Interwoven Tributaries

A Community Genetics Platform for Ecological Interactions

A thesis submitted to the University of Manchester for the degree of

Doctor of Philosophy

in the Faculty of Life Sciences

2012

Mouhammad Shadi Khudr

"Even in a gene-centered view of evolution, it is the armies of gene packages we call species that wage the genetic wars and create the genetic alliances."

John N. Thompson (1999)*

*Thompson, J. N. (1999). The evolution of species interactions. *Science*, *284*(5423), 2116-2118. (doi: 10.1126/science.284.5423.2116)

Table of Contents

Word count (including footnotes and endnotes): 67.628

List of Tables

List of Figures

Abstract

Community genetics research investigates the influence of intra-specific genetic variation on species interactions. This rapidly growing research field consists of more than one approach to explore how a significant portion of the environment of a focal species is differentially defined by the expressed genomes of other interacting species. While the basic concept of community genetics is well supported empirically, there is still a set of pertinent issues in need of further investigation.

The initial research addressed herein focused on the extent to which the magnitude of a community genetic effect can be moderated when acting in concert with other forces in nature, i.e. the interaction between community genetic effects and the effects of other eco-evolutionary processes such as competition and parasitism.

Subsequent research investigated the impact of genetic variation of host plants in agro-ecosystems on the performance (reproductive success) and behaviour (distribution and feeding-site choice) of plant-associated pests such as aphids, especially when pests and their hosts were subject to plant-mediated interactions.

In addition, the differential effects of Indirect Ecological Effects (IEEs) and Indirect Genetic effects (IGEs) on the emergence of shared (extended) phenotypes between natural enemies (i.e. biological control agents and phytophagous insects) were examined.

I provide clear evidence for significant effects of the genetic variation of host plant on aphid performance, behaviour and intra- and inter-specific competition. My findings also give credence to the concept of reciprocal moderation between plant genotype and aphid competition. I also provide observations on competition that segues into less antagonistic and possibly into a more cooperative form of interaction. In addition, I establish novel systems of economically important crop genotypes, noxious sap-feeding aphid species and root-galling nematodes. I also devise an amalgamated approach to interpret the interwoven set of mechanisms that underpin the observations presented and conclusions drawn. I also provide further investigation on the role of Indirect Ecological Effects (IEEs) between root-knot nematodes and sap-feeding aphids, and demonstrate the influence of in-plant variation on the interaction between the spatially separated plant consumers. Furthermore, I use a quantitative genetic experimental design in order to demonstrate a differential impact of parasitoid genotype on the behaviour of its aphid host. As such, I provide some of the clearest evidence to date that the phenotype of an organism can be the product of the genes expressed in another organism via Inter-specific Indirect Genetic Effects (IIGEs).

Finally, I conducted research on epiphytic bromeliads and their associated faunal communities in the tropics. Here I demonstrate that the influence of intra-specific genetic variation of the host plant on the associated ecological communities may be more universal than previously conceived, with a plausible role for such variation in the maintenance of biological diversity.

My research provides evidence for the genetic basis of species interactions and, interestingly, a genetic basis for the evolutionary arms-race between foragers and their hosts. My doctoral work adds new evidence to the increasing literature on the evolutionary importance of (Genotype x Genotype) interactions and (Genotype x Genotype x Environment) interactions in shaping the dynamics of pest communities, which in turn can affect plant phenotype and can influence the properties and services of the focal ecosystem in which the inter-players live and interact.

Declaration

No portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Study Rationale (Organisation of thesis chapters)

The five primary research papers of this thesis all attempt to examine how genetic variation within a single species may have larger effects on associated communities. The papers also, through a range of different approaches, shed light on the effects of (Genotype x Genotype) and (Genotype x Genotype x Environment) interactions on the performance (reproductive success) and behaviour (distribution and preference) of plant-associated invertebrates. I also endeavour to untangle the relative contributions of various factors involved in the interplay between community genetic effects and competition, parasitoidism and Indirect Ecological Effects.

In the general introduction, I explicate the novel synthesis of community genetics as a cutting-edge discipline in the sphere of evolutionary biology.

In chapter 1, I investigate the effect of broad bean cultivars (within-plant genetic variation) on the performance (reproductive success) and behaviour (on-plant distribution) of multiple genotypes of pea aphids.

In chapter 2, I contrast the community genetic effects and competition effects on confined hosts and under multiple host choice conditions. I focus on the performance and behaviour of a focal pea aphid genotype under both intra- and inter-specific competition.

Chapter 3 examines how the individual genetic differences in an aphid's natural enemy (parasitic wasp) influence the response of a focal aphid genotype in broad bean cultivation.

In chapter 4, I explore the reciprocal effect of above- and below-ground plant enemies, i.e. aphids and root-knot nematodes, on the fitness of the inter-players.

Finally, in chapter 5, in a three-year research collaboration with colleagues, I address the community genetic effects of an epiphytic tank-forming bromeliad (as an

ecologically important keystone species) on associated invertebrate communities in a tropical ecosystem.

The general discussion of the thesis forms the conclusion and provides insights on the intricacy of the genetics of ecological interactions within communities. Furthermore, I provide additional remarks on the advancement of community genetics as an amalgamating frontier in biology, considering some potential future directions for future research. The conclusion also contrasts community genetics with alternative schools of thought and empiricism. I envisage an integration of insights from niche construction theory, eco-evolutionary dynamics, complexity theory, and adaptive complex system dynamics, along with well established perspectives in the realm of community genetics in order to encourage the formulation of a hybrid approach encompassed and empowered by the amalgamative capacity of community genetics. Such ideation should pave the way for more efficacious dialogue between these burgeoning disciplines towards answering burning questions on the complexity of the interaction between ecology and evolution in natural and agricultural man-made systems.

Copyright Statement

The author of this thesis (including any appendices and/or schedules to this thesis) owns certain copyright or related rights in it (the "Copyright") and he has given The University of Manchester certain rights to use such Copyright, including for administrative purposes.

ii. Copies of this thesis, either in full or in extracts and whether in hard or electronic copy, may be made **only** in accordance with the Copyright, Designs and Patents Act 1988 (as amended) and regulations issued under it or, where appropriate, in accordance with licensing agreements which the University has from time to time. This page must form part of any such copies made.

iii. The ownership of certain Copyright, patents, designs, trade marks and other intellectual property (the "Intellectual Property") and any reproductions of copyright works in the thesis, for example graphs and tables ("Reproductions"), which may be described in this thesis, may not be owned by the author and may be owned by third parties. Such Intellectual Property and Reproductions cannot and must not be made available for use without the prior written permission of the owner(s) of the relevant Intellectual Property and/or Reproductions.

iv. Further information on the conditions under which disclosure, publication and commercialisation of this thesis, the Copyright and any Intellectual Property and/or Reproductions described in it may take place is available from the Head of School of Life Sciences (or the Vice-President) and the Dean of the Faculty of Life Sciences, for Faculty of Life Science's candidates.

Acknowledgements

Remarks on authorship

All chapters of this thesis were prepared for the submission to peer reviewed journals. I designed and executed all of the studies presented here in cooperation with my supervisor Dr. Preziosi. Details of authorship and my contribution to each study are provided accordingly at the cover page of each paper-chapter.

General Introduction

Note:

*This doctoral thesis is articulated under the new format Ph.D regulations (alternative format). The general introduction provides a general background on the novelty of the research conducted. The general introduction also summarises the major findings of each paper (chapter).

The thesis is concluded with a brief general discussion of the work including further insights and future directions.

General Introduction

What is Community Genetics, Why and How?

Community genetics is 'the study of the genetics of species interactions and their ecological and evolutionary consequences' (Antonovics 1992, Whitham et al. 2003, 2006). The field of community genetics has grown exponentially since first articulated by Antonovics in 1992 (Agrawal 2003, Johnson and Stinchcombe 2007, Rowntree et al. 2011). Community genetics operates at the interface among ecology, genetics and evolution (see the appendix to this general introduction for an illustrated overview). One of its main interests is the influence of intra-specific genetic variation of ecologically important species (i.e. keystone species) on the structure and composition of associated ecological communities (Fritz et al. 1996, Dungey et al. 2000, Whitham et al. 2003, Ito and Ozaki 2005, Johnson and Agrawal 2005, Whitham et al. 2006, 2008) and ecosystem processes (Driebe and Whitham 2000, Whitham et al. 2003, Schweitzer et al. 2005, Whitham et al. 2006).

Community genetics is a novel burgeoning frontier in evolutionary biology

One of the most prominent facets of community genetics, compared to other areas at the interface between ecology and evolution, is the scale at which experimental manipulations and community analyses take place. In community genetics, the genotype is the focus, i.e. exploring how genetically based variation within a species changes its ecological interactions with other species and the resulting consequences this has for the associated community assemblage. The other important point is that the community genetics approach is not constrained by co-evolution. So

while co-evolution may be a consequence of community genetic effects, it is not a prerequisite (Johnson and Stinchcombe 2007, Rowntree et al. 2011).

In addition, the geographic mosaic of co-evolution (Thompson 1994) and eco-evolutionary dynamics (Pelletier et al. 2009, Schoener 2011) tend to look at population level differences (where past evolutionary processes are the key). Community genetic effects may be the basis of some of these hot and cold spots of co-evolution and may be important in the formation of feedbacks, However, the focus of the geographic mosaic of co-evolution and eco-evolutionary dynamics is at a higher level (the population) and over a slightly longer time frame (see Johnson and Stinchcombe 2007, Rowntree et al. 2011).

Community genetics currently uses several different approaches to understand community level effects caused by intra-specific genetic variation (within-species genetic variation). The main approaches (Agrawal 2003, Johnson and Stinchcombe 2007, Rowntree et al. 2011) are:

First, the quantitative approach which addresses the effect of the genetics of 'species assemblages' on the phenotype of a focal species (e.g. Zytynska et al. 2011). Herein, community geneticists envisage the phenotype of a focal species to be determined by a combination of the genotype (G) of this focal species, an environmental input (E), and the (G×E) interaction. The environmental part (E) can be split into two elements: the biotic environment and the abiotic environment (Zytynska et al. 2011; see also the appendix to this general introduction for a broader overview).

Second is the perspective focusing on the ramifications of intra-specific genetic variation (within-species genetic variation) of a focal species or keystone species (i.e. fundamental or influential regardless of species biomass) on associated communities. This is described as the 'dominant species approach' (Whitham et al.

2003, Schweitzer et al. 2004, 2005, Whitham et al. 2006; see this general introduction appendix for a broader overview).

Third, conventionally, a phenotype represents a set of expressed traits encoded within the genome of an individual that is subject to environmental variability. The extended phenotype approach (which is in line with the second approach of community genetics, i.e. the 'dominant species approach'), proposes that the definition of a phenotype can be extended to encapsulate the plausible consequences of intra-specific genetic variation of fundamental species on ecological communities and ecosystem properties and services, and hence that genes operate on higher levels beyond the individual and population levels (Whitham et al. 2003, Schweitzer et al. 2004, 2005, Whitham et al. 2006; but see Dawkins 1982 and Laland 2004).

What gives credence to the emergence of extended and even shared phenotypes amongst species is the concept that a significant proportion of the environment of each species can be comprised by the expressed genomes of other interacting species (Dawkins 1982, Henter and Via 1995, Underwood and Rausher 2000, Ferrari et al. 2001, Whitham et al. 2003). This defines the intra-specific genetic effect or community genetic effect (CG).

In this regard, the extended and shared phenotypes can be considered as assembled phenotypic norms or traits more likely to emerge via antagonism, cooperation and symbiosis (Dawkins 1982, Jablonka 2004, Laland 2004, Whitham et al. 2003). As such, shared and extended phenotypes, serving to opt for conflict or conflict avoidance between species, may help species (interactors) to maximise fitness i.e. proliferation and survivorship (Dawkins 1976, 1982, Jablonka 2004, Araújo et al. 2008). This is particularly applicable when the influence of intra-specific genetic variation (CG effects) intensifies across levels in multi-trophic systems (Bailey et al. 2006, 2009).

Fourth, the eco-evolutionary approach of Neuhauser et al. (2003) exploring how evolution might occur on ecological time scales under the conditions of disequilibrium. They advocate the necessity to amalgamate ecological and evolutionary models to decode the tangled areas between CG effects and community processes. This approach is especially applicable for man-made agro-ecosystems (see Hersch-Green et al. 2011, Rowntree et al. 2011).

Genetic variation of reciprocal interactions (G×G) has been well documented for plant-herbivore associations and an expanding body of evidence has accredited the influence of community genetic effects (CG) as defining the context and composition of ecological communities (Service 1984, Whitham et al. 2003, Wimp et al. 2005, Bailey et al. 2006, Bangert et al. 2006, Whitham et al. 2006, 2008). Recently, attention has increased towards the effects of genetic diversity and inter-specific genetic variation on preference (host discrimination and selection for feeding sites) and performance (reproductive success) of plant associated faunas (e.g. Utsumi et al. 2010). Inter-specific interactions are supposed to depend on the genetic variation of other interacting species within the ecological network or trophic system (i.e. Genotype×Genotype×Environment (G×G×E); Abrams 1995, Astles et al. 2005, Tétard-Jones et al. 2007). This is also the case for plant-mediated interactions (Wootton 1994, 2002, Kaplan and Denno 2007, Kaplan et al. 2009). In such cases, community genetic effects (CG) might modulate or become modulated by other ecological processes and mechanisms such as IEEs (Indirect Ecological Effects; Wootton 1994, 2002, Astles et al. 2005). Furthermore, the magnitude of community genetic effects (CG) against other ecological processes, such as predation, competition and parasitoidism, is still poorly understood, particularly for systems composed of economic crops, insect pests and their natural enemies (but see Smith et al. 2008). Therefore, while there is now a good supporting body of evidence that community

genetic effects (CG) are common and influential (Johnson and Stinchcombe 2007, Rowntree et al 2011), the investigation of the interaction between CG and other ecological forces and processes (e.g. competition and parasitoidism) is ripe for exploration (Hersch-Green et al. 2011). There is a need to qualitatively as well as quantitatively examine the interactions between such effects. This is plausibly the case for the above-ground and below-ground associations that are mediated by shared host plants in both complex natural forest ecosystems and conventional agricultural ecosystems, where communities thrive within communities (Ferrari and Vavre 2011, Rowntree et al. 2011, Zytynska et al. 2012).

Ecological interactions

Communities are governed by the properties of their biotic components

Species and their interactions do not develop or evolve in voids. Instead, species and their activities reflect the dynamic biotic constituents of the systems where they exist and interact with other biotic (con- and hetero-specific) and abiotic (physical, chemical) constituents. In this context, within-species genetic variation can influence both pair-wise and multi-species interactions (Whitham et al. 2003, Bailey and Whitham 2006a , Bailey et al. 2006, Bangert et al. 2006, Whitham et al. 2006, Wimp et al. 2007, Mooney and Agrawal 2008, Mooney et al. 2008). Consequently, ecosystems will be affected by the dynamics of their constituents, *viz* phenotypic co-variation via bottom-up and top-down routes as mediated by the genetics of focal species (Whitham et al. 2003, Bailey and Whitham 2006a,b, Whitham et al. 2006, Johnson 2008, Lawrence et al. 2012). This should have consequences for the entire ecosystem and the mass-energy relationships within multi-trophic systems (Mejer and

Jørgensen 1979, King 2003, Whitham et al. 2003, Bonchev et al. 2005, Ulanowics 2005, Whitham et al. 2006).

It may be argued that evolution is dependent on the nature and nurture of the interacting species (Fordyce 2006, Pfennig and McGee 2009) as well as the relative frequency, intensity and diversity of species interactions (Paine 1980, Wolf et al. 1999, Kondoh 2003, Odling-Smee et al. 2003, Laland 2004, Beckerman et al. 2006, Montoya et al. 2006, Laland and Boogert 2008). This can be regarded in the light of 'community heritability' (see Whitham et al. 2003, 2006), and as articulated by Goodnight (1990): "...if the interactions among the members of the community are passed intact from the parent community, the interaction will be heritable at the community level, and thus contribute to a response to community selection". Therefore, species interactions can be predictable (Bailey et al. 2006; see also Wolf et al. 1999, Whitham et al. 2003).

Community genetics and mediated effects

Host plants and their associated herbivorous insects reciprocally influence one another (Strauss 1991, Jones et al. 1994, Miller and Travis 1996, Jones et al. 1997, Wright et al. 2002, Odling-Smee et al. 2003, Whitham et al. 2003, 2006, Mooney and Agrawal 2008). Such interplay takes place via direct and indirect pathways (Strauss 1991, Wootton 1994, Moore et al. 1997, Wootton 2002, Mooney and Agrawal 2008). Indirect effects arise when the interactions between inter-players are conveyed via the mediation of a 'vehicle' or an 'interactor' (Dawkins 1982, Jablonka 2004). This vehicle can be:

1) A third intervening species (e.g. IEEs; Wootton 1994, Astles et al. 2005).

2) A single genotype (e.g. Intra-Specific Indirect Genetic Effects, IIGEs; Moore et al. 1997).

3) Genetics of conspecifics or heterospecifics (e.g. Indirect Inter-Specific Genetic Effects, IIGEs; Wolf et al. 1998, Wolf 2000, but see Shuster et al. 2006).

An interesting example of indirect ecological effects is explained by the interactions between above- and below-ground herbivory that are mediated by host plant (Kaplan et al. 2009). This interaction entails feedback loops, both negative and positive, that spring up between root- and shoot-communities that never meet (Kaplan et al. 2008, 2009, Moens 2009).

A good body of evidence has accrued to support the supposition that the transmission of indirect effects through trophic levels is influenced by genetic variation within species (Bailey et al. 2006, Whitham et al. 2006). It can be argued that an interaction between two genotypes (G×G) co-varies with the environmental factors to which the two species are subject (Astles et al. 2005). Community genetics research endeavours to explore how within-species genetic variation and inter-specific genetic interactions can modulate the impact of indirect effects (e.g. Astles et al. 2005). However, there is a heightened need to investigate how community genetics effects interact with IEEs and IGEs within economic crop-dependent faunal community systems.

Doctoral work

The thesis of my work is that within-species genetic variation influences species interactions and *vice-versa*. To test this I define and use a variety of systems, both simple agricultural systems and complex natural rainforest systems. I especially focus on the importance of the mediating effects of genetic variation on competition. I also focus on the influence of indirect effects on the relationship between intra-specific genetic variation and species interaction. I provide evidence of the relative importance of CG effects relative to other ecological forces such as competition.

The research I have conducted throughout my doctoral studies involved laboratory and greenhouse experimental work at the University of Manchester facilities besides field work in the tropics of Central America (Belize).

