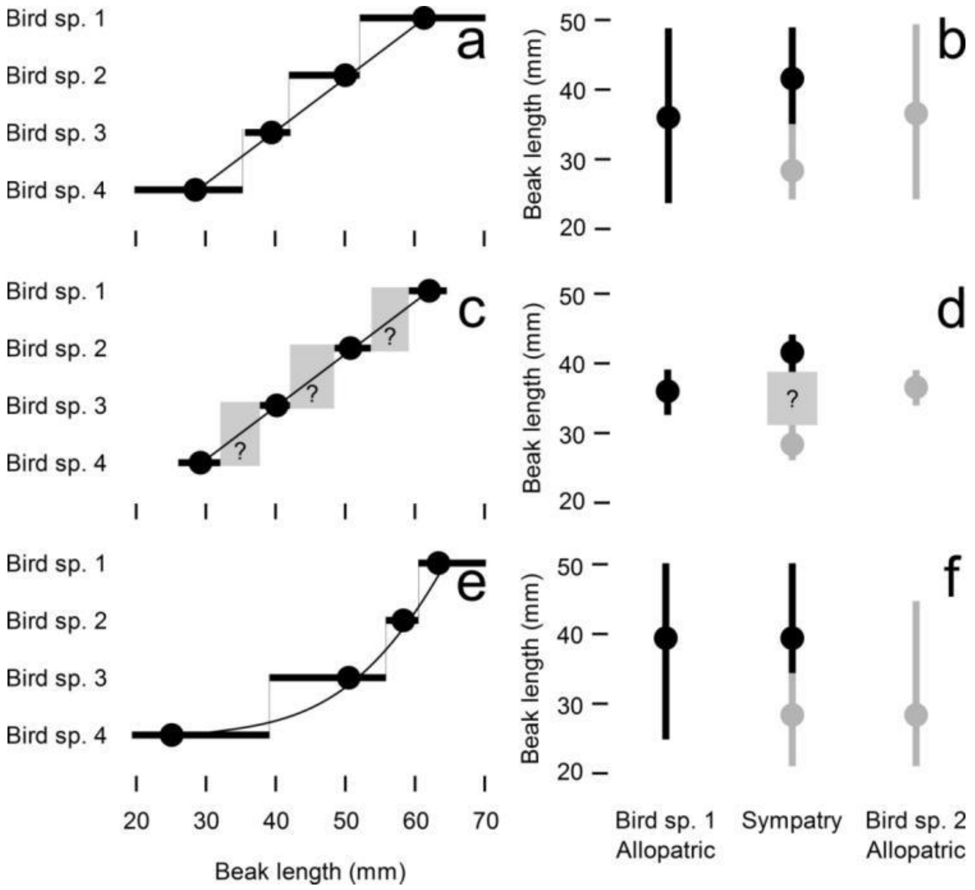
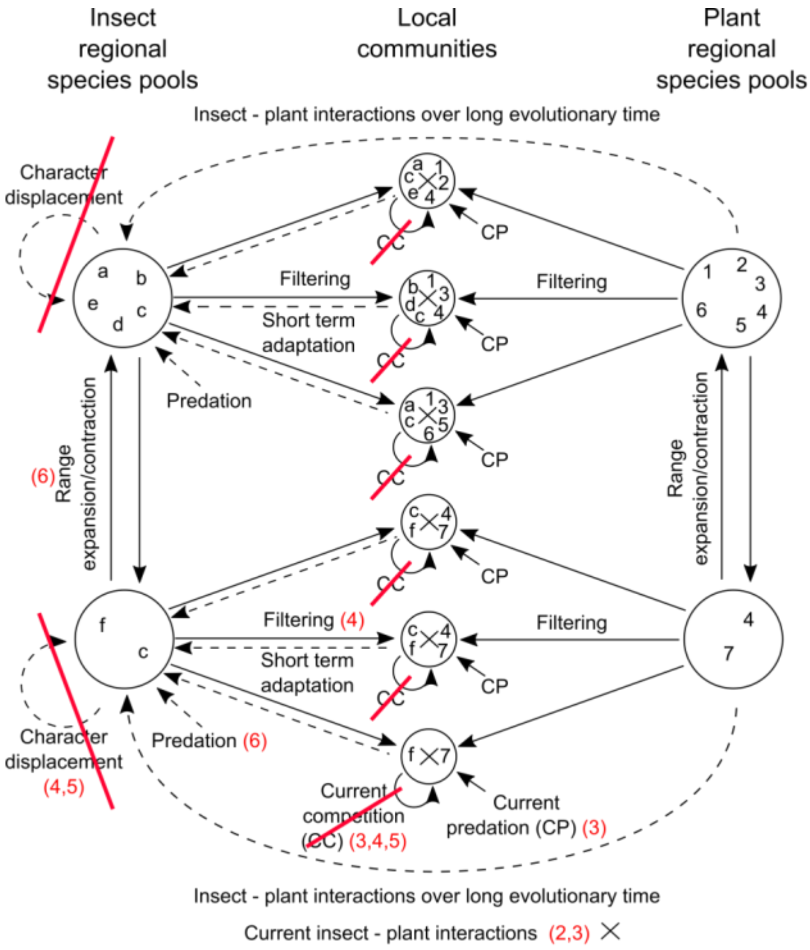


Figure 7.3: Plots on the left (a, c, and e) illustrate how evidence for community-wide ECD can be misinterpreted when trait variation (i.e. the degree of specificity) is ignored, plots on the right (b, d, and f) illustrate the same for species pairs. Dots represent means and bars represent the range of trait variation in a population. Assume that beak length in a group of birds determines how efficiently individual birds drink nectar from flowers with tubes (e.g. individuals forage best on flowers that have tubes which correspond to their beak sizes). The community in a) exhibits strong evidence for community-wide ECD because there is little overlap in trait variation between species. Note that trait means of species are also evenly spaced (hence the straight diagonal line). Therefore, the community also exhibits the signature pattern for community-wide ECD. The community in c), like in a), is characterised by species with evenly spaced means. However, trait variation is not continuous between species (note the large gaps). This suggests that a process other than interspecific competition (e.g. stabilizing selection on traits that maximise resource exploitation) is leading to decreased trait variation and might, in part or completely, explain the community-wide ECD-like pattern. Unlike both a) and c), the community in e) is not characterised by evenly spaced trait means (hence the curved line). Traditional methods would therefore not detect community wide ECD. However, non-overlap in trait variation strongly suggests community-wide ECD. The geographic evidence for character displacement between a species pair in b) suggests ECD. In sympatry trait variation has shifted so that trait variation and means do not overlap between bird sp. 1 and 2. The pattern in d) is poor evidence for ECD. Although trait means have shifted in the zone of sympatry, when considering trait variation, it appears as if both species evolved to avoid a resource that they used when allopatric. f) is an example where trait means did not shift between zones of allopatry and sympatry. Nonetheless, the

decrease in variation towards the zone of sympatry strongly suggests interspecific competition.