Spotlight on the studies comprising this thesis

Chapter1: Article for *Ecological Monographs*

Broad bean Varieties Differentially Impact the Performance of Aphid Genotypes

Mouhammad Shadi Khudr[1], Tomos Potter[1], Richard Preziosi[1]

[1]Faculty of Life Sciences, The University of Manchester

Synopsis

This work presents a novel model with high potential for community genetics research. The model assembles a matrix of four genotypes of broad bean as provision for six aphid genotypes (four green and two pink) in addition to vetch aphid and green peach aphids (one genotype of each). Infestations for each cultivar were replicated in enclosures within controlled growth cabinets (microcosms). Aphid performance (reproductive success measured through population growth rate per capita) and behaviour (preference for on-plant micro-feeding sites) were investigated. An assessment of aphid impact on plant fitness was also conducted. Interesting results were obtained pertaining to the influence of the interaction between host plant and pest genotypes. I found strong effects of broad bean genotype, aphid genotype and their interaction on traits of both aphids and broad beans. The influence of host plant genotype on aphid behaviour was clearly notable, as was the interaction between plant genotype and aphid genotype. The model system defined in this work provides novel

insights on aphid response to community genetic effects that can be applied to pest control efforts in agricultural systems.

Chapter Two: Article for *Animal Ecology*

Community Genetic Effects versus Competition Effects in a Model Pea aphid System

Mouhammad Shadi Khudr[1], Tomos Potter[1], Richard Preziosi[1]

[1]Faculty of Life Sciences, The University of Manchester

Synopsis

This work addresses questions on the interaction between community genetic effects (broad bean intra-specific genetic variation) and competition as an important eco-evolutionary force. The work investigated a plausible modulation of the influence of broad bean genotype (community genetic effect) on the performance (reproductive success) and behaviour (on- and in-between plant distribution) of a focal pink aphid genotype. The consistency of performance and behaviour of this aphid was assessed against four green conspecifics and two heterospecifics. Three broad bean cultivars were used in both choice and no-choice enclosures under stabilised temperate conditions. The research included three experimental protocols:

1) Choice, presence and absence of a competitor.

2) No choice, inter-specific competition.

3) No choice, intra-specific competition.

The results of this work indicated that the effects of inter-specific competition and community genetics on aphid behaviour are of a similar magnitude. The findings

contribute towards ongoing efforts to untangle the dynamics of the relationship between intra-specific genetic variation of host plants and pest-pest interactions in agricultural systems.

Chapter three: Article for *Biology Letters*

Parasitoid wasps influence where aphids die via an inter-specific indirect genetic effect.

Mouhammad Shadi Khudr[1], Johan Oldekop[1], David M. Shuker[2], Richard Preziosi[1]

[1]Faculty of Life Sciences, The University of Manchester

[2]School of Biology, The University of St. Andrews, Harold Mitchell Building, St Andrews, Fife, KY16 9TH, U.K of Life Sciences, The University of Manchester

Synopsis

A quantitative genetic half-sib experimental design of the parasitoid wasp (*Aphidius ervi*) was used to investigate the genetic variation basis of the manipulation of aphid host by the parasitoid in terms of the behaviour of aphids post-parasitism. It is demonstrated in this study that different genotypes of the parasitoid wasp cause differential responses in the genetically identical aphids under attack. Different sire families of the parasitoid exerted differential influence on their pea aphid hosts. This work integrates biological control schemes into community genetics, providing evidence for a genetic basis for species interactions on economic crops.

Chapter four: Article for *Oikos*

Plant Community Genetic Effect Mediates Spatially Separated Competition

Mouhammad Shadi Khudr[1], Nouri Baker[1], Richard Preziosi[1]

[1]Faculty of Life Sciences, The University of Manchester

Synopsis

Little is known about how effects between above- and below-ground levels of herbivory are mediated by plant genetic variation. Investigation of the interaction between shoot and root sap-feeding pests that never meet is of vital importance for understanding how a host plant mediates the interaction between spatially separated plant consumers. This work investigated the effects of multi-level herbivory on host plant vigour in the light of within-plant genetic variation and competition between two aphid species (focal green peach aphid and black bean aphid) and root-knot nematodes (RKN). In addition, the work explored the effects of the interaction between the indirect ecological effect (impact of RKN) and the community genetic effects (tomato intra-specific variation) on shoot-feeders. The influence of RKN on the performance (reproductive success) and behaviour (on-plant distribution) of the focal aphid genotype across six tomato cultivars was observed. The impact of above-ground herbivory on the abundance of RKN was also explored.

The results show that plant biomass depended not only on the plant genotype but also on the differential response of plant cultivar to herbivory. A highly significant influence of the interaction between tomato genotype and competition on the focal aphid genotype was recorded. Moreover, the nematodes (RKN) were influenced by competition with the aphids on the host plant but the influence was dependent on the tomato genotype mediating the interaction. The findings highlight the importance of

unraveling the interwoven branches of ecology and evolution of plant-pest dynamics in order to understand the possible modulation mechanisms that occur between intra-specific genetic variation and spatially separated competition within agro-ecosystems.

Chapter five: Article published in *Oecologia* 2012

Community genetic effects of tank-forming bromeliads on associated invertebrates in a tropical forest ecosystem

Sharon Zytynska[1], Mouhammad Shadi Khudr[1], Edwin Harris[1], Richard Preziosi[1]

[1]Faculty of Life Sciences, The University of Manchester

[2]John Dalton Building, Manchester Metropolitan University, M1 5GD, UK

Synopsis

This paper represents the tropical field work element of my research (Belize, Central America). The work was conducted in the summer (July) for three consecutive years (2008-2010). Epiphytic tank-forming bromeliads were collected on a fine geographic scale within three distinct sites in Chiquibul forest, Belize. Geographic data on height and location above sea level were recorded. Subsequently, geometric morphological data of the plants were obtained before they were dismantled leaf by leaf, with the associated invertebrate communities collected and systematically classified and categorised according to age (adults and juveniles). In parallel, DNA AFLP-fingerprinting was performed and differences amongst the plant samples collected were undertaken. Afterwards, correlation analyses between genetic, species and geographic data were processed.

The major findings indicated that the genetic variation within the bromeliad species did influence the associated invertebrate communities. Relatively, the overall species diversity the bromeliads harboured was contingent on the genetic variation of the bromeliads. In addition, more genetically similar bromeliads hosted similar invertebrate communities. This finding was statistically significant for the juvenile-stage invertebrates but not for the adults.

The findings suggest that within-species genetic variation in ecologically important species may constitute a supporting ecosystem service for maintaining community and ecological processes.

Concluding remarks

Through my research to date I infer that ecological communities are not only assemblages of species but are also 'spatiotemporal dynamic matrices' that develop and evolve in accordance with the rates of development of their species and the interactions between species. Such context can result from the numerous inherent interactions among the constituent species that form an integrated community (Paine 1963, Pimm and Lawton 1977, Paine 1980, Montoya and Solé 2002, Odling-Smee et al. 2003, Montoya et al. 2006, Whitham et al. 2006). Nevertheless, the integration is always subject to change at any level of its organisation (Levin 1998, Simberlof 1998, Odling-Smee et al. 2003, Juarrero 2010) as part of the bigger co-evolutionary mosaic (Thompson 1994, 1999). Therefore, communities can be defined by the internal as well as the external properties of their constituents and by the interactions between these constituents and the environment in which they cohabit (Levin 1998, King 2003, Odling-Smee et al. 2003, Whitham et al. 2003, Bonchev et al. 2005, Montoya et al. 2006, Whitham et al. 2006, Juarrero 2010). An example of this is the beaver, which

through its daily activities ecologically and genetically modifies its shared environment with other taxa (Bailey et al. 2004, Bailey and Whitham 2006b). The beaver also undergoes an ongoing process of cyclical interaction with the selective environment affecting other species, including even the beaver itself (Jones et al. 1994, 1997, Odling-Smee et al. 2003, Laland 2004, Brodie 2005, Bailey and Whitham 2006a,b; see also Lawrence et al. 2012).

My consensus is that community genetics, through its numerous and amalgamated perspectives, functions holistically just as Newton's colour-disc that blends light (here the inputs of pertinent CG perspectives) into a synthesis. At the same time, community genetics is also a form of a 'reductionist toolkit', analogous to the prism splitting light, through its analytical function, which elucidates the individual components of the unified whole.

My research and conclusions concord with the understanding that "Genetically based interactions among individuals of different species exist regardless of the unit at which selection is occurring; however, these interactions become heritable variation only when selection is acting at the community level" as articulated by Goodnight (1990).

References

Abrams, P. A. (1995). Implications of dynamically variable traits for identifying, classifying, and measuring direct and indirect effects in ecological communities. *American Naturalist*, *146*, 112-134.

Agrawal, A. A. (2003). Community genetics: New insights into community ecology by integrating population genetics. *Ecology*, *84*, 543-544.

Antonovics, J. (1992). Toward community genetics. In R. S. Fritz, & E. L. Simms (Eds.), *Ecology and Evolution of Plant Resistance to Herbivores and Pathogens: Ecology, Evolution, and Genetics* (pp. 426-449). USA: University Of Chicago Press.

Araújo, M. S., Guimarães, P. R., Svanbäck, R., Pinheiro, A., Guimarães, P., Dos Reis, S. F., & Bolnick, D. I. (2008). Network analysis reveals contrasting effects of intraspecific competition on individual vs. population diets. *Ecology*, *89*(7), 197-213.

Astles, P. A., Moore, A. J., & Preziosi, R. F. (2005). Genetic variation in response to an indirect ecological effect. *Proceedings of the Royal Society B*, *272*, 2577-2581.

Bailey, J. K., & Whitham, T. G. (2006a). Biodiversity is related to indirect interactions among species of large effect. In T. Ohgushi, T. Craig, & P.W. Price (Eds.), *Indirect interaction webs: Nontrophic linkages through induced plant traits* (pp. 306-328). UK: Cambridge University Press. Retrieved from http://web.utk.edu/~jbaile29/publications/Bailey%20and%20Whitham%20indi rect%20interaction%20webs.pdf

Bailey, J. K., & Whitham, T. G. (2006b). Indirect trait-mediated interactions between cottonwoods and beavers positively affect sawfly abundance. *Ecological Entomology*, *31*, 294-297.

Bailey, J. K., Hendry, A., Kennison, M., Post, D., Palkovacs, E., Pelletier, F., Harmon, L., & Schweitzer, J. A. (2009). From genes to ecosystems: An emerging synthesis of eco-evolutionary dynamics. *New Phytologist*, *184*, 746-749.

Bailey, J. K., Schweitzer, J. A., Rehill, B. J., Lindroth, R. L., Martinsen, G. D., & Whitham, T. G. (2004). Beavers as molecular geneticists: A genetic basis to the foraging of an ecosystem engineer. *Ecology*, *85*, 603-608.

Bailey, J. K., Wooley, S. C., Lindroth, R. L., & Whitham, T. G. (2006). Importance of species interactions to community heritability: A genetic basis to trophic-level interactions. *Ecology Letters*, *9*, 78-85.

Bangert, R. K., Turek, R. J., Rehill, B., Allan, G. J., Wimp, G. M., Schweitzer, J. A., Bailey, J. K., Martinsen, G., D., Keim, P., Lindroth, R., L., Whitham, T. G. (2006). A genetic similarity rule determines arthropod community structure. *Molecular Ecology*, *15*, 1379-1392.

Beckerman, A. P., Petchey, O. L., & Warren, P. H. (2006). Foraging biology predicts food web complexity. *Proceedings of the National Academy of Sciences*, *37*, 13745-13749.

Bonchev, D., Rouvray, D. H., & Mezey, P. G. (2005). *Complexity in chemistry, biology, and ecology.* London: Springer.

Brodie, E. D. (2005). Caution: Niche construction ahead. *Evolution*, *59*(1), 249-251.

Canadian Ministry of Natural Resources. (2005, April). Ontario biodiversity strategy. Retrieved from http://www.ontla.on.ca/library/repository/mon/10000/252160.pdf

Dawkins, R. (1976). *The selfish gene.* New York City: Oxford University Press.

Dawkins, R. (1982). The extended phenotype. Oxford: W.H. Freeman And Company.

Driebe, E. M., & Whitham, T. G. (2000). Cottonwood hybridization affects tannin and nitrogen content of leaf litter and alters decomposition. *Oecologia*, *123*, 99-107.

Dungey, M., Potts, H. S., Whitham, T. G., & Li, H. (2000). Plant genetics affects arthropod community richness and composition: Evidence from a synthetic eucalypt hybrid population. *Evolution*, *54*, 1938-1946.

Ferrari, J., & Vavre, F. (2011). Bacterial symbionts in insects or the story of communities affecting communities. *Philos Trans R Soc Lond B Biol Sci*, *366*(1569), 1389-1400.

Ferrari, J., Müller, C. B., Kraaijeveld, A. R. & Godfray, H. C. J. (2001). Clonal variation and covariation in aphid resistance to parasitoids and a pathogen. *Evolution*, *55* (9), 1805-1814.

Fordyce, J. A. (2006). The evolutionary consequences of ecological interactions mediated through phenotypic plasticity. *The Journal of Experimental Biology*, *209*, 2377-2383.

Fritz, R. S., Roche, B. M., Brunsfeld, S. J., & Orians, C. M. (1996). Interspecific and temporal variation in herbivore responses to hybrid willows. *Oecologia*, *108*, 121-129.

Goodnight, C. J. (1990). Experimental studies of community evolution I: The response to selection at the community level. *Evolution*, *44*(6), 1614-1624.

Henter, H. J., & S. Via. (1995). The potential for coevolution in a host-parasitoid system. I. Genetic variation within an aphid population in susceptibility to a parasitic wasp. *Evolution*, *49*(3), 427-438.

Hersch-Green, E. I., Turley, N. E., & Johnson, M. T. J. (2011). Community genetics: What have we accomplished and where should we be going? *Philosophical Transactions of the Royal Society B-Biological Sciences*, *366*, 1453-1460.

Ito, M., & Ozaki, K. (2005). Response of a gall wasp community to genetic variation in the host plant *Quercus crispula*: A test using half-sib families. *Acta Oecologica*, *27*, 17-24.

Jablonka, E. (2004). From replicators to heritably varying phenotypic traits. the extended phenotype revisited. *Biology & Philosophy*, *19*, 353-375.

Johnson, M. T. J. (2008). Bottom-up effects of plant genotype on aphids, ants and predators. *Ecology*, *89*, 145-154.

Johnson, M. T. J., & Agrawal, A. A. (2005). Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (*Oenothera biennis*). *Ecology*, *86*, 874-885.

Johnson, M. T. J., & Stinchcombe, J. R. (2007). An emerging synthesis between community ecology and evolutionary biology. *Trends in Ecology and Evolution*, *22*, 250-257.

Jones, C. G., Lawton, J. H., & Shachak, M. (1994). Organisms as ecosystem engineers. *Oikos*, *69*, 373-386.

Jones, C. G., Lawton, J. H., & Shachak, M. (1997). Positive and negative effects of organisms as physical ecosystem engineers. *Ecology*, *78*, 1946-1957.

Juarrero, A. (2010). Complex dynamical systems theory. "Cognitive Edge Network". Retrieved from http://cognitive-edge.com/uploads/articles/100608%20Complex_Dynamical_Systems_Theory.pdf

Kaplan, I., & Denno, R. F. (2007). Interspecific interactions in phytophagous insects revisited: A quantitative assessment of competition theory. *Ecology Letters*, *10*(10), 977-994.

Kaplan, I., Halitschke, R., Kessler, A., Sardanelli, A., & Denno, R. F. (2008). Constitutive and induced defenses to herbivory in above- and belowground plant tissues. *Ecology*, *89*(2), 392-406.

Kaplan, I., Sardanelli, S., & Denno, R. F. (2009). Field evidence for indirect interactions between foliar-feeding insect and root-feeding nematode communities on *Nicotiana tabacum*. *Ecol. Entomol, 34*, 262-270.

King, C. C. (2003). Biocosmology 2: Evolutionary epoch: Complexity, chaos and complementarity. *340*. Retrieved from http://www.dhushara.com/pdf/biocos2.pdf

Kondoh, M. (2003). Foraging adaptation and the relationship between food-web complexity and stability. *Science, 299*, 1388-1391.

Laland, K. N. (2004). Extending the extended phenotype. *Biology and Philosophy*, *19*, 313-325.

Laland, K. N., & Boogert, N. J. (2008). Niche construction, co-evolution and biodiversity. *Ecol Econ*, *69*(4), 731-736.

Lawrence, D., Fiegna, F., Behrends, V., Bundy, J. G., Phillimore, A. B., Bell, T., & Barraclough, T. G. (2012). Species interactions alter evolutionary responses to a novel environment. *PLoSBiol*, *10*(5): e1001330. (doi:10.1371/journal.pbio.1001330)

Levin, S. A. (1998). Ecosystems and the biosphere as complex adaptive systems. *Ecosystems*, *1*, 431-436.

Mejer, H. F., & Jørgensen, S. E. (1979). Energy and ecological buffer capacity, State-of-the-art of ecological modelling. In: S.E. Jørgensen, (Ed.), Environmental

sciences and applications, Proc. 7th Conf. Ecological modelling, 28 august–2 September 1978, Copenhagen international society for ecological modelling, Copenhagen.

Miller, T. E., & Travis, J. (1996). The evolutionary role of indirect effects in communities. *Ecology, 77*(5), 1329-1335.

Moens, M., Perry, R., & Starr, J. L. (2009). *Meloidogyne species* – a diverse group of novel and important plant parasites. *Root Knot Nematodes.* Wallingford, UK: CABI Publishing CAB International.

Montoya, J. M., & Solé, R. V. (2002). Small world patterns in food webs. *J. Theor. Biol., 214*(3), 405-412.

Montoya, J. M., Solé, R. V., & Pimm, S. L. (2006). Ecological networks and their fragility. *Nature, 442,* 259-264.

Mooney, K. A., & Agrawal, A. A. (2008). Plant genotype shapes ant-aphid interactions: Implications for community structure and indirect plant defense. *American Naturalist, 171*(6), E195-E205. Retrieved from http://www.bio-nica.info/biblioteca/Mooney2008Formicidae.pdf

Mooney, K. A., Jones, P., & Agrawal, A. A. (2008). Coexisting congeners: Demography, competition, and interactions with cardenolides for two milkweed-feeding aphids. *Oikos, 117,* 450-458.

Moore, A. J., Brodie, E. D., & Wolf, J. B. (1997). Interacting phenotypes and the evolutionary process: I. Direct and indirect genetic effects of social interactions. *Evolution, 51*(5), 1352-1362.

Neuhauser, C., Andow, D. A., Heimpel, G. E., Ruth, G. M., Shaw, G., & Wagenius, S. (2003). Community genetics: Expanding the synthesis of ecology and genetics. *Ecology, 84*(3), 545-558.

Odling-Smee, F. J., Laland, K. N., & Feldman, M. W. (2003). *Niche construction-the neglected process in evolution.* Princeton, NJ: Princeton University Press.

Paine, R. T. (1963). Trophic relationships of 8 sympatric predatory gastropods. *Ecology*, *44*, 63-73.

Paine, R. T. (1980). Food webs: linkage, interaction strength and community infrastructure, the third Tansley lecture. *J. Anim. Ecol.*, *49*, 667-685.

Pelletier, F., Garant, D., & Hendry, A. P. (2009). Eco-evolutionary dynamics. *Philos Trans R Soc Lond B Biol Sci*, *364*(1523), 1483-1489.

Pfennig, D. W., & McGee, M. (2009). Resource polyphenism increases species richness: A test of the hypothesis. *Phil. Trans. R. Soc. B*, *365*(1540), 577-591.

Pimm, S. L., & Lawton, J. H. (1977). Number of trophic levels in ecological communities. *Nature*, *268*(3), 329-331.

Robinson, C. J., Bohannan, B. J. M., & Young, V. B. (2010). From structure to function: The ecology of host-associated microbial communities. *Microbiol. Mol. Biol. Rev.*, *74*(3), 453-476. (doi: 10.1128/MMBR.00014-10)

Rowntree, J. K., Shuker, D. M., & Preziosi, R. F. (2011). Forward from the crossroads of ecology and evolution. *Phil. Trans. R. Soc. B*, *366*(1569), 1322-1328.

Schoener, T. W. (2011). The newest synthesis: Understanding the interplay of evolutionary and ecological dynamics. *Science*, *331*(6016), 426-429.

Schweitzer, J. A., Bailey, J. K., Hart, S. C., & Whitham, T. G. (2005). Nonadditive effects of mixing cottonwood genotypes on litter decomposition and nutrient dynamics. *Ecology*, *86*(10), 2834-2840.

Schweitzer, J. A., Bailey, J. K., Rehill, B. J., Martinsen, G. D., Martinsen, S. C., Hart, R., Lindroth, L., Keim, P. , & Whitham, T. G. (2004). Genetically based trait in a dominant tree affects ecosystem processes. *Ecology Letters*, *7*, 127-134.

Service, P. (1984). Genotypic interactions in an aphid-host plant relationship: *Uroleucon rudbeckiae* and *Rudbeckia laciniata*. *Oecologia*, *61*(2), 271-276.

Shuster, S. M., Lonsdorf, E. V., Wimp, G. M., Bailey, J. K., & Whitham, T. G. (2006). Community heritability measures the evolutionary consequences of indirect genetic effects on community structure. *Evolution*, *60*, 146-158.

Simberloff, D. (1998). Flagships, umbrellas, and keystones: Is single-species management passé in the landscape era? *Biological Conservation*, *83*(3), 247-257.

Smith, R. A., Mooney, K. A., & Agrawal, A. A. (2008). Coexistence of three specialist aphids on common milkweed, *Asclepias syriaca*. *Ecology*, *89*(8), 2187-2196.

Strauss, S. Y. (1991). Indirect effects in community ecology their definition study and importance. *Trends in Ecology and Evolution*, *6*, 206-210.

Tétard-Jones, C., Kertesz, M. A., Gallois, P., & Preziosi, R. F. (2007). Genotype-by-genotype interactions modified by a third species in a plant–insect system. *Am Nat*, *170*, 492-499.

Thompson, J. N. (1994). *The coevolutionary process*. Chicago: University Of Chicago Press.

Thompson, J. N. (1999). The evolution of species interactions. *Science*, *284*(5423), 2116-2118.

Ulanowicz, R. E. (2005). Fluctuations and order in ecosystem dynamics. *E:CO*, *7*(2), 14-20.

Underwood, N., & Rausher, M. D. (2000). The effects of host–plant genotype on herbivore population dynamics. *Ecology*, *81*, 1565-1576.

Utsumi, S., Ando, Y., & Miki, T. (2010). Linkages among trait-mediated indirect effects: A new framework for the indirect interaction web. *Population Ecology*, *52*(4), 485-497.

Wade, M. J. (2003). Community genetics and species interactions. *Ecology, 84*, 583-585.

Whitham, T. G., Bailey, J. K., Schweitzer, J. A., Shuster, S. M., Bangert, R. K., LeRoy, C. J., Lonsdorf, E. V., Allan, G. J., DiFazio, S. P., Potts, B. M., Fischer, D. G., Gehring, C. A., Lindroth, R. L., Marks, J. C., Hart, S. C., Wimp, G. M., & Wooley, S. C. (2006). A framework for community and ecosystem genetics: From genes to ecosystems. *Nat Rev Genet, 7*, 510-523.

Whitham, T. G., DiFazio, S. P., Schweitzer, J. A., Shuster, S. M., Allan, G. J., Bailey, J. K., & Woolbright, S. A. (2008). Extending genomics to natural communities and ecosystems. *Science, 320*, 492-495.

Whitham, T. G., Young, W. P., Martinsen, G. D., Gehring, C. A., Schweitzer, J. A., Shuster, S. M Wimp, G. M., Fischer, D. G., Bailey, J. K., Lindroth, R. L., Woolbright, S. , & Kuske, C. R. (2003). Community and ecosystem genetics: A consequence of the extended phenotype. *Ecology, 84*, 559-573.

Wimp, G. M., Martinsen, G. D., Floate, K., Bangert, R. K., & Whitham, T. G. (2005). Plant genetic determinants of arthropod community structure and diversity. *Evolution*, *59*(1), 61-69.

Wimp, G. M., Wooley, S., Bangert, R. K., Young, W. P., Martinsen, G. D., Keim, P., Rehill, B., Lindroth, R. L., & Whitham, T. G. (2007). Plant genetics predicts intra-annual variation in phytochemistry and arthropod community structure. *Mol Ecol, 16*, 5057-5069.

Wolf, B. J. (2000). Indirect genetic effects and gene interactions. In B. J. Wolf, E. D. Brodie, & M. J. Wade (Eds.), *Epistasis and the Evolutionary Process* (pp. 158-176). New York: Oxford University Press.

Wolf, J. B., Brodie, E. D., & Moore, A. J. (1999). Interacting phenotypes and the evolutionary process II. Selection resulting from social interactions. *Am Nat*, *153*, 254-266.

Wolf, J. B., Brodie, E. D., Cheverud, J. M., Moore, A. J., & Wade, M. J. (1998). Evolutionary consequences of indirect genetic effects. *Trends in Ecology & Evolution*, *13*, 64-69.

Wootton, J. T. (1994). The nature and consequences of indirect effects in ecological communities. *Annual Review of Ecology and Systematics*, *25*, 443-466.

Wootton, J. T. (2002). Indirect effects in complex ecosystems: Recent progress and future challenges, journal of sea research. *Journal of Sea Research*, *48*(1), 157-172.

Wright, J. P., Jones, C. G., & Flecker, A. S. (2002). An ecosystem engineer, the beaver, increases species richness at the landscape scale. *Oecologia*, *132*, 96-101.

Zytynska, S. E., Fay, M. F., Penney, D., & Preziosi, R. F. (2011). Genetic variation in a tropical tree species influences the associated epiphytic plant and invertebrate communities in a complex forest ecosystem. *Phil. Trans. R. Soc. B*, *366*(1569), 1329-1336.

Zytynska, S. E., Khudr, M. S., Harris, E., & Preziosi, R. F. (2012). Genetic effects of tank-forming bromeliads on the associated invertebrate community in a tropical forest ecosystem. *Oecologia*. (In press). Retrieved from http://www.springerlink.com/content/p700k6v52tt82xr7/fulltext.pdf

Eco-Evolutionary Dynamics

Extended Phenotypes

Intra- and Inter- Community feedback loops

Community Heritability

Perturbation

Pairwise and Diffuse (Co)Evolution

Anthropogenic Effect

Mechanistic reading for the influence of intra-specific genetic variation on community structure and composition

Reciprocal influence between species interactions and intra-specific genetic variation of keystone species

How genetic variation and diversity affect ecosystem processes and services

Can evolution occur on an ecological time scale within communities prone to disequilibrium?

Ecology

Evolution

Community Genetics

Genetics

Within communities, is a higher degree of genetic diversity a bonus for persistence vs. genetic uniformity?

IEEs

Indirect Ecological Effects

Inter-specific

Intra-specific

IGEs

Indirect Genetic Effects

To contrast CG effects with other eco-evolutionary processes such as competition

'Holistic + Reductionist' amalgamative approach

To bridge empirical and theoretical perspectives in evolutionary ecology

Mutualism

Trophic Relationships

Symbiosis

Competition

Parasitism

Parasitoidism

Predation

Niche Construction Theory

Population Genetics

Theory of Co-evolution

Community Ecology

Evolutionary Ecology

Figure 1`: At the interface of genetics, evolution and ecology.

This diagram illustrates the major points of focus within community genetics (CG). The field has burgeoned since founded in 1992 by Antonovics based on the advances in population genetics and community ecology (Agrawal 2003, Rowntree et al. 2011). The summary of CG scope and themes depicted here are derived and adapted from [*Ecology* special feature edited by Agrawal (2003), Johnson and Stinchcombe (2007), and the *Philosophical Transactions of The Royal Society B* theme issue compiled and edited by Rowntree et al. (2011)]. CG shares some common factors with similar schools of thought such as niche construction and eco-evolutionary dynamics (see thesis conclusion for further remarks on CG and niche construction).

Community genetics proposes that a significant proportion of each species' environment is defined by the relative expression of other species networking with this particular species

(Rowntree et al. 2011, Zytynska et al. 2011)

Species assemblage

P = Genotype + Environment + (Genotype x Environment)

$P = G_1 + E + G_1 \times E$

$E = E_A + E(P_2)$

$P_2 = G_2 + E + G_2 \times E$

Biotic Environment = Phenotype of other interacting organisms

$P = G_1 + G_2 + G_1 \times G_2 + E$

Constant reciprocal change between the environment and the inter-players embedded in that very environment

(Odling-Smee et al. 2003, Laland and Boogert 2008)

Resilience
Fitness
Existence
Change
Persistence
Resistance
Change

Functionality

(Bailey and Whitham 2006b, Laland and Boogert 2008, Juarrero 2010, Robinson et al. 2010, Zytynska et al. 2012)

Structure

Composition

Membership

Linkage
Geographical
Species
Genetic

(Zytynska et al. 2012)

Ecosystem engineers
(Jones et al. 1997, Bailey and Whitham 2006b)

Species association

Species Interactions

IEEs

IGEs

Communities within communities

DNA Fingerprinting

Community

(Antonovics 1992, Whitham et al. 2003, 2006)

Intra-specific genetic variation

Biodiversity

Alpha
Within one location

Beta
'Mediatory' between alpha and gamma

Gamma
Amongst locations

(Robinson et al. 2010)

(Wootton 1994, Moore et al. 1997, Rowntree et al. 2011)

(Whitham et al. 2003, Bailey and Whitham 2006a,b, Whitham et al. 2006, Zytynska et al. 2012)

Definition

"Biodiversity is the variability among living organisms from all sources, including inter-alia [among other things], terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are a part; this includes diversity within species, between species and of ecosystems."

Canadian Ministry of Natural Resources; [Ontario's Biodiversity Strategy (OBS); 2005]

43

Figure 2`. A bird's eye view on biodiversity, ecological communities and species interactions in the light of community genetics (CG).

This illustration is based on concepts derived from the works proposed by Thompson (1994, 1999), Wade (2003), Astles et al. (2005), Bonchev et al. (2005), Johnson and Stinchcombe (2007), and Rowntree et al. (2011), and the works cited in the figure. This is besides the *Ecology* special feature edited by Agrawal (2003). This overview emphasises the amalgamated perspective of CG including:

1) The 'quantitative' approach (e.g. Zytynska et al. 2011). As the equation displays, the environment (E) of a species can be defined as abiotic (A) and biotic [the effect of the genetics of other interacting species on the phenotype of a focal species (P)].

2) The 'dominant species approach' proposing that a significant portion of a species' environment is the expressed genomes of other interacting species (Rowntree et al. 2011). The extended phenotypes emerge when the genes of a keystone species (e.g. epiphytic bromeliads) affect associated faunal communities in the canopy beyond the individual and population levels of the epiphyte (Whitham et al. 2003, 2006, Zytynska et al. 2012).

This view also emphasises that species do not exist, develop and interact in a vacuum. Therefore, beyond the limitation of typological definitions of the putative interactions within and between species such as +, -, 0 or neutrality, a complexity of interactions and multi-level interplay can occur between species and their changeable environment in terms of Genotype × Genotype, Genotype × Genotype × Environment, Indirect Ecological Effects (IEEs), and Indirect Genetic Effects (IGEs); (Strauss 1991, Wootton 1994, Moore et al. 1997, Wolf et al. 1999, Wolf 2000, Wootton 2002, Odling-Smee et al. 2003, Whitham et al. 2003, Bailey and Whitham 2006a,b, Whitham et al. 2006).

*The vector images adapted in the illustration are free images to use under 'Creative Commons Attribution-Share Alike 3.0 License'; available at:

http://www.freevector.com/tree-bush-silhouettes

http://www.freevector.com/trees-vector-graphics/

http://www.freevector.com/free-insects-vectors/

Chapter 1: Broad bean varieties differentially impact the performance of aphid genotypes

My Contribution:

I designed and ran the experiment, collected, entered and carried out analysis and visualisation of the data, and produced the manuscript in collaboration with and under Dr. Richard Preziosi's supervision. Mr. Tomos Potter took part in maintenance and data recording, and analysed a portion of the data set as a part of his final year honours undergraduate project.

Article for *Ecological Monographs*

Broad bean varieties differentially impact the performance of aphid genotypes

Mouhammad Shadi Khudr[1], Tomos Potter[1], Richard Preziosi[1]

[1]Faculty of Life Sciences, The University of Manchester

Abstract

We can describe the relationship between leguminous plants and aphid herbivores as an evolutionary arms-race. However, little is known about how genetic variation within plants and aphids influence the outcome of their interactions. This work uses a community genetics approach to explore how population growth rates and distribution patterns of different species and genotypes of aphids are influenced by the genotypic variation of the legume they infest. We also examine how these inter-specific genotypic interactions influence host plant fitness. The model system defined in this study provides novel insights on performance and behavioural responses to community genetic effects.

Key words:

Aphid performance, aphid behaviour, community genetics, within-species genetic variation, broad bean cultivars.

Introduction

Community genetics, first articulated by Antonovics (1992), combines the investigation of the ecology and evolution of interacting species in order to explore

how within-species genetic variation influences species interactions and ecological assemblages. This research paradigm provides a frame of reference and a toolkit in order to determine whether the expressed genomes of interacting species, along with abiotic environmental factors, determine significant proportions of other species' environments (Rowntree et al. 2011). To such an extent, intra-specific genetic variation functions beyond the individual and population level (Whitham et al. 2003, 2006), and consequently affects community structure and ecosystem processes (Whitham et al. 2003, Schweitzer et al. 2004, Schweitzer et al. 2005, Whitham et al. 2006).

The theory of community genetics allows evolutionary ecologists to amalgamate opinions from related theories such as the niche construction perspective theorised by Lewontin (1983) and elaborated by Odling-Smee et al. (2003). As such, community genetics addresses a set of major ecological questions and brings us towards a better mechanistic understanding of what governs species interactions. An example of this is the case of the North American beaver (*Castor canadensis*) which has been classed as an ecosystem engineer (Jones et al. 1994, 1997). The beaver, through constructing its niche (Odling-Smee et al. 2003), modulates the availability of resources to numerous other organisms by cutting trees, building dams and creating canals and lodges. Furthermore, this ecologically important species shows the ability to selectively harvest specific tree genotypes. The beaver directly and indirectly influences the composition and development of the surrounding forest. Therefore, this species functions as a "molecular geneticist" (Bailey et al. 2004) through the reciprocal direct and indirect interactions between its individuals and their surroundings (Bailey and Whitham 2006). Consequently, the beavers alter the selection acting not only on the organisms exposed directly to the beavers' activities (Laland and Boogert 2008), but also the entire riparian ecosystem (Naiman et al. 1988,

Wright et al. 2002). In this context, the beaver's genotype functions beyond the individual and population level, creating an extended phenotype that affects other species and the entire ecosystem (Whitham et al. 2003). This is ultimately expected to result in community and ecosystem phenotypes that depend on a co-existing set of species (Bangert et al. 2006, Shuster et al. 2006, Whitham et al. 2006).

Empirically, community genetics provides a toolbox of approaches and may utilise conventional and novel experiments and systems. The three main approaches have been defined by Rowntree et al. (2011) as:

a) The extended phenotype approach where the focus is on the consequences of intra-specific genetic variation of fundamental species on ecological communities and ecosystems (e.g. Whitham et al. 2003, Schweitzer et al. 2005, Whitham et al. 2006).

b) The quantitative genetic approach examines how genetic interactions between species (inter-specific genotype by genotype interactions; G x G) can influence each interactors' phenotype (e.g. Zytynska et al. 2011). This interaction is also subject to the effect of environment (biotic and abiotic), e.g. Tétard-Jones et al. 2007, Zytynska et al. 2010), and to species-mediated effects, both genetic (IGEs; Moore et al. 1997; G x G x IIGEs; Wolf 2000, Wolf et al. 1998, Wolf et al. 1999) and ecological (G x G x IEEs; Astles et al. 2005).

c) The ecological and evolutionary approach of Neuhauser et al. (2003) examines how evolution might occur on ecological time scales. Their example looks at how anthropogenic effects may put ecological systems into disequilibrium and cause strong selection and rapid evolutionary response. Neuhauser et al. (2003) argue that neither ecological models nor

evolutionary approaches alone are sufficient to explain the link between within-species variation and community processes.

Defining a novel community genetics model system

As crop damaging pests causing substantial economic losses every year, aphids play an important role within natural and agricultural ecosystems (Oerke et al. 1994, Blackman and Eastop 2000, Van Emden and Harrington 2007). They act as major plant virus-vectors (Van Emden and Harrington 2007). They may also have an effect due to their propensity to multiply very rapidly on susceptible host plants; covering shoots with excreted honeydew and hence hinder photosynthesis and weaken their host plants (Dixon 1977, Hales et al. 1997, Powell et al. 2006). In favourable conditions, aphids reproduce parthenogenetically with populations growing at exponential rates and forming colonies of genetically identical individuals i.e. clones (Dixon 1987, 1998, Blackman and Eastop 2000, Van Emden and Harrington 2007). Despite being genetically identical, individuals within such colonies show high levels of plasticity and polyphenism in response to biotic and abiotic environmental stimuli (Weisser and Stadler 1994, Zera and Denno 1997). However, little is known about the effects of 'within-plant' genetic variation on the performance (reproductive success) and emergence of functional variants of the predominant asexual morphs (apterae) of these pests in temperate regions (but see Dixon 1985, Dombrovsky et al. 2009).