My findings suggest that restio leafhopper community structure emerges from the speciation process, and that niche partitioning is not a signature of interspecific competition between restio leafhopper species. This leads to the counterintuitive notion that studying restio leafhoppers in the local insect community context may provide little insight into the specialisation and speciation process. On the contrary, studying restio leafhoppers in a local community context has highlighted key aspects of the speciation process. For example, different species use different plants when they co-occur locally, suggesting that prezygotic reproductive isolation is maintained because of, in part, narrowly evolved host preference hierarchies (Chapter 4). Studying restio leafhoppers in a plant community context, particularly at regional scales, may provide important insight into both specialisation and speciation of restio leafhoppers (Fig 7.4). For example, future work could determine whether restio leafhopper populations are more likely to specialise on locally abundant restio species or those that are geographically widespread. More broadly, it would be interesting to know whether spatial heterogeneity in plant distributions promotes and/or inhibits specialisation and speciation of leafhoppers. In Chapter 5 I showed that spatial heterogeneity in plant distributions at large spatial scales might promote host shifts (Chapter 5), while the results of Chapter 4 suggest that too much small spatial scale turnover in plant species composition may impede specialisation and therefore speciation. Thus I think that explicitly exploring the influence of the plant speciation process itself (and their emergent community patterns) on divergence and diversification of restio leafhoppers would represent the most insightful and rewarding continuation of the work I have started here.



Chapter 2: Restio leafhopper species use one or few host species within a local community. They do not switch between host plants as plants cycle through different growth stages.

Chapter 3: A local community exhibits host plant partitioning. Host plant partitioning can be explained by host preference and performance linkage in the absence of current interspecific competition.

Chapter 4: Restio leafhopper communities generally exhibit niche partitioning. Niche partitioning is the result of narrow host preferences and not competitive exclusion. Restio leafhopper species do not narrow their host preferences in the presence of potential competitors.

Chapter 5: Narrow host preferences do not result from interspecific competition. Populations in restio leafhopper species rich regions are less host specific than those in species poor regions.

Chapter 6: *Cephalelus uncinatus* encounters different restio species across its distribution range. Allopatric and parapatric, but not sympatric populations exhibit locally adapted host preferences. Populations have diverged in morphological traits linked to camouflage against predators.

Figure 7.4: An insect-plant community framework for insect specialisation and speciation partly tested throughout my thesis. The processes excluded by my work are crossed off the diagram in red. Chapter numbers in red and chapter summaries indicate the basis for rejection of these processes as well as the grounds for accepting others. Large circles on the left represent regional species pools of insects and those on the right represent regional species pools of plants. Small circles represent local insect – plant communities that are, in part, random assemblages from insect and plant regional species pools. Local insect assemblages are, however, also determined by the plant species that are present within local communities (i.e. habitat filtering). Predation within local communities selects against insects that are not camouflaged on their host plants. Over long evolutionary time scales, evolutionary feedback from predation within many local communities leads to host specialisation. Direct interactions between insects and plants also affect specialisation over evolutionary time. That is, insects specialise physiologically to become better at exploiting host plants. In addition, insects do not become specialised to host plants that are not common in local communities across space and time. Allopatric speciation (between regions) lead to geographic range reduction (of an insect species), and changes in species composition in one region, but does not increase species richness within regional species pools. Furthermore, range expansion from one region to another (possibly leading to secondary contact between species that speciated allopatrically) leads to changes in both insect species composition and species richness within the region expanded to. Speciation, however, occurs under a combination of sympatry and allopatry. Therefore, speciation can be finished within one region increasing species richness and changing species composition simultaneously. Increased regional species richness leads to more completely filled plant niches within local communities, but does not force insects to specialise. Nevertheless, it increases the need to evolve pre-mating isolating mechanisms between species to prevent hybridisation that affects fitness negatively.

References

Agrawal, A. A. 1998. Induced Responses to Herbivory and Increased Plant Performance. Science 279:1201–1202.

Albert, A., and D. Schluter. 2004. Reproductive character displacement of male stickleback mate preference: reinforcement or direct selection? Evolution 58:1099–1107.

Anderson, B., P. Ros, T. J. Wiese, and A. G. Ellis. 2014. Intraspecific divergence and convergence of floral tube length in specialized pollination interactions. Proceedings of the Royal Society B: Biological Sciences 281.

Arakaki, S., and M. Tokeshi. 2011. Analysis of spatial niche structure in coexisting tidepool fishes: Null models based on multi-scale experiments. Journal of Animal Ecology 80:137–147.

Araya, Y. N., J. Silvertown, D. J. Gowing, K. J. McConway, H. P. Linder, and G. Midgley. 2011. A fundamental, eco-hydrological basis for niche segregation in plant communities. New Phytologist 189:253–258.

Armbruster, W. S., and N. Muchhala. 2009. Associations between floral specialization and species diversity: Cause, effect, or correlation? Evolutionary Ecology 23:159–179.

Augustyn, W. J., B. Anderson, M. Stiller, and A. G. Ellis. 2013. Specialised host-use and phenophase tracking in restio leafhoppers (Cicadellidae: Cephalelini) in the Cape Floristic Region. Journal of Insect Conservation 17:1267–1274.

Berlocher, S. H., and J. L. Feder. 2002. Sympatric speciation in phytophagous insects: moving beyond controversy? Annual review of entomology 47:773–815.

Bernays, E. A., and W. T. Wcislo. 1994. Sensory capabilities, information processing, and resource specialization. The quarterly review of biology 69:187–204.

Blüthgen, N., F. Menzel, and N. Blüthgen. 2006. Measuring specialization in species interaction networks. BMC ecology 6:9.

Boecklen, W. J., and M. T. Hoffman. 1993. Sex-Biased Herbivory in Ephedra trifurca: The Importance of Sex-by-Environment Interactions. Oecologia.

Boecklen, W. J., P. W. Price, and S. Mopper. 1990. Sex and drugs and herbivores: sex-biased herbivory in arroyo willow (*Salix lasiolepis*). Ecology 71:581–588.

Bolnick, D. I., P. Amarasekare, M. S. Araujo, R. Burger, J. M. Levine, M. Novak, V. H. W. Rudolf, S. J. Schreiber, M. C. Urban, and D. A. Vasseur. 2011. Why intraspecific trait variation matters in community ecology. Trends in Ecology and Evolution 26:183–192.