Due to their diet, sap feeding aphids are expected to be notably influenced by the variation in the phenotype of their host plants (Nottingham et al. 1992a,b, Powell et al. 2006, Johnson 2007). In this context, chemical and morphological variability in host plants, resulting from host genetic differences, and the differential response of host genotypes to their environment (which includes the aphids themselves) is expected to influence reproductive success and survivorship of aphids (Journet 1980,

Moran 1981, Service and Lenski 1982, Service 1984). Such an influence can be apparent in aphid performance (e.g. population growth rate or reproductive success) and behaviour (e.g. feeding site preference and aphid distribution on host plant; Journet 1980, Moran 1981, Service 1984; see also Smith and Boyko 2007, Smith et al. 2008). Contrasting intra- and inter-specific performance of naturally co-occurring aphids could provide insights into the way genotypes of a species respond differentially to the expressed genomes of their biotic environment (which, for aphids, is largely defined by their host plants). Therefore, aphids in general, and pea, vetch and green peach aphids in particular, provide excellent models for evolutionary ecological studies (Dixon 1998, Brisson and Stern 2006, Tagu et al. 2008; see also The International Aphid Genomics Consortium 2010). These aphid species are polyphagic, polymorphic, and show preference for specific hosts on which they can achieve higher reproductive rates when other environmental conditions are advantageous (Dixon 1977, Blackman and Eastop 2000).

Broad bean (*Vicia faba*) is an important worldwide crop (Saxena et al. 1993). It has been cultivated for thousands of years since the early Neolithic era (Cole 1970). The most ancient well preserved specimen, found in Syria, goes back to the late 10th millennium B.P. (Tanno and Willcox 2005) from where it probably radiated (see also Cubero and Suso 1981, Duc 1997, Duc et al. 2010). Broad beans are easy to grow, environmentally tolerant (e.g. Loss and Siddique 1997, Link et al. 1999). Broad beans are also beneficial in sustainable agriculture as green manures or cover crops (Duc 1997, HDRA 1998). Because broad beans associate with nitrogen-fixating bacteria (e.g. Jordan 1984) they are utilised as natural soil fertilisers (HDRA 1998; references therein). It has been suggested that the worldwide spread of broad beans (Duke 1981, Bond et al. 1985) is a result of their high level of intra-specific genetic variability (Perrino et al. 1991). Furthermore, broad bean is a globally important component of

human diet and major food industries (Bond et al. 1985, Nachi and Guen 1996). Broad beans are also increasingly being used as animal fodder and silage (e.g. Carpenter and Johnson 1968, Duke 1981, Antongiovanni et al. 2002, Volpelli et al. 2010) and may have potential as a source of medication (e.g. Rabey et al. 1992). Broad beans are also used in fighting erosion, soil improvement and as barriers against weeds and plant disease (e.g. HDRA 1998, Bellotti 2004). There are thousands of broad bean cultivars (genotypes; accessions, Duc et al. 2010) that are recorded and globally maintained (Bond et al. 1985, Perrino et al. 1991, Bond et al. 1994). However, this economic crop is associated with a diverse group of invertebrate pests including pea and vetch aphids along with their natural enemies (Muehlbauer and Tullu 1997, Nuessly et al. 2004).

Factors influencing aphid performance and behaviour

Phenotypic variation of traits in aphids is subject to the influence of the genetic variation in the host plant and the interaction between aphids and their host plants (Service 1984, Tétard-Jones and Leifert 2011, Tétard-Jones et al 2011, Zytynska and Preziosi 2011). There is a well demonstrated correlation between resource (host) quality and aphid population growth (Perrin 1976). However, it can be argued that both aphids and host plants have the capacity to influence (directly or indirectly, reciprocally or diffusely) each other's fitness (Dawkins 1982, Shuster et al. 2006). Within this context, intra-specific genetic variation of focal species can theoretically alter the evolutionary trajectories of associated species (Neuhauser et al. 2003, Whitham et al. 2003), the context and composition of entire ecological communities (Whitham et al. 2003, Johnson and Agrawal 2005, Bailey et al. 2006, Bangert et al. 2006, Whitham et al. 2006, Wimp et al. 2007), and probably the evolution of the entire system through the eco-evolutionary dynamics of its interacting species (e.g. Antonovics 2003, Whitham et al. 2008, Schoener 2011).

To some extent, the genetically determined differences in plant response to the environment maybe due to genes controlling plasticity. However, aphids may respond to environmental change through a combination of acclimatisation and adaptation (Diehl and Bush, 1984, Drés and Mallet 2002, Frantz et al. 2006). This may result in ecological specialisation (Walsh 1864, Muller 1981); a process through which a species shifts to new environments and adapts to novel plant resources in response to host variability (Frantz et al. 2009; but see Hoberg and Brooks 2008).

Janzen (1977) conceptualised the ability of a species to produce highly phenotypically adaptive (or versatile) individuals to ensure species survival. In a review on aphid population structure, Dixon (1985) envisaged a superior genotype of aphids to be the one which can switch on and off the production of functional morphs (e.g. thrifty, winged, braver, suicidal, sexual, asexual) in order to ensure maximal reproductive success not only on a single preferred host but also on a wide array of hosts (i.e. a highly phenotypically plastic clone).

Variation in aphid phenotypes arises through the synergy of a set of factors including; environmental cues (Walters and Dixon 1982, Dixon 1998), plant direct and indirect genetic effects (Tétard-Jones and Leifert 2011, Tétard-Jones et al. 2011), perception cues (e.g. Chapman 2003, Glinwood et al. 2009), density (group) effects (Sutherland 1969), and host plant influence (Tariq et al. 2010). This is besides maternal effects (Schuett et al. 2011, Cote et al. 2010) especially in the context of the 'telescoping of generations' that is common in aphids (Dixon 1987). Given the high level of plasticity aphids are capable of (Dixon 1985, Agrawala 2007, Dombrovsky et al. 2009); such a spectrum of cues is expected to stimulate the formation and generation of temperaments [differential inclinations; (Réale et al. 2007)] at the conspecific as well as heterospecific levels. Prone to the induction by a threat and/or feedback loops, these inclinations can be considered as idiosyncrasies characterising

the differences in preference and performance among genotypic identities belonging to a single species (Réale et al. 2007, Bergmüller and Taborsky 2010, Schuett et al. 2011).

The ability of a clone to produce individuals that vary in their functional ecological roles is at least partially achieved by a remarkable ability of aphids to activate and/or suppress gene expression in response to environmental stimuli (Dixon 1985, Stearns 1989, West-Eberhard 1989, Nijhout 1999, de Vos et al. 2010). Community genetics approaches allow a detailed exploration of this plasticity.

Interestingly, Dombrovsky et al. (2009) showed that molecular diversity (presumably at the level of the proteome or metabolome) can occur, in response to environmental cues, even within a single clone descending from a single aphid female. Morphologically identical, these aphids are different on the molecular level and show significant phenotypic variation in those physiological and behavioural traits that serve to create environmentally induced functional phenotypes a.k.a. 'repertoire' (Dombrovsky et al. 2009).

Here we examine the performance and behaviour of six different genotypes of pea aphid (*Acyrthosiphon pisum* Harris), a single genotype of vetch aphid (*Megoura viciae* Buckton) and a single genotype of green peach aphid (*Myzus persicae* Sulzer) on each of four cultivars (genotypes) of broad bean (*Vicia faba*). We assess the influence of host plant genotype, aphid genotype and species, and the interaction between aphid and host plant in order to explore the underlying community genetic effects in this model system.

Methods

Broad bean cultivars

We used four different cultivars (breeds) of broad beans (*Vicia faba*) of significant commercial importance. A cultivar is, by definition, a horticultural variety that has been deliberately and selectively produced by crossing two different varieties within a species in search for specific desirable characteristics i.e. yield of the crop or disease resistance (e.g. Flores et al. 1998). The new cultivated breeds are synthetics with beneficially significant heterosis (Duc 1997). These lines do not exist in the wild but do in agricultural systems, and are consistently maintained through cultivation for uniformity and agronomic stability (Duc 1997, Flores et al. 1998, Duc et al. 2010). Because of the long-term maintenance for uniformity, cultivars have been inbred for many generations (e.g. 'Aquadulce Claudia' has been maintained since 1850). Thus, genetic variation within cultivars will be vanishingly small compared to genetic variation among cultivars. Therefore, cultivars can be considered the equivalent of a recombinant inbred line that has been maintained for many generations and thus can be treated as a genotype (Cordeiro et al. 1983, Oyedokun 1985, Underwood and Rausher 2000, Sharpe and Lydiate 2003, Emebiri et al. 2005, Hash et al. 2006, Kan et al. 2010, Rose et al. 2010).

Broad bean is an ideal host plant for aphids (Blackman and Eastop 2000) and is easy to maintain in the lab. The cultivars of broad beans used in this work were Masterpiece Green Longpod (C1), Optica (C2), Aquadulce Claudia (C3), and Sutton (C4). These cultivars were obtained from commercial suppliers. Plants were approximately one month old seedlings germinated in a controlled green house facility at the University of Manchester Botanical Research Grounds (in 2008-2009) before being transferred to laboratory growth chambers [22°C and a 16:8 (L:D) photoperiod]. Steam sterilised compost was used as potting material in 6cm diameter x 10cm deep plastic pots. Pots were placed in plastic propagators with mesh windows to maintain proper ventilation.

In order to standardise plant height, plants were cropped consistently to a standard height (12 cm) prior to aphid infestation, which often resulted in multiple stem formation.

Aphid genotypes

We used six genotypes of pea aphid (*Acyrthosiphon pisum* Harris) including four green clones and two pink clones. Five of the genotypes were obtained from Imperial College (London) in 2007; hereafter **G1**, **G2**, **G3**, **G5** and **G6**) while the genotype (**G4**) was reared from a single female collected from the wild using a broad bean plant as a bait near Manchester, UK. Both vetch aphid (*Megoura Viciae* Buckton) and green peach aphid (*Myzus persicae* Sulzer) clones were established from single juvenile collected at an allotment in Manchester, UK for the former and from Buxton, UK for the latter. Clones of the aphid genotypes were maintained in multiple microcosms within growth cabinets under standardised conditions [16:8 (L:D) photoperiod, 16 – 22ºC].

To control for maternal effects we used young nymphs descending from one founder mother for each of the genotypes investigated. Furthermore, stocks of clones were maintained on a broad bean cultivar different from the cultivars used in the experiment to avoid conditioning effects on the aphids.

Experimental units

For each experimental unit, four 1st and 2nd instars of aphids were introduced to the bottom of a pot containing a one month old individual broad bean plant. Note that 1st and 2nd instars were balanced across all treatment replicates. For the vast majority of replicates we introduced two first instars of aphids and two second instars. However, because of the difficulty of distinguishing between first and second instars

of *Myzus persicae* it is possible that some replicates of this species had three first or three second instars.

Plants were covered with plastic sleeves supplied with fine-meshed windows for proper ventilation. Ten replicates of each of the 32 combinations (8 aphid treatments on each of 4 cultivars) were individually and randomly placed in growth cabinets (**Figure 1**). Plants were watered every other day. After fourteen days, plants were divided into three sections (bottom, middle and top thirds) using a ruler and the numbers of aphids, on each section, were counted. We also recorded the number of winged (alate) morphs present. Plants were then harvested, root volumes were estimated and rootlets counted, before plants were divided into roots and shoots. Afterwards, plants were placed in a drying oven at 40ºC for several days before weighing. (Note that using differential plant root system volumes as an indirect fitness measure in order to study aphid impact is not the main focus of this paper and thus data in this regard are not illustrated herein.).

Aphid fitness measures

For the majority of our analyses we used aphid population growth rate as a measure of clonal fitness. For a sexually reproducing species this would confound selection acting on the different genotypes of parents and offspring. However, for a clonally reproducing organism it is the cumulative reproduction for the entire clone that best reflects the fitness of the clonal genotype. In this regard, population growth rates provide a useful measure of fitness (Murray 1985, Murray 1990, Giske et al. 1993, Agrawal et al. 2004). (See the methods in Chapter 2 and the annex to chapter 2 for further details on fitness).

Aphid population growth rates: Relative aphid genotypic reproductive success (population growth rates) was calculated after Agrawal et al. (2004), using the equation:

$$GR = \frac{\left(\ln(N_2) - \ln(N_1)\right)}{t}$$

where **GR** is Population Growth rate, N_1 is the Initial number of aphids, and N_2 is the final clone size at day 14.

Relative polyphenism: (production of alate morphs): Because aphid clones may produce winged individuals in response to stress we also measured the number of winged individuals produced. The rate of the production of winged individuals can also be interpreted as a measure of fitness, although there is more error associated with this estimate because of the naturally low rate at which winged individuals are produced in the absence of stress related to overcrowding, host depletion and presence of natural enemies.

We recorded the number of winged aphids produced at day 14 in each replicate. The number of winged versus wingless aphids can also be interpreted as a measure of phenotypic plasticity (Whitman and Agrawal 2009) or polyphenism. This reflects the relative investment in energetically costly dispersal morphs (production of winged individuals) versus reproduction (wingless and more fecund individuals) as influenced by plant genotype (Groeters and Dingle 1989, Dixon 1998).

Aphid Behaviour

On-plant distribution of aphids was observed as an indication of aphid preference to congregate on specific plant micro-feeding sites (classified as bottom third, middle third, and top third of the host plant). The dilemma between preference

and performance for aphids in confined enclosures is expected to affect the choices of each aphid genotype in terms of reproductive success, and niche specialisation (Bergmüller and Taborsky 2010, Zytynska and Preziosi 2011).

Plant fitness

Our primary measure of plant fitness was total dry weight because it is one of the most reliable measures of inter-cultivar vigour or performance (Silim and Saxena 1992). Shoot-length to root-length percentages were used as secondary measures of plant fitness that reflect the differential investment in growth by plants exposed to a sub-lethal attack.

Statistical analyses

Performance data (growth rate of aphids and dry weights of plants) were analysed using a two-way ANOVA where the first factor was aphid genotype or aphid species (the mean for *A. pisum* genotypes was used for the species level comparisons) and the second factor was broad bean cultivar. To correct for simple plant size effects on aphid number, plant dry weight was included as a covariate in the aphid performance analyses. Behavioural (positional) data for aphids was analysed using a nominal logistic analysis in a two-way ANOVA design. Both aphid genotypes and plant genotypes were treated as random effects. Analyses were carried out using JMP® Version 8. For each response variable we first compared the three aphid species (inter-specific) and then compared the 6 pea aphid genotypes (intra-specific). Illustration of data was carried out using and MS Excel 2003 and Open source SVG graphics editor Inkscape (Ver. 0.48.01 under GPL).

Experimental Design

Confined Resources

Performance (population growth rate) and on plant distribution (behaviour) for 8 Aphid Genotypes
[6 *Acyrthosiphon pisum* (four greens and two pinks) + 2 other species genotypes: (*Megoura viciae* and *Myzus persicae*)]
across four different cultivars of *Vicia Faba*

G3: Green *A.pisum*

G4: Green *A.pisum*

G5: Pink *A.pisum*

G2: Green *A.pisum*

Initial Infection always
with 4 aphids

C 1   C 2   C 3   C 4

10 Isolated Replicates
per Cultivar

G1: Green *A.pisum*

G6: Pink *A.pisum*

**Pea Aphid
Genotypes**

G8: *M.persicae*

G7: *M.viciae*

Green Peach Aphid

Vetch Aphid

G 1   G 8

C 1

Genetically Identical daughters
were randomly selected
from one clone for each of
the genotypes examined

C 4

Random
320 position-matrix of
8 aphid genotypes X 4 broad bean cultivars

Figure 1: Experimental Design. Both genotypes of *Acyrthosiphon pisum* and two different species (*Megoura viciae* and *Myzus persicae*) of aphids were individually raised on four cultivars of *Vicia faba* providing 32 possible combinations of aphid and host genotypes. Ten replicates of each combination were raised simultaneously for a total of 320 plants. The cultivars of broad beans used were Masterpiece Green Longpod (C1), Optica (C2), Aquadulce Claudia (C3), and Sutton (C4). Each replicate was infected with four early instars of the relevant focal aphid genotype at day 1 of the experiment.

Results

Aphid Performance

Analysis of aphid species performance indicated that aphid growth rate was significantly influenced by aphid species ($F_{2,6}$ = 17.95, P = 0.0024) but not by broad bean cultivar ($F_{3,6}$ = 2.67, P = 0.1111) or the interaction of aphid species and broad bean cultivar ($F_{6,273}$ = 0.83, P = 0.5521; **Figure 2**).

In our comparisons of the six pea aphid genotypes, performance was significantly influenced by aphid genotype ($F_{5,15}$ = 6.83, P = 0.0015) and the interaction of aphid and broad bean cultivar ($F_{15,188}$ = 2.96, P = 0.0015) and was marginally significantly influenced by broad bean cultivar ($F_{3,15}$ = 2.59, P = 0.0640; **Figure 2**).

Aphid genotypes showed differential reproductive success across broad bean cultivars with notable superiority of pea aphid **G1**, **G4** and the heterospecific **G7**. They interchangeably shared the three top spots of the three highest population growth rates displayed in (**Table 1**).

Within *A. pisum*, green genotypes showed greater clonal growth rates than pink genotypes (**G5** and **G6**) on average. However, two green genotypes displayed similar performance patterns to those shown by pink genotypes, but the green genotype **G3** was relatively inferior in performance compared to all other pea aphid genotypes. In contrast, inter-specifically, the green peach aphid **G8** showed the lowest performance rank across broad beans (**Table 1**).

Because plant dry weight was used as a covariate in both of these analyses the differences in aphid numbers are not simply a response to plant size and are likely due to some other distinct property that varies among broad bean cultivars.
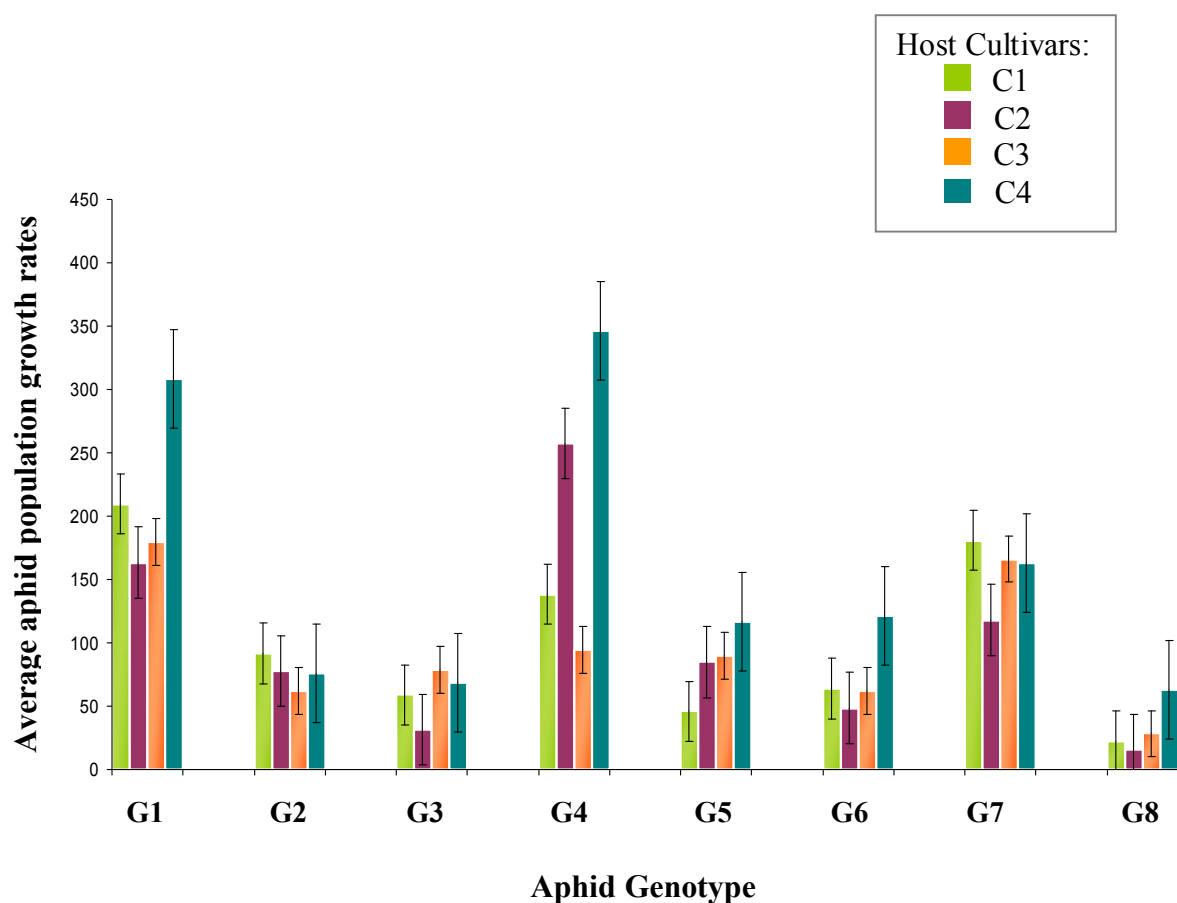
Figure 2: Genotypic reproductive rates. Aphid population growth rates (numbers at day 14; mean +/- SE) for six pea aphid genotypes (**G1** to **G6**) and two heterospecifics (**G7** and **G8**) on each of four host cultivars. Host broad bean cultivars are depicted in different colours as illustrated via the figure key.

Ranked abundance (Intra- and Inter-specific)							

	1st	2nd	3rd	4th	5th	6th	7th	8th
G1	2	2						
G4	1	1	2					
G7	1	1	2					
G2				2		2		
G6				1	1	1	1	
G3				1		1	2	
G5					3		1	
G8								4

Table 1: Frequencies of rankings of aphid clonal performance across four broad bean cultivars. Aphids (both within pea aphids and among aphid species) varied in performance rank. While the amount of variation is considerable there is clearly a performance hierarchy among genotypes that is reasonably consistent across host plant genotypes. The numbers within the cells of the table indicate the frequency of the rank of aphid reproductive success i.e. population growth rate or superiority and inferiority in performance across broad bean cultivars. For instance, **G8** was a weak performer displaying the lowest reproductive rate on all cultivars, whereas its counterparts (**G1**, **G4** and **G6**) achieved the highest tallies (rates) of reproductive success across host plant cultivars.

[Pea aphids (**G1**…**G6**), *M. viciae* (**G7**), *M. persicae* (**G8**)].

Aphid Polyphenism

The production of alates (winged aphids) implied a similar pattern of being influenced by aphid genotype and the interaction between aphid genotype and

cultivar. Because the numbers of alates are so low it was not possible to statistically analyse this data but consistent patterns can be seen (**Figure 3**). Overall, **G3** had the largest number of winged aphid production (113 in total) while **G8** produced the least (1 alate). There were large numbers of **G1** on cultivars 1 and 4 with no induction of winged individuals. Moreover, extremely few alates were produced by **G1** on C2 (2 alates) and C3 (1 alate). A similar pattern is apparent for vetch aphid (**G7**) that interestingly showed noticeable consistency in abundance across broad bean cultivars. On the other hand, pea aphid (**G4**), ranking second in terms of overall abundance, showed a notable increase in alate production especially on cultivars C2 and C4. Compared with other aphids, **G4** produced the largest number of alates (58) on a single cultivar (C2). Numbers of alates (10 vs. 1) for the two pink genotypes **G5** and **G6** flipped between (C1, C2) and (C3, C4).

Aphid Genotype across broad bean cultivars
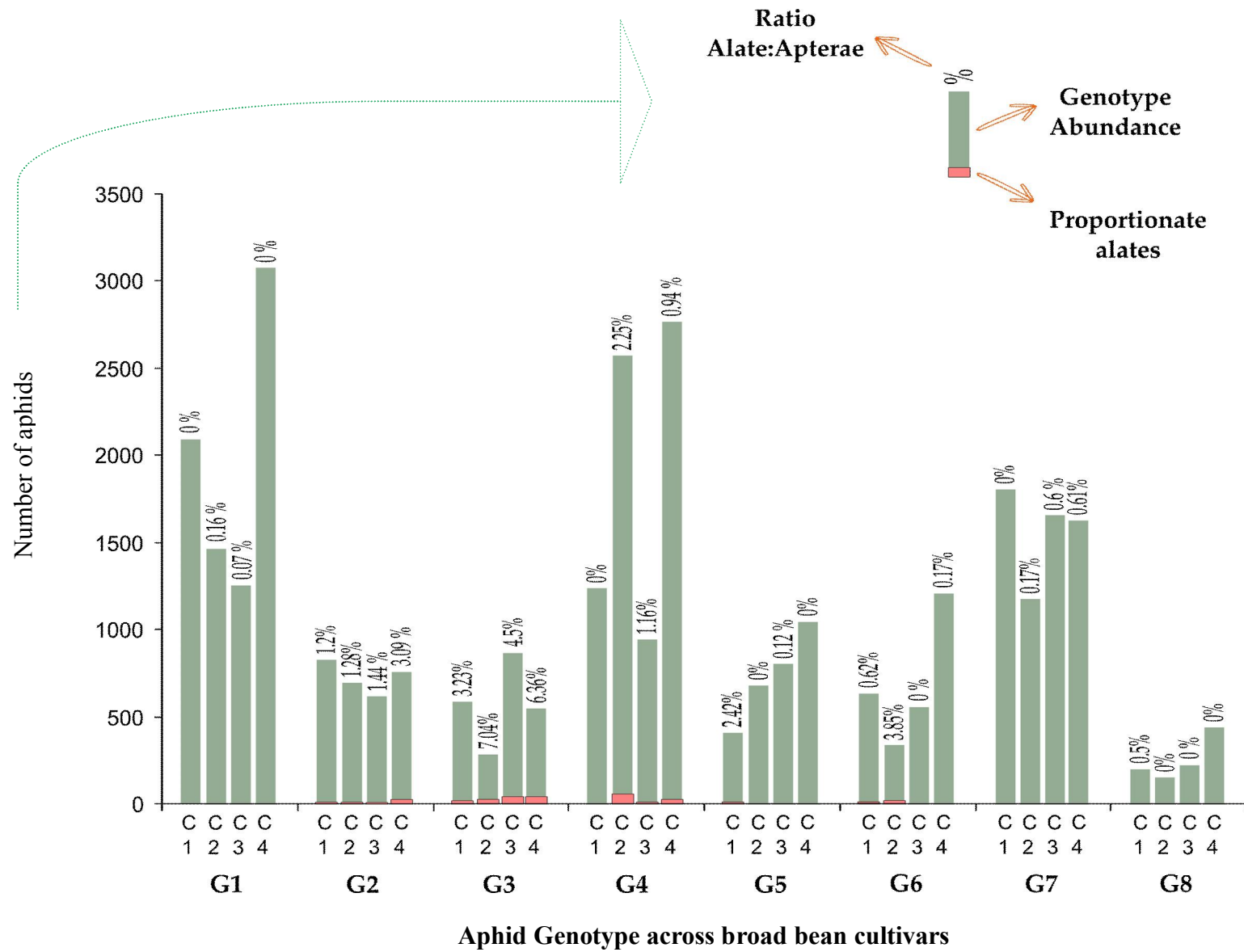
Figure 3: Alate-morph production. Production of winged progeny reflects the differential investment in reproduction (non-winged) vs. dispersal (winged). Proportions of alates (in red) versus apterae (in green) for each of the three aphid species (eight genotypes) across four broad bean cultivars are depicted. While the incidence of winged individuals is rare there is a clear difference in the likelihood of alate production among aphid genotypes and an apparent differential response of aphid genotypes to broad bean cultivars.

[Pea aphids (**G1**…**G6**), *M. viciae* (**G7**), *M. persicae* (**G8**)].

Aphid behaviour

We measured behaviour as the distribution of aphids on the top, middle and bottom thirds of the plant. We used aphid numbers (totals) for the statistical test and illustrated the distribution proportionally. There were very strong aphid species effects (nominal logistic regression; χ^2 = 81.73, P< 0.0001), broad bean cultivar effects (χ^2 =106.73, P<0.0001) and an interaction between aphid species and cultivar (χ^2 = 572.86, P<0.0001, **Figure 4**).

Likewise, there were very strong effects of aphid genotype (χ^2 = 512.61, P<0.0001), broad bean cultivar (χ^2 = 78.40, P<0.0001) and the interaction of genotype and cultivar (χ^2 = 890.59, P<0.0001, **Figure 4**). Aphids showed a propensity to congregate in the bottom third of the plant, except for **G4** where this was notably reduced on all cultivars excluding C4, and also for **G5** (on all cultivars except C3). In **G4**, aggregation was highest in the middle third of the host plant.

Plant section (third):

☐ Top   ☐ Mid   ☐ Bottom

M. viciae

G 1   C1 C2 C3 C4   G 3   C1 C2 C3 C4   G 5   C1 C2 C3 C4   G 7   C1 C2 C3 C4

Proportions of aphids

100%
80%
60%
40%
20%
0%

C1 C2 C3 C4   G 2   C1 C2 C3 C4   G 4   C1 C2 C3 C4   G 6   C1 C2 C3 C4   G 8

A. pisum: 6 Genotypes

M. persicae

Aphid Genotype across cultivars

66
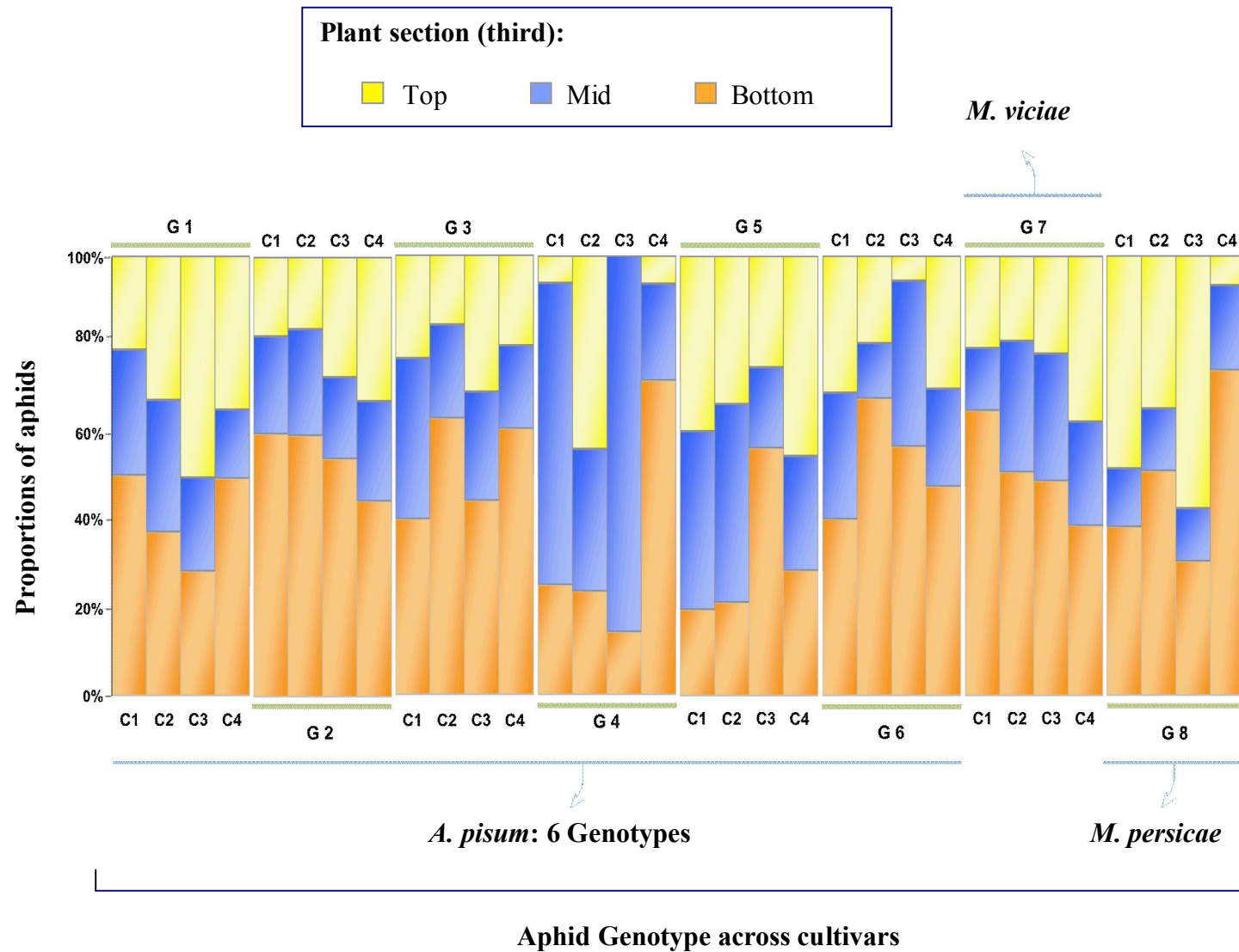
Figure 4: On-plant distribution of aphid genotypes across four broad bean cultivars. Intra-plant variation, aphid genotype and their interaction notably influenced aphid distribution on both intra- and inter-specific levels.

Bottom, Middle and top thirds (sections) of the plant have different colours as illustrated and numbers of aphids that congregated on each third (section) are depicted proportionally.

[Pea aphids (**G1**…**G6**), *M. viciae* (**G7**), *M. persicae* (**G8**)].

Plant performance

As expected, plant dry weight was significantly influenced by cultivar ($F_{3,6} = 11.30$, P = 0.0004), but was also significantly influenced by aphid genotype ($F_{5,6} = 5.09$, P = 0.0062) and marginally significantly influenced by the interaction of aphid genotype and cultivar ($F_{15,2} = 1.69$, P = 0.0564, **Figure 5**).

Shoot length-root length ratios showed no significant response to variation in broad bean cultivar, aphid genotype or the interaction of both when only *A.pisum* genotypes were compared. In contrast, the interaction between aphid species and broad bean cultivars was significant for the shoot-root ratio ($F_{2,6} = 17.95$, P=0.0353). (See also the appendix to this chapter for further insights on the ranks of broad bean cultivars according to aphid reproductive success).
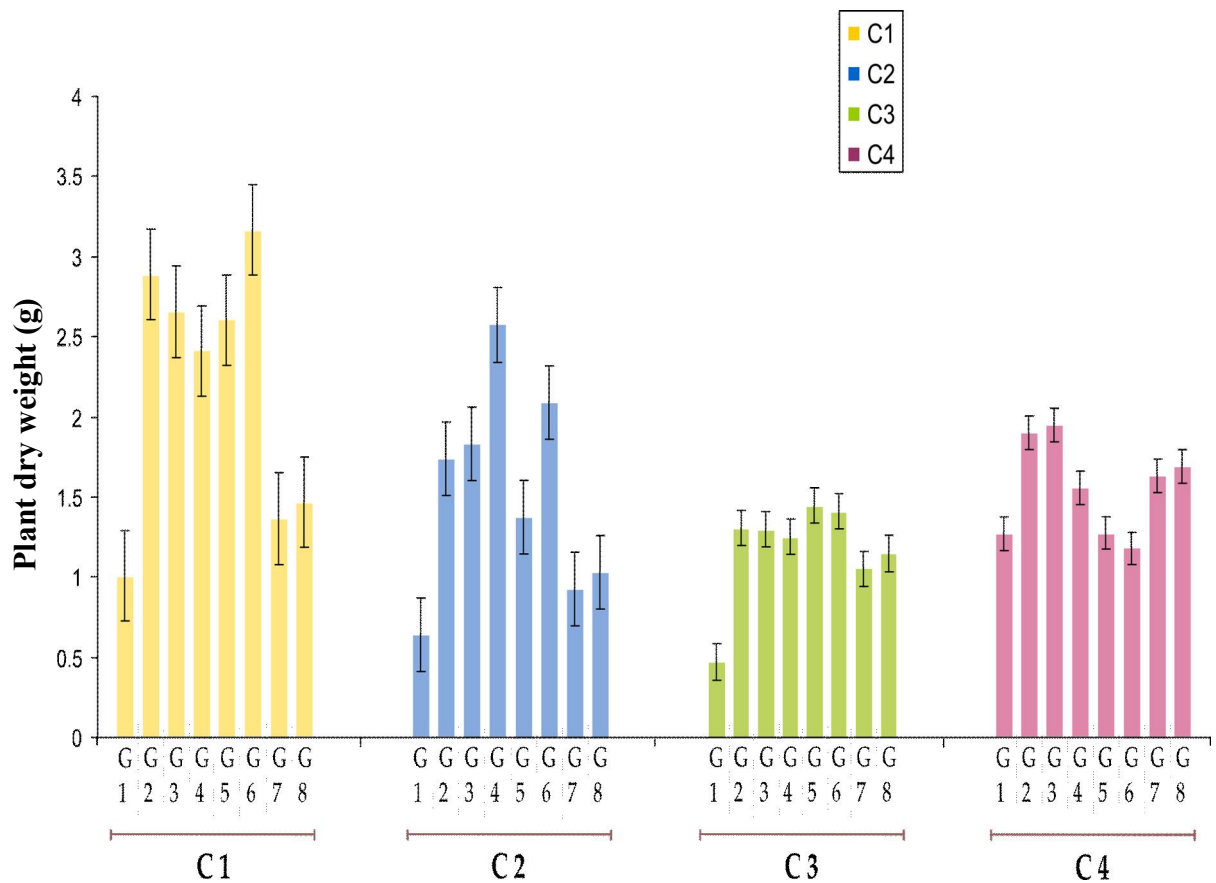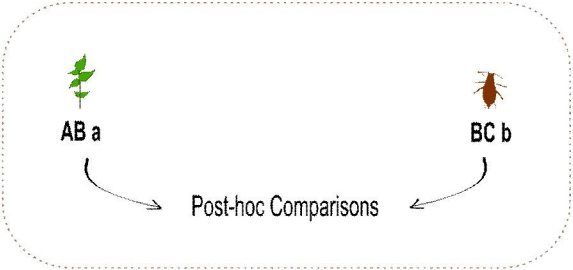
Broad bean cultivars across aphid genotypes

Figure 5: Broad bean cultivar biomass (mean +/- SE) across eight aphid genotypes. While there is a strong pattern of differences among cultivars, the variation due to aphid genotype spans a broader range. The differential response of aphid genotypes to cultivars can also be seen in the profile differences among the four cultivars. Four broad bean cultivars (C1, C2, C3, and C4) are assorted and depicted in different colours corresponding with the biomass of cultivar per aphid genotype.