Bolnick, D. I., T. Ingram, W. E. Stutz, L. K. Snowberg, O. L. Lau, and J. S. Paull. 2010. Ecological release from interspecific competition leads to decoupled changes in population and individual niche width. Proceedings of the Royal Society B: Biological Sciences 277:1789–1797.

Bolnick, D. I., and P. Nosil. 2007. Natural selection in populations subject to a migration load. Evolution 61:2229–2243.

Bond, W. J., F. I. Woodward, and G. Midgely. 2005. The global distribution of ecosystems in a world without Fire. New Phytologist 165:525–537.

Briggs, B. G., and H. P. Linder. 2009. A new subfamilial and tribal classification of Restionaceae (Poales). Telopea 12:333–345.

Brower, L. 1958. Bird Predation and Foodplant Specificity in Closely Related Procryptic Insects. The American Naturalist 92:183–187.

Brown, W. L., and E. O. Wilson. 1956. Character displacement. Systematic Zoology 5:49–64.

Bush, G. 1969a. Mating Behavior, Host Specificity, and the Ecological Significance of Sibling Species in Frugivorous Flies of the Genus *Rhagoletis* (Diptera, Tephritidae). The American Naturalist 130:669–672.

Bush, G. L. 1969b. Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera, Tephritidae). Evolution 23:237–251.

Bush, G. L. 1975. Modes of Animal Speciation. Annual Review of Ecology and Systematics 6:339–364.

Butlin, R. K., J. Galindo, and J. W. Grahame. 2008. Review. Sympatric, parapatric or allopatric: the most important way to classify speciation? Philosophical transactions of the Royal Society of London. Series B, Biological sciences 363:2997–3007.

Carroll, S. P., S. P. Klassen, and H. Dingle. 1998. Rapidly evolving adaptations to host ecology and nutrition in the soapberry bug. Evolutionary Ecology 12:955–968.

Chambers, J. Q., N. Higuchi, and J. P. Schimel. 1998. Ancient trees in Amazonia. Nature 391:135–136.

Claridge, M. F. 1985. Acoustic Signals In The Homoptera: Behaviour, Taxonomy, and Evolution. Ann. Rev. Entomol. 30:297–317.

Coetzee, J. H., M. G. Wright, and J. H. Giliomee. 1997. Antiherbivore mechanisms of economically important Proteaceae species in the African Cape Fynbos. Journal Of Applied Entomology 121:367–372.

Condon, M. A., S. J. Scheffer, M. L. Lewis, and S. M. Swensen. 2008. Hidden neotropical diversity: greater than the sum of its parts. Science 320:928–931.

Connell, J. H. 1961. The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. Ecology 42:710–723.

Cornelissen, T., and P. Stiling. 2005. Sex-biased herbivory: a meta-analysis of the effects of gender on plant-herbivore interactions. Oikos 111:488–500.

Cornell, H. V, and J. H. Lawton. 1992. Species interactions, local and regional processes, and limits to the richness of ecological communities: a theoretical perspective. Journal of Animal Ecology 61:1–12.

Cottrell, C. B. 1985. The absence of coevolutionary associations with capensis floral element plants in the larval/plant relationships of Southwestern Cape Butterflies. Transvaal Museum Monographs 4:115–124.

Courtney, S. P. 1981. Coevolution of Pierid Butterflies and Their Cruciferous Foodplants. III. *Anthocharis cardamines (L.)* Survival, Development and Oviposition on Different Host plants. Oecologia 51:91–96.

Cowling, R., and P. Rundel. 1996. Plant diversity in Mediterranean-climate regions. Trends in Ecology and Evolution 11:362–366.

Cozzolino, S., and A. Widmer. 2005. Orchid diversity: An evolutionary consequence of deception? Trends in Ecology and Evolution 20:487–494.

Davies, D. M. 1986. A taxonomic description of the leafhopper fauna (Homoptera: Cicadellidae) in association with Restionaceae. MSc. University of Stellenbosch.

Davies, D. M. 1988. Leafhoppers (Homoptera: Cicadellidae) associated with the Restionaceae, 1. The tribe Cephalelini (Ulopinae). Journal of the Entomological Society of Southern Africa 51:31–64.

Dayan, T., and D. Simberloff. 2005. Ecological and community-wide character displacement: the next generation. Ecology Letters 8:875–894.

Dayan, T., D. Simberloff, E. Tchernov, and Y. Yom-Tov. 1989. Inter-and intraspecific character displacement in Mustelids. Ecology 70:1526–1539.

Denno, R. F., S. Larsson, and K. L. Olmstead. 1990. Role of enemy-free space and plant quality in host-plant selection by willow beetles. Ecology 71:124–137.

Denno, R. F., M. S. McClure, and J. R. Ott. 1995. Interspecific Interactions in Phytophagous Insects: Competition Reexamined and Resurrected. Annual Review of Entomology 40:297–331.

Després, L., J.-P. David, and C. Gallet. 2007. The evolutionary ecology of insect resistance to plant chemicals. Trends in Ecology and Evolution 22:298–307.

Després, L., and N. Jaeger. 1999. Evolution of oviposition strategies and speciation in the globeflower flies *Chiastocheta spp.*(Anthomyiidae). Journal of Evolutionary Biology 12:822–831.

Devictor, V., J. Clavel, R. Julliard, S. Lavergne, D. Mouillot, W. Thuiller, P. Venail, S. Villéger, and N. Mouquet. 2010. Defining and measuring ecological specialization. Journal of Applied Ecology 47:15–25.

Diamond, J. M. 1975. Assembly of species communities. Pages 342–444 *in* M. L. Cody and J. M. Diamond, editors. Ecology and evolution of communities. Harvard University Press, Cambridge, Massachusetts.

Dieckmann, U., and M. Doebeli. 1999. On the origin of species by sympatric speciation. Nature 400:354–357.

Dilcher, D. 2000. Toward a new synthesis: major evolutionary trends in the angiosperm fossil record. Proceedings of the National Academy of Sciences 97:7030–7036.

Dormann, C. F., and R. Strauss. 2014. A method for detecting modules in quantitative bipartite networks. Methods in Ecology and Evolution 5:90–98.

Dyer, L. A., M. S. Singer, J. T. Lill, and J. O. Stireman. 2007. Host specificity of Lepidoptera in tropical and temperate forests. Nature 448:696–699.

Ehrlich, P., and P. Raven. 1964. Butterflies and plants: a study in coevolution. Evolution 18:586–608.

Ellis, A. G., A. Verboom, T. van der Niet, S. D. Johnson, and H. P. Linder. 2014. Speciation and extinction in the Greater Cape Floristic Region. Pages 119–141 *in* N. Allsopp, J. F. Colville, and G. A. Verboom, editors. Fynbos: ecology, evolution, and conservation of a megadiverse region. Oxford University Press, New York.

Erwin, T. 1982. Tropical forests: their richness in Coleoptera and other arthropod species. Coleopterists Bulletin 36:74–75.

Feder, J., S. Opp, B. Wlazlo, K. Reynolds, W. Go, and S. Spisak. 1994. Host fidelity is an effective premating barrier between sympatric races of the apple maggot fly. Proceedings of the National Academy of Sciences of the United States of America 91:7990–7994.

Feder, J., K. Reynolds, W. Go, and E. Wang. 1995. Intra-and interspecific competition and host race formation in the apple maggot fly, *Rhagoletis pomonella* (Diptera: Tephritidae). Oecologia 101:416–425.

Fitzpatrick, B. M., J. A. Fordyce, and S. Gavrilets. 2009. Pattern, process and geographic modes of speciation. Journal of Evolutionary Biology 22:2342–2347.

Flamm, R. O., T. L. Wagner, S. P. Cook, P. E. Pulley, R. N. Coulsen, and T. M. McArdle. 1987. Host colonization by cohabiting *Dendroctonus frontalis*, *Ips avulsus*, and *I. calligraphus* (Coleoptera: Scolytidae). Environmental Entomology 16:390–399.

Forister, M. L., L. A. Dyer, M. S. Singer, J. O. Stireman III, and J. T. Lill. 2012. Revisiting the evolution of ecological specialization, with emphasis on insect-plant interactions. Ecology 93:981–991.

Forister, M. L., V. Novotny, A. K. Panorska, L. Baje, Y. Basset, P. T. Butterill, L. Cizek, P. D. Coley, F. Dem, I. R. Diniz, P. Drozd, M. Fox, A. E. Glassmire, R. Hazen, J. Hrcek, J. P. Jahner, O. Kaman, T. J. Kozubowski, T. A. Kursar, O. T. Lewis, J. Lill, R. J. Marquis, S. E. Miller, H. C. Morais, M. Murakami, H. Nickel, N. A. Pardikes, R. E. Ricklefs, M. S. Singer, A. M. Smilanich, J. O. Stireman, S. Villamarín-Cortez, S. Vodka, M. Volf, D. L. Wagner, T. Walla, G. D. Weiblen, and L. A. Dyer. 2014. The global distribution of diet breadth in insect herbivores. Proceedings of the National Academy of Sciences of the United States of America 112:442–447.

Fox, L. R., and P. A. Morrow. 1981. Species Specialization: Species property or Local Phenomenon? Science 211:887–893.

Funk, D., K. Filchak, and J. Feder. 2002. Herbivorous insects: model systems for the comparative study of speciation ecology. Genetica 116:251–267.

Futuyma, D. J. 2008. Sympatric Speciation: Norm or Exception? Pages 136–148 Specialization, Speciation, and Radiation: The Evolutionary Biology of Herbivorous Insects.

Futuyma, D., and G. Moreno. 1988. The evolution of ecological specialization. Annual Review of Ecology and Systematics 19:207–233.

Gause, G. F., O. K. Nastokova, and W. W. Alpatov. 1934. The influence of biologically conditioned media on the growth of mixed population of *Paramecium caudatum* and *P. aurelia*. Journal of Animal Ecology 3:222–230.

Gerber, G. P., and A. C. Echternacht. 2000. Evidence for asymmetrical intraguild predation between native and introduced Anolis lizards. Oecologia 124:599–607.

Giliomee, J. H. 2003. Insect diversity in the Cape Floristic Region. African Journal of Ecology 41:237–244.

Glyphis, J. P., and G. M. Puttick. 1988. Phenolics in some Southern African mediterranean shrubland plants. Phytochemistry 27:743–751.

Goldblatt, P. 1997. Floristic diversity in the Cape Flora of South Africa. Biodiversity and Conservation 6:359–377.

Gosden, T. P., and E. I. Svensson. 2009. Density-dependent male mating harassment, female resistance, and male mimicry. The American naturalist 173:709–721.

Gotelli, N. J., and A. M. Ellison. 2013. EcoSimR.

Gould, G. G., and M. H. Sweet II. 2000. The Host Range and Oviposition Behavior of Ochrimnus mimulus (Hemiptera: Lygaeidae). The Southwestern Naturalist. 45:15-23

Grant, P., and B. Grant. 2006. Evolution of character displacement in Darwin’s finches. Science 313:224–226.

Gravendeel, B., A. Smithson, F. J. W. Slik, and A. Schuiteman. 2004. Epiphytism and pollinator specialization: drivers for orchid diversity? Philosophical transactions of the Royal Society of London. Series B, Biological sciences 359:1523–1535.

Grether, G. F., N. Losin, C. N. Anderson, and K. Okamoto. 2009. The role of interspecific interference competition in character displacement and the evolution of competitor recognition. Biological Reviews 84:617–635.

Gripenberg, S., P. J. Mayhew, M. Parnell, and T. Roslin. 2010. A meta-analysis of preference–performance relationships in phytophagous insects. Ecology Letters 13:383–393.

Guimarães, P. R., V. Rico-Gray, P. S. Oliveira, T. J. Izzo, S. F. dos Reis, and J. N. Thompson. 2007. Interaction intimacy affects structure and coevolutionary dynamics in mutualistic networks. Current biology 17:1797–1803.

Harborne, J. 1979. Correlations between flavonoid chemistry, anatomy and geography in the Restionaceae. Phytochemistry 18:1323–1327.

Hardin, G. 1960. The competitive exclusion principle. Science 131:1292–1297.

Hardy, N. B., and S. P. Otto. 2014. Specialization and generalization in the diversification of phytophagous insects: tests of the musical chairs and oscillation hypotheses. Proceedings of the Royal Society B: Biological Sciences 281:1–10.

Harrington, L. A., A. L. Harrington, N. Yamaguchi, M. D. Thom, P. Ferreras, T. R. Windham, and M. DW. 2009. The impact of native competitors on an alien invasive: temporal niche shifts to avoid interspecific aggression? Ecology 90:1207–1216.

Hereford, J. 2009. A quantitative survey of local adaptation and fitness trade-offs. The American naturalist 173:579–588.

Herppich, M., W. B. Herppich, and D. J. von Willert. 2002. Leaf nitrogen content and photosynthetic activity in relation to soil nutrient availability in coastal and mountain fynbos plants (South Africa). Basic and Applied Ecology 3:329–337.