[Pea aphids (**G1**…**G6**), *M. viciae* (**G7**), *M. persicae* (**G8**)].

We also created a matrix to allow comparison of each combination of aphid and host genotypes. The matrix indicates significant differences (based on Tukey's post-hoc following 1-way ANOVA) among cultivars for each aphid clone or species, and among aphid clones or species for each cultivar (**Table 2**).

	Acyrthosiphon pisum Genotypes						**Megoura viciae Genotype**	**Myzus persicae Genotype**
	G 1	**G 2**	**G 3**	**G 4**	**G 5**	**G 6**	**G 7**	**G 8**
C 1	AB a / ABC	Ab cd / Aa	A cd / A ab	A abc / A ab	A cd / A ab	A cd / A a	AB bc / A ab	A d / AB bc
C 2	B ab / BC b	A bc / AB ab	A ab / A bc	B a / B ab	A bc / A a	A bc / AB ab	B ab / C b	A c / B b
C 3	cb / B ab	A b / B a	A ab / A a	A ab / B a	A ab / Ba	A ab / B a	AB b / Bc ab	A a / B b
C 4	Aa / A ab	A c / AB a	A c / A a	B a / B a	B a / A c	AC / B a	AB bc / A a	B c / A a

Table 2: Matrix of differences in performance for all combinations of aphid genotypes and host plant cultivars. For both aphid performance and plant dry weight, lower case letters indicate significant differences (Tukey's post-hoc following 1-way ANOVA) among cells within each row, whereas upper case letters indicate significant differences among cells within each column. Cells sharing the same letter in a row or column are not significantly different.

Discussion

A new model system for Community Genetics

We found strong effects of broad bean cultivar, aphid genotype and their interactions on traits of both aphids and broad beans. Due to the presence of significant inter-specific GxG interactions, this model system is highly suitable for the investigation in community genetics. Agricultural model systems are easy to manipulate. However, while several systems are currently used in community genetics (e.g. Dungey et al. 2000, Neuhauser et al. 2003, Whitham et al. 2003, Bailey et al. 2006, Whitham et al. 2006, Johnson and Stinchcombe 2007, Keith et al. 2010), there are few that represent agricultural systems. An example in this regard is the 'Poplar' and 'Eucalyptus' tree systems (e.g. Dungey et al. 2000, Whitham et al. 2003, and Barbour et al. 2009). These systems are based on cultivated tree species and have been used very successfully to examine large scale questions. However, trees are not as easily manipulated as annual crops. In contrast, the barley-aphid system established by Tétard-Jones et al. 2011 represented an agricultural system using a grass and a major economic pest.

Here we define a legume-based system using pea aphids (*Acyrthosiphon pisum*) and stable cultivars (genotypes) of broad bean (*Vicia faba*). Both aphids and broad beans have many defined genotypes that are easily available and the occurrence of large groups of single genotypes represents a real world situation for both species. Both species have fast generation times and are easily maintained in controlled conditions in the laboratory. This system has the added advantage that pea aphid genotypes vary in colour (green and pink) thus allowing visual identification of mixed clones on a single plant. Further, this system could easily be expanded to include additional species of aphids (shown in this study), host plants and a well-studied array of predators, pathogens and soil communities.

The use of agricultural systems may not be regarded as representing a 'natural' system; however, agricultural systems now cover a significant portion of the landscape and represent an agro-ecosystem where associated communities of pests, predators and symbionts interact, develop and may evolve. Indeed, the current practice of monoculture planting is likely to cause intense selection on the associated communities and can result in rapid evolutionary response due to lack of selective disruption e.g. overcoming resistance (see the proceedings on sweet potato by Mackay et al. 1989). Additionally, the use of community genetics approaches to study agricultural systems allows us to address agricultural problems that may depend on the interaction of crop and pest genotypes.

Aphid performance

Inter-specific: There are now several examples of the influence of the within-species genetic variation of host plants on the diversity and fitness of associated faunal communities (e.g. Journet 1980, Moran 1981, Fritz et al. 1996, Dungey et al. 2000, Ito and Ozaki 2005). The three aphid species we examined showed significant variation in their reproductive rates. Such effects can be due to differences in intrinsic reproductive capacity or environmental cues (e.g. variation in the resistance of host plant), although we observed no evidence of host plant size effects. We used six polymorphic pea aphid genotypes across four broad bean genotypes. The use of the other two species i.e. vetch aphid and green peach aphid (one genotype each) was to create a frame of comparative reference for pea aphid genotypes given the fact that one and/or both of the above mentioned alien species often cohabit the same plant infested by pea aphids in the field (pers. obs.). All aphid identities in the experiment are analysed as random effects, thus they are treated as representatives of their species in general. While we used only single genotypes of the other aphid species (vetch

aphid and green peach aphid), they are treated as representatives at the species level only. The use of additional genotypes of those species would of course provide more information about intra-specific variation in those species and their interactions with pea aphids, but that was not the focus of this study.

Intra-specific: As expected, *A. pisum* genotypes varied in their performance, however, performance was also strongly influenced by host plant genotype and the specific combination of aphid and plant genotypes. It is well reported that different aphid genotypes vary in response to host strains (e.g. Fritz et al. 1994, Whitham et al. 1994, Fritz et al. 1996). This result adds to the increasing evidence of the evolutionary importance of GxG interactions in shaping pest communities, which in turn can affect plant phenotypic plasticity (Tétard-Jones and Leifert 2011).

Aphid polyphenism

Polyphenism (a form of phenotypic plasticity, Whitman and Agrawal 2009) refers to the capacity of one genotype to produce distinctive discrete phenotypes in response to environmental stimuli (Mayr 1963, Nijhout 2003, West-Eberhard 2003, Whitman and Agrawal 2009). Here, we use this term to refer to the production of winged aphids (alates). Surprisingly, higher aphid densities did not trigger alate production on some host cultivars but did induce very small numbers of alates on other ones. For those cultivars, where alate production was seen, there appears to be an interaction between cultivar and aphid genotype that influenced the rate of alate production. However, we were unable to test this speculation statistically because of the rarity of alate production.

Acyrthosiphon pisum is polymorphic (Caillaud and Losey 2010) with green and pink morphs which vary in their propensity to produce winged morphs in response to crowding (Sutherland 1969, Muller et al. 2001), parasitoids (Sloggett and

Weisser 2002), aphid alarm pheromones (Hatano et al. 2010) and host deterioration (Muller et al. 2001, but see Mashanova et al. 2008). This in turn suggests differential fitness and survival capacities for these morphs (Hille Ris Lambers 1966, Zera and Denno 1997). Aphid polymorphism (Dixon 1977) can be influenced by aphid diet (i.e. plant host nutrients; Harrewijn 1978) and photoperiod (Dixon 1985). Winged morphs are dispersive, but, in contrast to the wingless aphids, are less fecund, take longer to reach maturity and require and are energetically costly to produce (Dixon 1977, 1985, Zera and Denno 1997). However, production of winged morphs is a response to specific stimuli and hence predictable (Stearns 1989, Nijhout 1999). The induction of alates can be influenced by preconditioning in pea aphids, through environmentally context-dependent maternal signalling (Beckerman et al. 2006a,b). In other words, a mother aphid's phenotype and even a grandmother's (through telescoping of generations) influence the offspring's phenotypes and environment (Mousseau and Fox 1998, Zehnder et al. 2007). The low production of winged morphs in this study reflects the stability of the biotic environment experienced by aphids since more stable environments usually correlate with lower levels of alate production (e.g. Artachoa et al. 2011). The variation we observed in alate production may reflect an underlying variation in life-history strategies. For example, Dixon (1985) addressed differential strategies in aphids and defined "risk takers" characterised by highly reproductive clones in contrast to ''risk averse'' ones that invest more heavily in alate production under environmental influences such as the presence of a natural enemy.

Myzus persicae which, although highly polyphagous and adaptable to a spectrum of host plants including broad bean (e.g. Ashby et al. 1982, Blackman and Eastop 2000, Margaritopoulos et al. 2000, Kuroli and Lantos 2008), curiously, exhibited the lowest densities on all broad bean cultivars. It also had the lowest rate of alate production.

It was expected that *M. persicae* would show a tendency to disperse from a plant genotype (i.e. produce alates) when experiencing plant hosts with high chemical resistance and hence negatively affecting the reproductive success of this aphid. However, in this study, this point could be attributed to *Myzus persicae's* subdued reproductive success on broad beans and possibly the response of offspring to maternal effect (e.g. Lowe 1973). Consequently, the quality of the host cultivars above the depletion threshold (aphids were not allowed to overcrowd) induced changes in the fitness of the progeny (e.g. Harrewijn 1978, Awmack and Leather 2002). In contrast, in the absence of natural enemies, production of winged individuals in *Megoura viciae* may be more contingent on changes in photoperiod and related maternal effects rather than tactile and crowding effects (Lees 1967). Under the favourable photoperiod and provision of preferable hosts in this study, the aforementioned postulation about vetch aphid (*Megoura viciae*) can explain the very low numbers of alates produced by this species. *Megoura viciae* thrived on all broad bean cultivars (i.e. displayed high levels of population growth rate) but its exponential rate of reproduction did correlate with extremely reduced production of winged morphs).

Aphid behaviour

The influence of host plant genotype on aphid behaviour was very strong as was the interaction between genotypes of the plant and aphid. Aphid genotypes displayed higher densities on selected parts of the host plants. Strong (1967) examined this behaviour of aphids and showed that such aggregations are not simply occurring by chance (called pseudo-aggregation). The repeatability of patterns of aggregation across replicates also supports the idea that this response is a result of active aggregation (i.e. choice) by aphid species. We argue that the plant genotype, and its

interaction with aphid genotype, underlies the consistent differences between the conspecifics of pea aphid across contexts; here a set of broad bean genotypes (Bergmüller and Taborsky 2010, Schuett et al. 2011). Therefore, the consistency in the differential behaviour (choice to congregate on specific parts of he host plant) showed by aphid genotypes is likely a product of the community genetics effect. It should be noted that, while aphid lines in this work were not exposed to natural enemies, it is expected that aggregative behaviour would be influenced by predation*.

What is a trait or a character?

A trait in this context can also be a plastic behavioural phenotypic trait that could be a shared feature/phenotype (*sensu* Dawkins 1982) between two distinct species influencing one another. As such, the co-evolutionary arms-race scenarios between plants and their phloem-feeding insects, or both the direct and indirect interplay of aphids and their natural enemies make very good examples in this regard (e.g. Berenbaum and Zangerl et al. 1998, Oliver et al. 2012).

Since aphids are ultimately dependent on their hosts, prominent bottom up effects (Bailey et al. 2006, Johnson 2008) are thought to influence aphids in a manner that is likely to induce the formation of distinct phenotypes with specific environmentally cued characteristics (see also Dixon 1977, 1998, Awmack and Leather 2002, Park and Hardie 2002). Such comprehension is concordant with the definition proposed by Réale et al. (2007) for 'temperament' as a concept of a behavioural trait that is adjustable and important in an eco-evolutionary context (see also Bergmüller and Taborsky 2010).

---

*The work presented in chapter 3 examines the effects of parasitoid genotype on aphids' behaviour using a quantitative genetic approach.

In this regard, the recent work by Dombrovsky et al. (2009) provides a mechanism by which this might occur by showing the capacity of aphids to produce genetically identical yet molecularly different individuals that display significant phenotypic variation in physiological as well as behavioural traits. We think that this diversity of phenotypes can be attributed to intra-specific genetic variation in aphids. Furthermore, the differential aphid reactions in response to a wide array of environmental cues are always exposed to the community genetic effects of host plants. Concomitantly, the outcome is a phenotypic gradient (temperament) or differential reproductive and behavioural consistency displayed by each generalist aphid genotype across a range of hosts (see Ferrari et al. 2006, Bergmüller and Taborsky 2010). Such argument is useful to further the interpretation of the findings in our experiment.

The G x G interaction between aphid and host should significantly affect generalist aphids' fitness. For example, although green peach aphid (*Myzus persicae*) is a generalist, it might have suffered from host resistance i.e. "antixenosis" (Kogan and Ortman 1978). Consequently, this negatively affected fecundity rates and hindered colonisation success. Thus, the sizes of the clone of this species were comparatively small.

Analysis of the *A.pisum* intra-specific response indicated distinct effects of aphid genotype and the interaction between aphid genotype and host cultivar on aphid distribution on the plant. We observed different levels of resistance for the four broad bean cultivars used. The variation in resistance relying on genetic differences among broad bean cultivars was reflected in the relative reproductive success of aphid genotypes. Thus, the 'plant perspective' definition of aphid resistance that treats all aphids as equal fails when considered from a community genetics perspective. This along with the dissimilarities in dry weight, root-shoot ratios and root-mass volume

(data are not presented here for the latter) in infested plants, highlights the importance of selecting more resistant, in-bred plant strains for agriculture.

Under the stabilised temperate conditions of the experiment, the involved species were maintained to reproduce parthenogenetically. It was notable that investment by aphid genotypes/species was oriented by aphid mothers towards fecundity (production of apterae) rather than dispersal (production of alate morphs) for most of the aphid genotypes [Dixon 1977; see also the annex to chapter 2; bet-hedging and risk-spreading tactics]. Furthermore, Lushai et al. (1997) reported an unusual phenomenon of intra-clonal variability within exclusively parthenogenetic clones of grain aphid (*Sitobion avenae*) and bird-cherry aphid (*Rhopalosiphum padi*). Lushai et al. (1997) related the findings to intrinsic aphid genomic factors rather than 'organismic lateral DNA transfer' and hence implied versatile behavioural and ecological specialisation in functionality within the focal clones (see also Dombrovsky et al. 2009).

Conclusion

Community genetics supports, with increasing evidence, the argument that within-species genetic variation in host plants plays an important ecological role by influencing the abundance, composition and diversity of associated communities (Whitham et al. 2003, 2006, Johnson and Stinchcombe 2007, Tétard-Jones et al. 2007, Hughes et al. 2008, and Bailey et al. 2009a,b). Here we have shown how multiple genotypes (cultivars) of broad beans can differentially affect performance (population sizes) and behaviour (on-plant distribution) of several aphid genotypes and how host plants may be reciprocally influenced. We also present a framework for combining other ecological effects into a community genetics framework. The need for such an

amalgamating approach of ecology and evolution has been emphasised in a review of the current and future directions of community genetics by Hersch-Green et al. (2011).

Selective stressors can be destabilising, within and across integrated communities, and thus potentially able to vary in correspondence with the genetic variation of inter-players on a very fine scale (Gould 1977; but see Thompson 1994). We believe that studies on aphid ecology should focus on the variation in host genetics as well as the interaction between host and aphid genotypes as contributory factors to the ecology and evolution of aphids and their host plants in agro-ecosystems.

References

Agrawal, A. A., Underwood, N., & Stinchcombe, J. R. (2004). Intraspecific variation in the strength of density dependence in aphid populations. *Ecological Entomology*, *29*, 521-526.

Agrawala, B. K. (2007). Phenotypic plasticity in aphids (Homoptera: Insecta): components of variation and causative factors. *Current Science, 93*, 308-313.

Antongiovanni, M., Acciaioli, A., Franci, O., Ponzetta, M. P., Pugliese, C., Buccioni, A., & Badii, M (2002). Field bean (*Vicia faba* var. minor) as a protein feed for

growing lambs with and without protected lysine and methionine supplementation. *Ital. J. Anim. Sci*, *1*, 229-238.

Antonovics, J. (1992). Toward community genetics. In R. S. Fritz, & E. L. Simms (Eds.), *Ecology and Evolution of Plant Resistance to Herbivores and Pathogens: Ecology, Evolution, and Genetics* (pp. 426-449). USA: University Of Chicago Press.

Antonovics, J. (2003). Toward community genomics. *Ecology*, *84*, 598-601

Artachoa, P., Figueroaa, C. C., Cortesa, P. A., Simon, J. C., & Nespoloa, R. F. (2011). Short-term consequences of reproductive mode variation on the genetic architecture of energy metabolism and life-history traits in the pea aphid. *Journal of Insect Physiology*, *57*(7), 986-994.

Ashby, J. W., Fletcher, J. D., Farrell, J. A. K., & Stufkens, M. R. (1982). Observations on host preferences and epidemiology of aphid species associated with legume crops. *New Zealand Journal of Agricultural Research*, *25*, 267-272.

Astles, P. A., Moore, A. J., & Preziosi, R. F. (2005). Genetic variation in response to an indirect ecological effect. *Proceedings of the Royal Society B*, *272*, 2577-2581.

Awmack, C. S., & Leather, S. R. (2002). Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology*, *47*, 817-844.

Bailey, J. K., & Whitham, T. G. (2006). Indirect trait-mediated interactions between cottonwoods and beavers positively affect sawfly abundance. *Ecological Entomology*, *31*, 294-297.

Bailey, J. K., Hendry, A., Kennison, M., Post, D., Palkovacs, E., Pelletier, F., Harmon, L., Schweitzer, J. A. (2009a). From genes to ecosystems: An emerging synthesis of eco-evolutionary dynamics. *New Phytologist*, *184*, 746-749.

Bailey, J. K., Schweitzer, J. A., Rehill, B. J., Lindroth, R. L., Martinsen, G. D., & Whitham, T. G. (2004). Beavers as molecular geneticists: A genetic basis to the foraging of an ecosystem engineer. *Ecology*, *85*, 603-608.

Bailey, J. K., Schweitzer, J. A., Úbeda, F., Koricheva, J., LeRoy, C. J., Madritch, M. D., Rehill,B. J., Bangert, R.K., Fisher, D.G., Allen, G., Whitham, T. G. (2009b). From genes to ecosystems: An emerging synthesis of eco-evolutionary dynamics. *Phil. Trans. R. Soc. B*, *364*(1523), 1607-1616.

Bailey, J. K., Wooley, S. C., Lindroth, R. L., & Whitham, T. G. (2006). Importance of species interactions to community heritability: A genetic basis to trophic-level interactions. *Ecology Letters*, *9*, 78-85.

Bangert, R. K., Turek, R. J., Rehill, B., Allan, G. J., Wimp, G. M., Schweitzer, J. A., Bailey, J. K., Martinsen, G., D., Keim, P., Lindroth, R., L., Whitham, T. G. (2006). A genetic similarity rule determines arthropod community structure. *Molecular Ecology*, *15*, 1379-1392.