Herrel, A., G. J. Measey, B. Vanhooydonck, and K. A. Tolley. 2011. Functional consequences of morphological differentiation between populations of the Cape Dwarf Chameleon (*Bradypodion pumilum*). Biological Journal of the Linnean Society 104:692–700.

Hillebrand, H. 2004. On the generality of the latitudinal diversity gradient. The American Naturalist 163:192–211.

Hochkirch, A., and J. Gröning. 2012. Niche overlap in allotopic and syntopic populations of sexually interacting ground-hopper species. Insect Science 19:391–402.

Hochkirch, A., J. Gröning, and A. Bücker. 2007. Sympatry with the devil: reproductive interference could hamper species coexistence. The Journal of animal ecology 76:633–642.

Hopkins, K. P., and K. A. Tolley. 2011. Morphological variation in the Cape Dwarf Chameleon (*Bradypodion pumilum*) as a consequence of spatially explicit habitat structure differences. Biological Journal of the Linnean Society 102:878–888.

Hughes, J. B., G. C. Daily, and P. R. Ehrlich. 2000. Conservation of Insects: a Habitat Approach. Conservation Biology 14:1788–1797.

Irschick, D. J., and J. B. Losos. 1999. Do lizards avoid habitats in which performance Is submaximal? The relationship between sprinting capabilities and structural habitat use in Caribbean anoles. The American Naturalist 154:293–305.

Ives, A. R., and H. C. J. Godfray. 2006. Phylogenetic analysis of trophic associations. The American Naturalist 168:E1–E14.

Janz, N., and S. Nylin. 2008. The oscillation hypothesis of host-plant range and speciation. Pages 203–215 *in* K. J. Tilmon, editor. Specialization, Speciation, and Radiation: The Evolutionary Biology of Herbivorous Insects. University of California Press, Berkeley.

Janzen, D. 1985. On ecological fitting. Oikos 45:308–310.

Jing, S. W., and P. D. Coley. 1990. Dioecy and herbivory: the effect of growth rate on plant defense in Acer negundo. Oikos 58:369–377.

Joern, A., T. Provin, and S. T. Behmer. 2012. Not just the usual suspects: Insect herbivore populations and communities are associated with multiple plant nutrients. Ecology 93:1002–1015.

Johnson, S. 1992. Plant–animal relationships. Pages 175–205 *in* R. M. Cowling, editor. The Ecology of Fynbos: Nutrients, Fire and Diversity. Oxfort University Press, Cape Town.

Johnson, S. D., H. P. Linder, and K. E. Steiner. 1998. Phylogeny and radiation of pollination systems in *Disa* (Orchidaceae). American Journal of Botany 85:402–411.

Jordal, B. H., B. C. Emerson, and G. M. Hewitt. 2006. Apparent “sympatric” speciation in ecologically similar herbivorous beetles facilitated by multiple colonizations of an island. Molecular Ecology 15:2935–2947.

Kameda, Y., A. Kawakita, and M. Kato. 2009. Reproductive character displacement in genital morphology in Satsuma land snails. The American naturalist 173:689–697.

Kaplan, I., and R. F. Denno. 2007. Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory. Ecology letters 10:977–994.

Kassen, R. 2002. The experimental evolution of specialists, generalists, and the maintenance of diversity. Journal of Evolutionary Biology 15:173–190.

Kawecki, T. T. J., and D. Ebert. 2004. Conceptual issues in local adaptation. Ecology letters 7:1225–1241.

Kemp, J. E. 2014. Untangling mechanisms structuring insect diversity patterns in the Cape Floristic Region: the Restionaceae and their herbivores. MSc thesis. Stellenbosch University.

Klein, D. R. 1990. Variation in quality of caribou and reindeer forage plants associated with season, plant part, and phenology. Rangifer:123–130.

Kuhlmann, M. 2009. Patterns of diversity, endemism and distribution of bees (Insecta: Hymenoptera: Anthophila) in Southern Africa. South African Journal of Botany 75:726–738.

Lawlor, L. R. 1980. Structure and stability in natural and randomly constructed competitive communities. American Naturalist 116:394–408.

Lawton, J. H., and D. R. Strong. 1981. Community patterns and competition in folivorous insects. American Naturalist 118:317–338.

Linder, H. 2011. The African Restionaceae: an Intkey identification and description system.

Linder, H. P. 2001. Two new species of Ceratocaryum (Restionaceae). Kew Bulletin 56:465–477.

Linder, H. P. 2003. The radiation of the Cape flora, southern Africa. Biological reviews of the Cambridge Philosophical Society 78:597–638.

Linder, H. P., P. Eldenäs, and B. G. Briggs. 2003. Contrasting patterns of radiation in African and Australian Restionaceae. Evolution 57:2688–2702.

Linder, H. P., and J. H. Vlok. 1991. The morphology, taxonomy and evolution of *Rhodocoma* (Restionacea) 175:139–160.

Lister, B. 1976. The nature of niche expansion in West Indian Anolis lizards I: ecological consequences of reduced competition. Evolution 30:659–676.

Losos, J. B. 1992. The evolution of convergent structure in Caribbean Anolis communities. Systematic Biology 41:403–420.

Losos, J. B. 1998. Contingency and Determinism in Replicated Adaptive Radiations of Island Lizards. Science 279:2115–2118.

Losos, J. B. 2009a. Five anole faunas, Part one: Greater Antillean Ecomorphs. Pages 29–57. Lizards in an evolutionary tree: Ecology and adaptive radiation of anoles. University of California Press, Berkeley.

Losos, J. B. 2009b. Ecology and adaptive radiation. Pages 205–232. Lizards in an evolutionary tree: Ecology and adaptive radiation of anoles. University of California Press, Berkeley.

Lovette, I. J., E. Bermingham, and R. E. Ricklefs. 2002. Clade-specific morphological diversification and adaptive radiation in Hawaiian songbirds. Proceedings of the Royal Society B: Biological Sciences 269:37–42.

De Luca, P., and G. Morris. 1998. Courtship Communication in Meadow Katydids: Female Preference for Large Male Vibrations. Behaviour 135:777–794.

MacColl, A. D. C. 2011. The ecological causes of evolution. Trends in Ecology and Evolution 26:514–522.

Mahler, D. L., L. J. Revell, R. E. Glor, and J. B. Losos. 2010. Ecological opportunity and the rate of morphological evolution in the diversification of Greater Antillean anoles. Evolution 64:2731–2745.