Barbour, R. C., Forster, L. G., Baker, S., Steane, D. A., & Potts, B. M. (2009). Biodiversity Consequences of Genetic Variation in Bark Characteristics within a Foundation Tree Species. *Conservation Biology*, *23*(5), 1146–1155.

Beckerman, A. P., Benton, T. G., Lapsley, C. T., & Koesters, N. (2006a). Talkin'Bout My Generation: Environmental variability and Cohort effects. *Am. Nat*, *162*, 754-767.

Beckerman, A. P., Benton, T. G., Lapsley, C. T., & Koesters, N. (2006b). How effective are maternal effects at having effects? *Proc. R. Soc. B*, *273*, 485-493.

Bellotti, W. D. (2004). Improving the productivity and sustainability of rainfed farming systems for the loess plateau of Gansu province. In International conference in Yangling, Shaanxi province, P.R. China, September 20-22, 2004. Ecological Book series-3. Revegetation and sustainable land use for soil

erosion control, the Loess plateau in Central China: Ecological restoration and management, issued by UNESCO in 2006 (pp. 252-277). China: ERSEC, UNESCO.

Berenbaum, M. R. & Zangerl, A. R. (1998). Chemical phenotype matching between a plant and its insect herbivore. *Proc. Natl Acad. Sci. 95*, 13743–13748. (doi: 10.1073/pnas.95.23.13743)

Bergmüller, R., & Taborsky, M. (2010). Animal personality due to social niche specialisation. *Trends in Ecology & Evolution, Trends in Ecology and Evolution-1267*, 1-8. (doi:10.1016/j.tree.2010.06.012)

Blackman, R. L., & Eastop, V. F. (2000). *Aphids of the world's crops - an identification and information guide,* 2nd ed. New York: John Wiley.

Bond, D. A., Jellis, G. J., Rowland, G. G., Le Guen, J., Robertson, L. D., Khalil, S. A., & Li-Juan, L. (1994). Present status and future strategy in breeding faba beans (*Vicia faba* L.) for resistance to biotic and abiotic stresses. *Euphytica*, *73*, 151-166.

Bond, D. A., Lawes, D. A., Hawtin, G. C., Saxena, M. C., & Stephens, J. S. (1985). Faba bean (*Vicia faba* L.). In: R. J. Summerfield & E. H. Roberts (Eds.), *Grain legume crops* (pp. 199-265). London, UK: William Collins Sons Co. Ltd..

Brisson, J. A., & Stern, D. L. (2006). The pea aphid, *Acyrthosiphon pisum*: An emerging model system for the molecular investigation of ecologically-relevant trait variation. *BioEssays*, *28*, 747-755.

Caillaud, M. C., & Losey, J. E. (2010). Genetics of color polymorphism in the pea aphid, *Acyrthosiphon pisum*. *Journal of Insect Science*, *10*(95), 754-767. Retrieved from http://insectscience.org/10.95

Carpenter, K. J., & Johnson, C. L. (1968). The metabolizable energy of field beans (*Vicia faba* L.) for poultry. *The Journal of Agricultural Science*, *70*, 391-392.

Chapman, R. F. (2003). Contact chemoreception in feeding by phytophagous insects. *Annu. Rev. Entomol.*, *48*, 455-484.

Cole, S. (1970). *The Neolithic revolution.* London: Trustees of The British Museum (Natural History).

Cordeiro, C. M. T., Maluf, W. R., & Miranda, J. E. C., (1983). Analysis of stability and genotypic similarity in a set of potato (*Solanum tuberosum* L.) cultivars. *Rev. Brazil. Genet.*, *2*, 279-294.

Cote, J., Clobert, J., Brodin, T., Fogarty, S., & Sih, A. (2010). Personality-dependent dispersal: Characterization, ontogeny and consequences for spatially structured populations. *Phil. Trans. R. Soc*, *365*, 4065-4076.

Cubero, J. I., & Suso, M. J. (1981). Primitive and modern forms of *Vicia faba. Die Kulturpflanze*, *29*, 137-145.

Dawkins, R. (1982). *The extended phenotype.* Oxford: W.H. Freeman And Company.

de Vos, M., Cheng, W. Y., Summers, H. E., Raguso, R. A., & Jander, G. (2010). Alarm pheromone habituation in *Myzus persicae* has fitness consequences and causes extensive gene expression changes. *PNAS*, *107*(33), 14673-14678.

Diehl, S. R., & Bush, J. L. (1984). An evolutionary and applied perspective of insect biotypes. *Ann. Rev. Entomol*, *29*, 471-504.

Dixon, A. F. G. (1977). Aphid ecology: Life cycles, polymorphism, and population regulation. *Ann. Rev. Ecol. Syst.*, *8*, 329-353.

Dixon, A. F. G. (1985). Structure of aphid populations. *Annual Review of Entomology*, *30*, 155-174.

Dixon, A. F. G. (1987). Parthenogenetic reproduction and the rate of increase in aphids. In A. K. Minks & P. Harrewijn (Eds.), *Aphids, their biology, natural enemies and control* (pp. 269-287). Amsterdam: Elsevier.

Dixon, A. F. G. (1998). *Aphid ecology.* London: Chapman and Hall.

Dombrovsky, A. A., Arthaud, L. L., Ledger, T. N., Tares, S., & Robichon, A. (2009). Profiling the repertoire of phenotypes influenced by environmental cues that occur during asexual reproduction. *Genome Research*, *19*, 2052-2063. (doi: 10.1101/gr.091611.109)

Drés, M., & Mallet, J. (2002). Host-races in plant-feeding insects and their importance in sympatric speciation. *Philos Trans R Soc Lond B Biol Sci*, *357*, 471-492.

Duc, G. (1997). Faba bean (*Vicia faba* L.). *Field Crops Res*, *53*, 99-109.

Duc, G., Bao, S., Baum, M., Redden, B., Sadikie, M., Suso, M. J., Vishniakova, M. & Zong, X. (2010). Diversity maintenance and use of *Vicia faba* L. genetic resources. *Field Crops Research*, *15*(3), 270-278.

Duke, J. A. (1981). *Handbook of legumes of world economic importance.* New York: Plenum Press.

Dungey, M., Potts, H. S., Whitham, T. G., & Li, H. (2000). Plant genetics affects arthropod community richness and composition: Evidence from a synthetic eucalypt hybrid population. *Evolution*, *54*(6), 1938-1946.

Emebiri, L. C., Matassa, V., & Moody, D. B. (2005). GENSTAT programs for performing Muir's alternative partitioning of genotype-by-environment interaction. *Journal of Heredity*, *96*(1), 78-79.

Ferrari, J., Godfray, H. J., Faulconbridge, A., Prior, K., & Via, S. (2006). Population differentiation and genetic variation in host choice among pea aphids from eight host plant genera. *Evolution*, *60*, 1574-1584.

Flores, F., Moreno, M. T., & Cubero, J. J. (1998). A comparison of univariate and multivariate methods to analyze g x e interaction. *Field Crops Res*, *56*, 271-286.

Frantz, A., Calcango, V. S., Mieuzet, L. G., Plantegenest, M., & Simon, J. (2009). Complex trait differentiation between host-populations of the pea aphid:

Implications for the evolution of ecological specialisation. *Biological Journal of the Linnean Society, 97*(4), 718-727.

Frantz, A., Plantegenest, M., Mieuzet, L., & Simon, J. C. (2006). Ecological specialization correlates with genotypic differentiation in sympatric host-populations of the pea aphid. *J. Evol. Biol, 19*(2), 392-401.

Fritz, R. S., Nichols-Orians, C. M., Brunsfeld, S. J., & Colin, M. (1994). Interspecific hybridization of plants and resistance to herbivores: Hypotheses, genetics, and variable responses in a diverse community. *Oecologia, 97,* 106-117.

Fritz, R. S., Roche, B. M., Brunsfeld, S. J., & Orians, C. M. (1996). Interspecific and temporal variation in herbivore responses to hybrid willows. *Oecologia, 108,* 121-129.

Giske, J., Aksnes, D. L., & Førland, B. G. (1993). Variable generation times and Darwinian fitness measures. *Evolutionary Ecology, 7,* 233-239.

Glinwood, R., Ahmed, E., Qvarford, E., Ninkovic, V., & Pettersson, J. A. (2009). Airborne interactions between undamaged plants of different cultivars affect insect herbivores and natural enemies. *Arthropod–Plant Interactions, 3*(4), 215-224.

Gould, S. J. (1977). *Ontogeny and phylogeny.* Cambridge, MA: Harvard University Press.

Groeters, F. R., & Dingle, H. (1989). The cost of being able to fly in the milkweed oleander aphid, *Aphis nerii* (Homoptera, Aphididae). *Evolutionary Ecology, 3,* 313-326.

Hales, D. F., Tomiuk, D. F. J., Ohrmann, K. W., & Sunnucks, P. (1997). Evolutionary and Genetic Aspects of Aphid Biology: a Review. *Eur. J. Entomol., 94,* 1-55.

Harrewijn, P. (1978). The role of plant substances in polymorphism of the aphid *Myzus persicae. Entomologia Experimentalis et Applicata, 24,* 198-214.

Hash, C. T., Blummel, M., & Bidinger, F. R. (2006). Genotype × environment interactions in food-feed traits in pearl millet cultivars. *International Sorghum and Millets Newsletter*, *47*, 153-157.

Hatano, E., Kunert, G., Weisser, W. W., & Sunnucks, P. (2010). Aphid wing induction and ecological costs of alarm pheromone emission under field conditions. *PLoS ONE*, *5*(6): e11188. (doi:10.1371/journal.pone.0011188)

HDRA. (1998). Green manures/cover crops. Retrieved from Garden Organic website: http://w.gardenorganic.org.uk/pdfs/international_programme/GreenMan.pdf

Hersch-Green, E. I., Turley, N. E., & Johnson, M. T. J. (2011). Community genetics: What have we accomplished and where should we be going? *Phil. Trans. R. Soc. B*, *366*(1569), 1453-1460.

Hille Ris Lambers, D. (1966). Notes on Californian aphids, with descriptions of new genera and new species (Homoptera, Aphididae). *Hilgardia*, *37*, 569-623.

Hoberg, E. P., & Brooks, D. R. (2008). A macroevolutionary mosaic: Episodic host-switching, geographical colonization and diversification in complex host–parasite systems. *Journal of Biogeography*, *35*(9), 1533-1550.

Hughes, A. R., Inouye, B. D., Johnson, D., T, M. T. J., Underwood, N., & Vellend, M. (2008). Ecological consequences of genetic diversity. *Ecology Letters*, *11*, 609-623.

Ito, M., & Ozaki, K. (2005). Response of a gall wasp community to genetic variation in the host plant *Quercus crispula*: A test using half-sib families. *Acta Oecologica*, *27*, 17-24.

Janzen, D. H. (1977). What are dandelions and aphids? *The American Naturalist*, *111*, 586-589.

Johnson, M. T. J., & Agrawal, A. A. (2005). Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (*Oenothera biennis*). *Ecology*, *86*, 874-885.

Johnson, M. T. J., & Stinchcombe, J. R. (2007). An emerging synthesis between community ecology and evolutionary biology. *Trends in Ecology and Evolution*, *22*, 250-257.

Johnson, M. T. J., (2007). Genotype-by-environment interactions leads to variable selection on life-history strategy in common evening primrose (*Oenothera biennis*). *Journal of Evolutionary Biology*, *20*, 190-200.

Johnson, M. T. J., (2008). Bottom-up effects of plant genotype on aphids, ants and predators. *Ecology*, *89*, 145-154.

Jones, C. G., Lawton, J. H., & Shachak, M. (1994). Organisms as ecosystem engineers. *Oikos*, *69*, 373-386.

Jones, C. G., Lawton, J. H., & Shachak, M. (1997). Positive and negative effects of organisms as physical ecosystem engineers. *Ecology*, *78*, 1946-1957.

Jordan, D. (1984). Rhizobaceae. In: D. P. Hendricks, H. A. Sneath & J. H. Halt (Eds). *Bergey's manual of systematic bacteriology*. *2*, 234-256.

Journet, A. R. P. (1980). Intraspecific variation in food plant favourability to phytophagous insects: psyllids on *Eucalyptus blakelyi* M. *Ecol Entomol*, *5*(3), 249-261.

Kan, A., Kaya, M., Gürbüz, A., Sanli, A., Özcan, K., & Çiftçi, C. Y. (2010). A study on genotype x environment interaction in chickpea cultivars (*Cicer arietinum* L.) grown in arid and semi-arid conditions. *Scientific Research and Essays*, *5*(10), 1164-1171.

Keith, A. R., Bailey, J. K., & Whitham, T. G. (2010). A genetic basis to community repeatability and stability. *J. Insect Physiol*, *11*, 3398-3406.

Kogan, M., & Ortman, E. F. (1978). Antixenosis-a new term proposed to define Painter's "nonpreference" modality of resistance. *Entomol. Soc. America Bull*, *24*, 175-176.

Kuroli, G., & Lantos, Z. (2008). Changes in abundance of aphids flying over and feeding on broad bean in a period of 20 years. *Archives of Phytopathology and Plant Protection*, *41*(4), 261-272.

Laland, K. N., & Boogert, N. J. (2008). Niche construction, co-evolution and biodiversity. *Ecol Econ*, *69*, 731-736. (doi:10.1016/j.ecolecon.2008.11.014)

Lees, A. D. (1967). The production of the apterous and alate forms in the aphid *Megoura viciae* Buckton, with special reference to the role of crowding. *J. Insect Physiol*, *13*, 289-318.

Lewontin, R. C. (1983). Gene, organism, and environment. In D. S. Bendall (Ed.), *Evolution from molecules to men* (pp. 273-285). Cambridge: Cambridge University Press.

Link, W. J., Abdelmula, A. A., Kittlitz, E. V., Bruns, S., Riemer, H., & Stelling, D. (1999). Genotypic variation for drought tolerance in *Vicia faba*. *Plant Breed*, *118*, 477-483.

Loss, S. P., & Siddique, K. H. M., (1997). Adaptation of faba bean (*Vicia faba* L.) to dryland Mediterranean-type environments. I. seed yield and yield components. *Field Crops Res*, *52*, 17-28.

Lowe, H. J. B., (1973). Variation in *Myzus persicae* (Sulzer) (Hemiptera, Aphididae) reared on different host plants. *Bull. Ent. Res*, *62*, 549-556.

Lushai, G., Sherratt, T. N., David, O., De Barro, P. J., & Maclean, N. (1997). Investigation of host selection by winged summer females of the aphid *Sitobion avenae* (Homoptera: Aphididae). *Entomologia Experimentalis et Applicata*, *85*, 199-209.

Mackay, K. T., Palomar M. K., & Sanico, R. T. (Eds). (1989). Sweet Potato Research and Development for Small Farmers. *Proceedings of the International Sweet Potato Symposium*. May 20-26, 1987 at the Visayas State College of Agriculture (VISCA), Leyte, the Philippines. SEAMO-SEARCA.

Margaritopoulos, J. T., Tsitsipis, J. A., Zintzaras, E., & Blackman, R. L. (2000). Host-correlated morphological variation of *Myzus persicae* (Hemiptera: Aphididae) populations in Greece. *Bulletin of Entomological Research*, *90*, 233-244.

Mashanova, A., Gange, A. C., & Jansen, V. A. A., (2008). Density-dependent dispersal may explain the mid-season crash in some aphid populations. *Popul Ecol*, *50*(3), 285-292.

Mayr, E. (1963). *Animal species and evolution.* Cambridge, MA: Harvard University Press.

Moore, A. J., Brodie, E. D., & Wolf, J. B. (1997). Interacting phenotypes and the evolutionary process: I. Direct and indirect genetic effects of social interactions. *Evolution*, *51*(5), 1352-1362.

Moran, N. (1981). Intraspecific viability in herbivore performance and host quality: A field study of *Uroleucon caligatum* (Homoptera: Aphididae) and its Solidago (Asteraceae) hosts. *Ecological Entomology*, *6*, 301-306.

Mousseau, T. A., & Fox, C. W. (1998). The adaptive significance of maternal effects. *Trends Ecol. Evol.*, *13*, 403-407.

Muehlbauer, F. J., & Tullu, A. (1997). *Cicer arietinum* L. Retrieved from NewCROP FactSHEET Center for New Crops and Plant Products. Purdue University, West Lafayette, IN website: http://www.hort.purdue.edu/newcrop/cropfactsheets/Chickpea.html

Muller, C. B., Williams, I. S., & Hardie, J. (2001). The role of nutrition, crowding and interspecific interactions in the development of winged aphids. *Ecol. Entomol, 26*, 330-340.

Muller, F. P. (1981). Biotype formation and sympatric speciation in aphids. *Proceedings of the International Symposium at Jablonna, 26*, 135-166. Polska Akademia Nauk, Instytut Zoologii.

Murray, B. G. (1985). Population growth rate as a measure of individual fitness. *Oikos, 44*, 509-511.

Murray, B. G. (1990). Population dynamics, genetic change, and the measurement of fitness. *Oikos, 59*(2), 189-199.

Nachi, N., & Guen, J. L. (1996). Dry matter accumulation and seed yield in faba bean (*Vicia faba* L.) genotypes. *Agronomie, 16*, 47-59.

Naiman, R. J., Johnston, C. A., & Kelley, J. C. (1988). Alteration of North American streams by beaver. *Bioscience, 38*, 753-762.

Neuhauser, C., Andow, D. A., Heimpel, G. E., Ruth, G. M., Shaw, G., & Wagenius, S. (2003). Community genetics: Expanding the synthesis of ecology and genetics. *Ecology, 84*, 554-558.

Nijhout, H. F. (1999). Control mechanisms of polyphenic development in insects. *Bioscience, 49*, 181-192.

Nijhout, H. F. (2003). The control of body size in insects. *Dev. Biol, 261*, 1-9.

Nottingham, S. F., Hardie, J., & Wadhams, L. J. (1992a). Aphid host finding behaviour: The role of plant volatiles. XIX International Congress of Entomology, Beijing, China, June 28th-July 4th, (1992), 1:172.

Nottingham, S. F., Hardie, J., & Wadhams, L. J. (1992b). Flight behaviour of the black bean aphid, *Aphis fabae*, and the cabbage aphid, *Brevicoryne brassicae*, in host and non-host plant odour. *Physiological Entomology, 18*, 389-394.

Nuessly, G. S., Hentz, M. G., Beiriger, R., & Scully, B. T. (2004). Insects associated with faba bean, *Vicia faba* (Fabales: Fabaceae), in Southern Florida. *Florida Entomologist, 87*(2), 204-211.

Odling-Smee, F. J., Laland, K. N., & Feldman, M. W. (2003). *Niche construction: The neglected process in evolution.* Princeton, NJ: Princeton University Press.