Mallet, J., A. Meyer, P. Nosil, and J. L. Feder. 2009. Space, sympatry and speciation. Journal of evolutionary biology 22:2332–2341.

Matsubayashi, K. W., I. Ohshima, and P. Nosil. 2010. Ecological speciation in phytophagous insects. Entomologia Experimentalis et Applicata 134:1–27.

Mayhew, P. J. 2007. Why are there so many insect species? Perspectives from fossils and phylogenies. Biological reviews of the Cambridge Philosophical Society 82:425–454.

McClure, M. S., and P. W. Price. 1975. Competition among Sympatric *Erythroneura* Leafhoppers (Homoptera: Cicadellidae) on America Sycamore. Ecology 56:1388–1397.

McClure, M. S., and P. W. Price. 1976. Ecotope characteristics of coexisting *Erythroneura* leafhoppers (Homoptera; Cicadellidae) on sycamore. Ecology 57:928–940.

McLain, D. K. 1981. Resource partitioning by three species of hemipteran herbivores on the basis of host plant density. Oecologia 48:414–417.

Meiri, S., D. Simberloff, and T. Dayan. 2011. Community-wide character displacement in the presence of clines: A test of Holarctic weasel guilds. Journal of animal ecology 80:824–834.

Mittelbach, G. G., D. W. Schemske, H. V Cornell, A. P. Allen, J. M. Brown, M. B. Bush, S. P. Harrison, A. H. Hurlbert, N. Knowlton, H. A. Lessios, C. M. McCain, A. R. McCune, L. A. McDade, M. A. McPeek, T. J. Near, T. D. Price, R. E. Ricklefs, K. Roy, D. F. Sax, D. Schluter, J. M. Sobel, and M. Turelli. 2007. Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. Ecology letters 10:315–31.

Mitter, C., B. Farrell, and B. Wiegmann. 1988. The phylogenetic study of adaptive zones: has phytophagy promoted insect diversification? American Naturalist 132:107–128.

Moen, D. S., and J. J. Wiens. 2009. Phylogenetic evidence for competitively driven divergence: Body-size evolution in caribbean treefrogs (Hylidae: Osteopilus). Evolution 63:195–214.

Mooney, H. A., and S. L. Gulmon. 1982. Constraints on Leaf Structure and Function in Reference to Herbivory. BioScience 32:198–206.

Morris, R. J., S. Gripenberg, O. T. Lewis, and T. Roslin. 2014. Antagonistic interaction networks are structured independently of latitude and host guild. Ecology letters 17:340–349.

Neu, C. W., C. R. Byers, and J. M. Peek. 1974. A technique for analysis of utilization-availability data. Journal of Wildlife Management 38:541–545.

Nosil, P. 2002. Transition rates between specialization and generalization in phytophagous insects. Evolution 56:1701–1706.

Nosil, P. 2004. Reproductive isolation caused by visual predation on migrants between divergent environments. Proceedings. Biological sciences 271:1521–1528.

Nosil, P. 2008. Ernst Mayr and the integration of geographic and ecological factors in speciation. Biological Journal of the Linnean Society 96:26–46.

Nosil, P. 2012. The geography of ecological speciation. Pages 141–168. Ecological speciation. Oxfort University Press, Oxfort.

Nosil, P. 2013. Degree of sympatry affects reinforcement in Drosophila. Evolution; international journal of organic evolution 67:868–72.

Nosil, P., and B. J. Crespi. 2006. Experimental evidence that predation promotes divergence in adaptive radiation. Proceedings of the National Academy of Sciences of the United States of America 103:9090–9095.

Nosil, P., B. J. Crespi, and C. P. Sandoval. 2003. Reproductive isolation driven by the combined effects of ecological adaptation and reinforcement. Proceedings of the Royal Society B: Biological Sciences 270:1911–8.

Nosil, P., L. J. Harmon, and O. Seehausen. 2009. Ecological explanations for (incomplete) speciation. Trends in Ecology and Evolution 24:145–156.

Nosil, P., and A. Mooers. 2005. Testing hypotheses about ecological specialization using phylogenetic trees. Evolution 59:2256–2263.

Nosil, P., C. Sandoval, and B. Crespi. 2006. The evolution of host preference in allopatric vs. parapatric populations of *Timema cristinae* walking sticks. Journal of evolutionary … 19:929–942.

Nosil, P., and C. P. Sandoval. 2008. Ecological niche dimensionality and the evolutionary diversification of stick insects. PLoS ONE 3:1–11.

Nosil, P., T. H. Vines, and D. J. Funk. 2005. Reproductive isolation caused by natural selection against immigrants from divergent habitats. Evolution 59:705–719.

Novotny, V., and Y. Basset. 2005. Host Specificity of Insect Herbivores in Tropical Forests. Proceedings of the Royal Society B: Biological Sciences 272:1083–1090.

Novotny, V., P. Drozd, S. E. Miller, M. Kulfan, M. Janda, Y. Basset, and G. D. Weiblen. 2006. Why are there so many species of herbivorous insects in tropical rainforests? Science 313:1115–1118.

Novotny, V., S. E. Miller, J. Hulcr, R. a I. Drew, Y. Basset, M. Janda, G. P. Setliff, K. Darrow, A. J. a Stewart, J. Auga, B. Isua, K. Molem, M. Manumbor, E. Tamtiai, M. Mogia, and G. D. Weiblen. 2007. Low beta diversity of herbivorous insects in tropical forests. Nature 448:692–695.

Nuhardiyati, M., and W. Bailey. 2005. Calling and duetting behavior in the leafhopper Balclutha incisa (Hemiptera: Cicadellidae: Deltocephalinae): Opportunity for female choice? Journal of Insect Behavior 18:259–280.

Nyman, T., V. Vikberg, D. R. Smith, and J.-L. Boevé. 2010. How common is ecological speciation in plant-feeding insects? A “Higher” Nematinae perspective. BMC evolutionary biology 10:266.

Olesen, J. M., J. Bascompte, Y. L. Dupont, and P. Jordano. 2007. The modularity of pollination networks. Proceedings of the National Academy of Sciences 104:19891–19896.

Osborn, H. 1903. An interesting case of mimicry. PSYCHE 9:327.

Pfennig, D. W., and P. J. Murphy. 2000. Character displacement in polyphenic tadpoles. Evolution 54:1738–1749.

Pianka, E. R. 1973. The structure of lizard communities. Annual Review of Ecology and Systematics 4:53–74.

Picker, M. D., and M. J. Samways. 1996. Faunal diversity and endemicity of the Cape Peninsula, South Africa ? a first assessment. Biodiversity and Conservation 5:591–606.

Poisot, T., J. D. Bever, A. Nemri, P. H. Thrall, and M. E. Hochberg. 2011. A conceptual framework for the evolution of ecological specialisation. Ecology letters 14:841–851.

Prendini, L. 1995. Parallel cladogenesis, coevolution, and insect-plant associations: evidence from leafhoppers (Cicadellidae, Cephalelini) and Restionaceae. Hons. thesis. University of Cape Town.

Prendini, L. 1997. Two new host-restricted restioid leafhoppers of the genus *Cephalelus* Percheron (Cicadellidae: Cephalelelini), with descriptions of the females of *C. brevipilus* Davies, *C. daviesi* Davies and *C. rawsonia* Davies. African Entomology 5:273–281.

Prendini, L., and H. P. Linder. 1998. Phylogeny of the South African species of restioid leafhoppers, tribe Cephalelini (Homoptera: Cicadellidae, Ulopinae). Entomologica scandinavica 29:11–18.

Procheş, Ş., and R. M. Cowling. 2006. Insect diversity in Cape fynbos and neighbouring South African vegetation. Global Ecology and Biogeography 15:445–451.

Procheş, Ş., F. Forest, R. Veldtman, S. L. Chown, R. M. Cowling, S. D. Johnson, D. M. Richardson, V. Savolainen, and S. Procheş. 2009. Dissecting the plant-insect diversity relationship in the Cape. Molecular Phylogenetics and Evolution 51:94–99.

Pryke, J. S., and M. J. Samways. 2008. Conservation of invertebrate biodiversity on a mountain in a global biodiversity hotspot, Cape Floral Region. Biodiversity and Conservation 17:3027–3043.

Rathcke, B. J. 1976. Competition and Coexistence with A Guild of Herbivorous Insects. Ecology 57:76–87.

Raupp, M. J., F. R. Milan, P. Barbosa, and B. A. Leonhardt. 1986. Methylcyclopentanoid monoterpenes mediate interactions among insect herbivores. Science 232:1408–1410.

Rezende, E. L., P. Jordano, and J. Bascompte. 2007. Effects of phenotypic complementarity and phylogeny on the nested structure of mutualistic networks. Oikos 116:1919–1929.

Rice, A. M., and D. W. Pfennig. 2005. Character displacement: in situ evolution of novel phenotypes or sorting of pre-existing variation? Journal of Evolutionary Biology 20:448–459.

Richards, M. B., W. D. Stock, and R. M. Cowling. 1997. Soil Nutrient Dynamics and Community Boundaries in the Fynbos Vegetation of South Africa. Plant Ecology 130:143–153.

Richmond, J. Q., and E. L. Jockusch. 2007. Body size evolution simultaneously creates and collapses species boundaries in a clade of scincid lizards. Proceedings of the Royal Society B: Biological Sciences 274:1701–1708.

Ricklefs, R. E. 2012. Naturalists, natural history, and the nature of biological diversity. The American Naturalist 179:423–35.

Ricklefs, R. E., and R. J. Marquis. 2012. Species richness and niche space for temperate and tropical folivores. Oecologia 168:213–220.

Robinson, B. W., and D. Schluter. 2000. Natural Selection and the Evolution of Adaptive Genetic Variation in Northern Freshwater Fishes. Pages 65–94 *in* T. A. Mousseau, B. Sinervo, and J. A. Endler, editors. Adaptive Genetic Variation in the Wild. Oxford University Press, New York.

Rodríguez, R. L., K. Ramaswamy, and R. B. Cocroft. 2006. Evidence that female preferences have shaped male signal evolution in a clade of specialized plant-feeding insects. Proceedings of the Royal Society B: Biological Sciences 273:2585–2593.

Roets, F., P. W. Crous, M. J. Wingfield, and L. L. Dreyer. 2009. Mite-mediated hyperphoretic dispersal of Ophiostoma spp. from the infructescences of South African Protea spp. Environmental entomology 38:143–152.

Ronsted, N., M. R. Symonds, T. Birkholm, S. Brøgger Christensen, A. W. Meerow, M. Molander, P. Mølgaard, G. Petersen, N. Rasmussen, J. van Staden, G. I. Stafford, and A. K. Jäger. 2012. Can phylogeny predict chemical diversity and potential medicinal activity of plants? A case study of amaryllidaceae. BMC Evolutionary Biology 12:182.

Rundle, H. D., and P. Nosil. 2005. Ecological speciation. Ecology letters 8:336–352.

Scheirs, J., L. De Bruyn, and R. Verhagen. 2000. Optimization of adult performance determines host choice in a grass miner. Proceedings of the Royal Society B: Biological Sciences 267:2065–2069.

Schemske, D. W., G. G. Mittelbach, H. V. Cornell, J. M. Sobel, and K. Roy. 2009. Is There a Latitudinal Gradient in the Importance of Biotic Interactions? Annual Review of Ecology, Evolution, and Systematics 40:245–269.

Schleuning, M., L. Ingmann, R. Strauß, S. a Fritz, B. Dalsgaard, D. M. Dehling, M. Plein, F. Saavedra, B. Sandel, J.-C. Svenning, K. Böhning-Gaese, and C. F. Dormann. 2014. Ecological, historical and evolutionary determinants of modularity in weighted seed-dispersal networks. Ecology letters 17:454–463.

Schluter, D. 1984. A variance test for detecting species associations, with some example applications. Ecology 65:998–1005.

Schluter, D. 1990. Species-for-species matching. The American Naturalist 136:560–568.

Schluter, D. 1994. Experimental evidence that competition promotes divergence in adaptive radiation. Science 266:798–801.

Schluter, D. 2000. The Ecology of Adaptive Radiation. Oxfort University Press, Oxfort.

Schluter, D. 2000b. Ecological character displacement in adaptive radiation. The American Naturalist 156:S4–S16.

Schluter, D., and J. D. McPhail. 1992. Ecological character displacement and speciation in sticklebacks. The American Naturalist 140:85–108.

Scott, A. C. 2000. The Pre-Quaternary history of fire. Palaeogeography, Palaeoclimatology, Palaeoecology 164:281–329.

Seehausen, O., and D. Schluter. 2004. Male-male competition and nuptial-colour displacement as a diversifying force in Lake Victoria cichlid fishes. Proceedings of the Royal Society B: Biological Sciences 271:1345–1353.

Servedio, M. R., G. S. Van Doorn, M. Kopp, A. M. Frame, and P. Nosil. 2011. Magic traits in speciation: “magic” but not rare? Trends in Ecology and Evolution 26:389–397.

Singer, M. C., and B. Wee. 2005. Spatial pattern in checkerspot butterfly – host plant association at local , metapopulation and regional scales:347–361.

Singer, M. S., and J. O. Stireman. 2005. The tri-trophic niche concept and adaptive radiation of phytophagous insects. Ecology Letters 8:1247–1255.

Slingsby, J., and G. Verboom. 2006. Phylogenetic relatedness limits co-occurrence at fine spatial scales: evidence from the Schoenoid sedges (Cyperaceae: Schoeneae) of the Cape Floristic Region, South. The American Naturalist 168:14–27.

Stephens, P. R., and J. J. Wiens. 2003. Explaining species richness from continents to communities: the time-for-speciation effect in emydid turtles. The American naturalist 161:112–128.

Stewart, A. J. A., and A. F. Wright. 1995. A new inexpensive suction apparatus for sampling arthropods in grassland. Ecological Entomology 20:98–102.

Stockhoff, B. A. 1991. Starvation resistance of gypsy moth, Lymantria dispar (L.) (Lepidoptera, Lymantriidae): tradeoffs among growth, body size, and survival. Oecologia 88:422–429.

Strong, D. R., J. H. Lawton and S. R. Southwood. 1984. Insects on Plants. Community Patterns and Mechanisms. Blackwell Scientific, Oxfort.

Strong, D. R., L. A. Szyska, and D. S. Simberloff. 1979. Test of community-wide character displacement against null hypotheses. Evolution 33:897–913.

Stuart, Y. E., and J. B. Losos. 2013. Ecological character displacement: glass half full or half empty? Trends in ecology & evolution 28:402–408.

Stuart-Fox, D., A. Moussalli, and M. J. Whiting. 2007. Natural selection on social signals: signal efficacy and the evolution of chameleon display coloration. The American Naturalist 170:916–30.

Tack, A. J. M., O. Ovaskainen, P. J. Harrison, and T. Roslin. 2009. Competition as a structuring force in leaf miner communities. Oikos 118:809–818.

Taylor, E. B., J. W. Boughman, M. Groenenboom, M. Sniatynski, D. Schluter, and J. L. Gow. 2006. Speciation in reverse: Morphological and genetic evidence of the collapse of a three-spined stickleback (Gasterosteus aculeatus) species pair. Molecular Ecology 15:343–355.

Thompson, A. R., T. C. Adam, K. M. Hultgren, and C. E. Thacker. 2013. Ecology and evolution affect network structure in an intimate marine mutualism. The American Naturalist 182:E58–72.

Thompson, J. N. 1993. Preference hierarchies and the origin of geographic specialization in host use in swallowtail butterflies. Evolution 47:1585–1594.

Thompson, J. N. 1998. Rapid evolution as an ecological process. Trends in Ecology & Evolution 13:329–332.

Toju, H., P. R. Guimarães, J. M. Olesen, and J. N. Thompson. 2014. Assembly of complex plant–fungus networks. Nature Communications 5:5273.

Tolley, K. A., R. N. V Raw, R. Altwegg, and G. J. Measey. 2010. Chameleons on the move: survival and movement of the Cape dwarf chameleon, *Bradypodion pumilum*, within a fragmented urban habitat. African Zoology 45:99–106.

Tukey, J. W. 1977. Exploratory Data Analysis. Addison-Wesley Publication Company. Reading, Massachusetts.

Vázquez, D. P., N. Blüthgen, L. Cagnolo, and N. P. Chacoff. 2009. Uniting pattern and process in plant-animal mutualistic networks: a review. Annals of botany 103:1445–1457.

Veech, J. A. 2013. A probabilistic model for analysing species co-occurrence. Global Ecology and Biogeography 22:252–260.

Verboom, G. A., J. K. Archibald, F. T. Bakker, D. U. Bellsedt, F. Conrad, L. Dryer, F. Forest, C. Galley, P. Goldblatt, J. F. Henning, K. Mummenhoff, H. P. Linder, M. Muasya, K. C. Oberlander, V. Savolainen, D. A. Snijman, T. van der Niet, and T. L. Nowell. 2009. Origin and diversification of the Greater Cape flora: Ancient species repository, hot-bed of recent radiation, or both? Molecular Phylogenetics and Evolution 51:44–53.

Via, S. 2001. Sympatric speciation in animals: the ugly duckling grows up. Trends in Ecology and Evolution 16:381–390.

Via, S. 2012. Divergence hitchhiking and the spread of genomic isolation during ecological speciation-with-gene-flow. Philosophical Transactions of the Royal Society B: Biological Sciences 367:451–460.

Wasserman, S. S., S. S. Wasserman, D. J. Futuyma, and D. J. Futuyma. 1981. Evolution of Host Plant Utilization in Laboratory Populations of the Southern Cowpea Weevil, *Callosobruchus maculatus* Fabricius (Coleoptera, Bruchidae). Evolution 35:605–617.

Wennström, A., and L. Hjulström. 2010. Mother really knows best: host choice of adult phytophagous insect females reflects a within-host variation in suitability as larval food. Chemoecology 20:35–42.

Whittall, J. B., and S. A. Hodges. 2007. Pollinator shifts drive increasingly long nectar spurs in columbine flowers. Nature 447:706–709.

Wiens, J. J., C. H. Graham, D. S. Moen, S. A. Smith, and T. W. Reeder. 2006. Evolutionary and ecological causes of the latitudinal diversity gradient in hylid frogs: treefrog trees unearth the roots of high tropical diversity. The American Naturalist 168:579–596.

Wiese, T. J. 2014. The evolution of fynbos-endemic Cephalelini leafhoppers specialising on Restionaceae. MSc thesis. University of Stellenbosch

Wilson, S. W., J. L. Smith, and A. H. Purcell. 1993. An inexpensive vacuum collector for insect sampling. Entomological News 104:203–208.

Winemiller, K. O., and E. R. Pianka. 1990. Organization in natural assemblages of desert lizards and tropical fishes. Ecological Monographs 60:27–55.

Winkler, I. S., and C. Mitter. 2008. The phylogenetic dimension of insect-plant interactions: a review of recent evidence. Pages 240–263 *in* K. J. Tilmon, editor. Specialization, speciation, and radiation: the evolutionary biology of herbivorous insects. University of California Press, Berkeley.

Wright, M. G., and M. J. Samways. 1998. Insect species richness tracking plant species richness in a diverse flora: gall-insects in the Cape Floristic Region, South Africa. Oecologia 115:427–433.

Wright, M. G., and M. J. Samways. 1999. Plant characteristics determine insect borer assemblages on Protea species in the Cape Fynbos, and importance for conservation management. Biodiversity and Conservation 8:1089–1100.