Oerke, E. C., Dehne, H. W., Schonbeck, F., & Weber, A. (1994). *Crop production and crop protection – estimated losses in major food and cash crops.* Amsterdam: Elsevier Science.

Oliver, K. M., Noge, K., Huang, E. M., Campos, J., M., Becerra, J., & Hunter, M., S. (2012). Parasitic wasp responses to symbiont-based defense in aphids. *BMC Biology, 10*(11). Retrieved from http://www.biomedcentral.com/1741-7007/10/11

Oyedokun, J. B. (1985). Genotype environment interactions and the identification of adatable cultivars of upland rice (*Oryza sativa*) in South-Western Nigeria. *Exp. Agric, 21*, 53-58.

Park, K. C., & Hardie, J. (2002). Functional specialisation and polyphenism in aphid Olfactory sensilla. *J. Insect Physiol, 48(5)*, 527-535.

Perrin, R. M. (1976). The population dynamics of the stinging nettle aphid *Microlophiurn carnosum* (Burkt.). *Ecological Entomology, 1*, 31-40.

Perrino, P., Maruca, G., Lester, R. N., Linsalata, V., Lattanzio, V., & Hanelt, P. (1991). Chemotaxonomie relationship among species of *Vicia* section *faba*. *Feddes Repertorium, 102*, 319-334.

Powell, G., Tosh, C. R., & Hardie, J. (2006). Host plant selection by aphids: Behavioral, evolutionary, and applied perspectives. *Annu. Rev. Entomol., 51*, 309-330.

Rabey, J. M., Vered, Y., Shabtai, H., Graff, E., & Korczyn, A. D. (1992). Improvement of parkinsonian features correlate with high plasma levodopa values after broad bean (*Vicia faba*) consumption. *J Neurol Neurosurg Psychiatry*, *55*(8), 725-727.

Réale, D., Reader, S. M., Sol, D., McDougall, P., & Dingemanse, N. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews*, *82*, 291-318.

Rose, T. J., Damon, P., & Rengel, Z. (2010). Phosphorus-efficient faba bean (*Vicia faba* L.) genotypes enhance subsequent wheat crop growth in an acid and an alkaline soil. *Crop & Pasture Science*, *61*(2), 1009-1016.

Rowntree, J. K., Shuker, D. M., & Preziosi, R. F. (2011). Forward from the crossroads of ecology and evolution. *Phil. Trans. R. Soc. B*, *366*(1569), 1322-1328.

Saxena, N. P., Krishnamurthy, L., & Johansen, C. (1993). Registration of a drought-resistant chickpea germplasm. *Crop Science*, *33,* 1424.

Schoener, T. W. (2011). The newest synthesis: Understanding the interplay of evolutionary and ecological dynamics. *Science*, *331*, 426-429.

Schuett, W., Dall, S. R. X, Baeumer, J., Kloesener, M. H., Nakagawa, S., Beinlich, F., & Eggers, T. (2011). 'Personality' variation in a clonal insect: The pea aphid, *Acyrthosiphon pisum. Developmental Psychobiology*, *53*, 631-640.

Schweitzer, J. A., Bailey, J. K., Hart, S. C., Wimp, G. M., Chapman, S. K., & Whitham, T. G. (2005). The interaction of plant genotype and herbivory decelerate leaf litter decomposition and alter nutrient dynamics. *Oikos*, *110*, 133-145.

Schweitzer, J. A., Bailey, J. K., Rehill, B. J., Martinsen, G. D., Martinsen, S. C., Hart, R., Lindroth, L., Keim, P. , & Whitham, T. G. (2004). Genetically based trait in a dominant tree affects ecosystem processes. *Ecology Letters*, *7, *127-134.

Service, P. (1984). Genotypic interactions in an aphid-host plant relationship: *Uroleucon rudbeckiae* and *Rudbeckia laciniata*. *Oecologia, 61*(2), 271-276.

Service, P. M., & Lenski, R. E. (1982). Aphid genotypes, plant phenotypes, and genetic diversity: A demographic analysis of experimental data. *Evolution, 36*, 1276-1282.

Sharpe, A. G., & Lydiate, D. J. (2003). Mapping the mosaic of ancestral genotypes in a cultivar of oilseed rape (*Brassica napus*) selected via pedigree breeding. *Genome, 46*, 461-468.

Shuster, S. M., Lonsdorf, E. V., Wimp, G. M., Bailey, J. K., & Whitham, T. G. (2006). Community heritability measures the evolutionary consequences of indirect genetic effects on community structure. *Evolution, 60*, 146-158.

Silim, S. N., & Saxena, A, M. C. (1992). Comparative performance of some faba bean (*Vicia faba*) cultivars of contrasting plant types. 1. Yield, yield components and nitrogen fixation. *Journal of Agricultural Science, Cambridge, 118*, 325-332.

Sloggett, J. J., & Weisser, W. W. (2002). Parasitoids induce production of the dispersal morph in the pea aphid, *Acyrthosiphon pisum. Oikos, 98*, 323-333.

Smith, C. M., & Boyko, E. V. (2007). The molecular bases of plant resistance and defense responses to aphid feeding: Current status. *Entomol. Exp. Appl, 122*, 1-16.

Smith, R. A., Mooney, K. A., & Agrawal, A. A. (2008). Coexistence of three specialist aphids on common milkweed, *Asclepias syriaca. Ecology, 89*(8), 2187-2196.

Stearns, S. C. (1989). The evolutionary significance of phenotypic plasticity. *BioScience, 39*, 436-445.

Strong, F. E. (1967). Aggregation behavior of pea aphids, *Acyrthosiphon pisum. Entomol. Exp. Appl., 10*, 463-475.

Sutherland, O. R. W. (1969). The role of crowding in the production of winged forms by two strains of the pea aphid, *Acyrthosiphon pisum. J. Insect Physiol, 15*, 1385-1410.

Tagu, D., Klingler, J. P., Moya, A., & Simon, J. C. (2008). Early progress in aphid genomics and consequences of plant-aphid interaction studies. *Mol. Plant Microbe Interact, 21*, 701-708.

Tanno, K., & Willcox, G. (2006). The origins of cultivation of *Cicer arietinum* L. and *Vicia faba* L.: Early finds from Tell El-Kerkh, North-west Syria, late 10th millennium B.P. *Vegetation History and Archaeobotany.* Retrieved from http://www.springerlink.com/content/713213743p4t6480/fulltext.html

Tariq, M., Wright, D. J., & Staley, J. T. (2010). Maternal host plant effects on aphid performance: Contrasts between a generalist and a specialist species on Brussels sprout cultivars. *Agricultural and Forest Entomology, 12*, 107-112.

Tétard-Jones, C., & Leifert, C. (2011). Plasticity of yield components of winter wheat in response to cereal aphids. *NJAS -Wageningen Journal of Life Sciences, 58*(3-4), 139-143. (doi:10.1016/j.njas.2011.01.003)

Tétard-Jones, C., Kertesz, M. A., & Preziosi, R. F. (2011). Quantitative trait loci mapping of phenotypic plasticity and genotype-environment interactions in plant and insect performance. *Phil. Trans. R. Soc. B, 366*, 1368-1379.

Tétard-Jones, C., Kertesz, M. A., Gallois, P., & Preziosi, R. F. (2007). Genotype-by-genotype interactions modified by a third species in a plant–insect system. *Am Nat, 170*, 492-499.

The International Aphid Genomics Consortium. Genome sequence of the pea aphid *Acyrthosiphon pisum* (2010). *PLoS Biol*, *8*(2): e1000313, 106-117. (doi:10.1371/journal.pbio.1000313)

Thompson, J. N. (1994). *The coevolutionary process.* Chicago: University Of Chicago Press.

Underwood, N., & Rausher, M. D. (2000). The effects of host–plant genotype on herbivore population dynamics. *Ecology*, *81*, 1565-1576.

Van Emden, H. F., & Harrington, R. (2007). *Aphids as crop pests.* London: CABI Publishing.

Volpelli, L. A., Comellini, M., Masoero, F., Moschini, M., Pietro, D., Fiego, L., & Scipioni, R. (2010). Faba beans (*Vicia faba*) in dairy cow diet: Effect on milk production and quality. *Italian Journal of Animal Science*, *9*(1). Retrieved from http://www.aspajournal.it/index.php/ijas/article/view/ijas.2010.e27/1289

Walsh, B. J. (1864). On phytophagous varieties and phytophagous species. *Proc. Entomol. Soc.* Philadelphia, *3*, 403-430.

Walters, K. F. A, & Dixon, A. F. G. (1982). The effect of host quality and crowding on the settling and take-off of cereal aphids. *Ann. Appl. Biol*, *101*(1), 211-218.

Weisser, W. W., & Stadler, B. (1994). Phenotypic plasticity and fitness in aphids. *Eur.J.Entomol*, *91*, 71-78.

West-Eberhard, M. J. (1989). Phenotypic plasticity and the origins of diversity. *Annu. Rev. Ecol. Syst.*, *20*, 249-278.

West-Eberhard, M. J. (2003). *Developmental plasticity and evolution.* Oxford, UK: Oxford University Press.

Whitham, T. G., Bailey, J. K., Schweitzer, J. A., Shuster, S. M., Bangert, R. K., LeRoy, C. J., Lonsdorf, E. V., Allan, G. J., DiFazio, S. P., Potts, B. M. Fischer, D. G., Gehring, C. A., Lindroth, R. L., Marks, J. C., Hart, S. C., Wimp, G. M.,

& Wooley, S. C. (2006). A framework for community and ecosystem genetics: From genes to ecosystems. *Nat Rev Genet*, *7*, 510-523.

Whitham, T. G., DiFazio, S. P., Schweitzer, J. A., Shuster, S. M., Allan, G. J., Bailey, J. K., & Woolbright, S. A. (2008). Extending genomics to natural communities and ecosystems. *Science*, *320*, 492-495.

Whitham, T. G., Morrow, P. A., & Potts, B. M. (1994). Plant hybrid zones as centers of biodiversity: The herbivore community of two endemic Tasmanian eucalypts. *Oecologia*, *97*, 481-490.

Whitham, T. G., Young, W. P., Martinsen, G. D., Gehring, C. A., Schweitzer, J. A., Shuster, S. M Wimp, G. M., Fischer, D. G., Bailey, J. K., Lindroth, R. L., Woolbright, S. , & Kuske, C. R. (2003). Community and ecosystem genetics: A consequence of the extended phenotype. *Ecology*, *84*, 559-573.

Whitman, D. W., & Agrawal, A. A. (2009). What is phenotypic plasticity and why is it important? In D. W. Whitman & T. N. Ananthakrishnan (Eds), *phenotypic plasticity of insects: Mechanisms and consequences* (pp. 1-54). Moscow, USA: Science.

Wimp, G. M., Wooley, S., Bangert, R. K., Young, W. P., Martinsen, G. D., Keim, P., Rehill, B., Lindroth, R. L., & Whitham, T. G. (2007). Plant genetics predicts intra-annual variation in phytochemistry and arthropod community structure. *Mol Ecol*, *16*, 5057-5069.

Wolf, B. J. (2000). Indirect genetic effects and gene interactions. In B. J. Wolf, E. D. Brodie, & M. J. Wade (Eds.), *Epistasis and the Evolutionary Process* (pp. 158-176). New York: Oxford University Press.

Wolf, J. B., Brodie, E. D., & Moore, A. J. (1999). Interacting phenotypes and the evolutionary process ii: Selection resulting from social interactions. *Am Nat*, *153*, 254-266.

Wolf, J. B., Brodie, E. D., Cheverud, A. J., Moore, A. J., & Wade, M. J. (1998). Evolutionary consequences of indirect genetic effects. *Trends Ecol. Evol.*, *13*, 64-69.

Wright, J. P., Jones, C. G., & Flecker, A. S. (2002). An ecosystem engineer, the beaver, increases species richness at the landscape scale. *Oecologia*, *132*, 96-101.

Zehnder, C. B., Parris, M. A., & Hunter, M. D. (2007). Effects of maternal age and environment on offspring vital rates in the Oleander aphid (Hemiptera: Aphididae). *Environ Entomol*, *36*, 910-917.

Zera, A. J., & Denno, R. F. (1997). Physiology and ecology of dispersal polymorphism in insects. *Annu Rev Entomol*, *42*, 207-230.

Zytynska, S. E., & Preziosi, R. F. (2011).Genetic interactions influence host preference and performance in a plant-insect system. *Evolutionary Ecology*, *25*(6), 1321-1333. (doi:10.1007/s10682-011-9493-7)

Zytynska, S. E., Fay, M. F., Penny, D., & Preziosi, R. F. (2011). Genetic variation in a tropical tree species influences the associated epiphytic plant and invertebrate communities in a complex forest ecosystem. *Phil Trans R Soc B*, *25*, 1329-13361333. (doi:10.1007/s10682-011-9493-7)

Zytynska, S. E., Fleming, S., Tétard-Jones, C., Kertesz, M. A., & Preziosi, R. F. (2010). Community genetic interactions mediate indirect ecological effects between a parasitoid wasp and rhizobacteria. *Ecology*, *91*, 1563-1568.

Additional Analyses

Ranking broad bean cultivars in the light of aphid reproductive success and aphid-cultivar interactions:

There were consistent patterns in performance of aphid genotypes, regardless of the host cultivar (**Table 1`**). Intra-specific variation in performance rank for pea aphids spanned a wide range across the rankings of the two other species, although *M. persicae* (**G8**) always ranked the lowest in front of other counterparts.

Rankings of cultivars based on aphid performance showed a distinct pattern with C4 consistently host to most aphid genotypes with considerable variation in host suitability among other cultivars (**Table 1`**).

Inter-specific	C4 > C1 > C2 > C3
Intra-specific	C4 > C2 > C1 > C3
Green *A. pisum*	C4 > C2 > C1 > C3
Pink *A. pisum*	C4 > C3 > C1 > C2

Table 1`: Comparison of ranked suitability of broad bean cultivars based on aphid reproductive success.

Broad bean genotypes were contrasted according to intra- and inter-specific population growth rates of aphids and *A.pisum* colour.

Such comparison reflects the extent to which broad bean cultivars differentially promoted reproductive success of aphids [(eight genotypes belonging to three aphid species; six pea aphid genotypes (four greens and two pinks) in addition to one vetch aphid and one green peach aphid genotypes].

There were notable differences in variability of aphid clonal growth rates. The highest difference between the maximum and minimum rates of aphid population growth across broad bean cultivars was recorded for **G4** (17.99) and the lowest for **G2** (2.1; **Table 2`**). However, the variation within a genotype appeared unrelated to mean-performance rank as expected under a significant GxG interaction.

Genotype	G4	G1	G5	G6	G7 *M.v.*	G8 *M.p.*	G3	G2
Difference	17.99	10.35	5.21	5.04	4.52	3.4	3.37	2.1
Mean rank	2	1.5	5.25	5.25	2.5	7.75	6	5.25

Table 2`: **Mean performance rank and largest differences (Max-Min) in growth rate across the plant cultivars tested for each of the aphid genotypes**. The values displayed in the table indicate the differences between the minimum and maximum rates of population growth rates (performance) for the eight aphid genotypes used, across four broad bean cultivars. As such, this set of data implies differences in plasticity (in terms of reproduction) among aphid counterparts. [Pea aphids (**G1**…**G6**), *M. viciae* (***M.v.***; **G7**), *M. persicae* (***M.p.***; **G8**)].

Chapter 2: Community genetic effects versus competition effects in a model pea aphid system

*Statement of the nature and extent of my own contribution:

I designed, performed and developed the experimental work. I also collected, entered and visualised the data and wrote the manuscript under Dr.Richard Preziosi's supervision.

Mr.Tomos Potter helped maintaining part of the system in the laboratory and used a portion of the data for a project.

Article for *Animal Ecology*

Community genetic effects versus competition effects in a model pea aphid system

Mouhammad Shadi Khudr[1], Tomos Potter[1], Richard F. Preziosi[1]

[1]Faculty of Life Sciences, The University of Manchester, M13 9PT

Abstract

The effect of within-species genetic variation on associated ecological communities (community genetic effects) has been well documented. However, little is known about the relationship between community genetic effects and other ecological forces. Community genetic effects are likely to be especially important in clonal systems such as aphids where individuals experience pressures exerted by both heterospecifics and cons-specifics that will occur in the context of plant variation. Here we contrast the effects of competition and community genetics using a model system of aphids and their broad bean host plants. We observe the effects on a focal aphid experiencing both intra-specific and inter-specific competition. Observations were recorded when aphids had a choice among host plants or were confined to a single host. The moderation effect of competition on community genetic effects, and *vice versa,* on the reproductive success of aphids (i.e. performance measured as population growth rate) was not clear. However, we found highly significant interactions between community genetic effects and both inter-specific and intra-specific competition effects on aphid behaviour (i.e. on-plant distribution in terms of the affinity to congregate on specific parts of the plant). This suggests that the relative effect sizes are similar for both

ecological forces and implies an impact of modulated competition effects by community genetic effects on aphid response. We discuss the mechanisms by which such forces may evolve and be maintained in a system of complex community interactions. Our findings highlight the importance of unravelling the interwoven branches of ecology and evolution in agro-ecosystems.

Keywords:

Community genetic effects, competition, intra-specific genetic variation, pest behaviour, host choice, feeding-site preference, broad bean, pea aphid, microcosm, confinement.

Introduction

There is a growing recognition that the synthesis of ecology and evolution is essential for understanding how species interact and develop through time and space (Thompson 1994, Odling-Smee et al. 2003, Whitham et al. 2003, 2006, Pelletier et al. 2009, Schoener 2011). When ecology and evolution are examined relative to one another, it becomes evident that we need to investigate how multi-level processes (genetic, evolutionary and ecological), as well as the interactions, influence the interacting species within and amongst communities (Johnson and Stinchcombe 2007, Hughes et al 2008, Hersch-Green et al. 2011). The rapidly growing field of community genetics considers that the environment of a species may be significantly defined by the expression of other interacting species' genomes (community genetic effect); (Antonovics 1992, Agrawal 2003, Rowntree et al. 2011). This perspective presents a clear framework for the integration of ecology, genetics and evolution (Rowntree et al. 2011).

Species (interactors), while constructing their niches modify the selective environments where they exist and interact (Odling-Smee et al. 2003). Species activities will affect the growth, abundance, diversity, distribution and co-existence of the interactors a well as the structure of their communities (Karban 1989, Hunter and Price 1992, Odling-Smee et al. 2003, Smith et al. 2008). Organisms under selection, through a wide array of ecological interactions, may respond in a number of ways: they may attack and resist, and thus strive to manipulate resources (e.g. Dixon 1998, Powell et al. 2006). Alternatively, interacting species may become more cooperative in order to maximise fitness and thus co-exist (Inbar and Wool 1995), or they vary in superiority and competitive capacity and hence one interactor may displace another interactor (Dawkins and Krebs 1979, Odling-Smee et al. 2003). Therefore, the interactors, through their activities, will modulate shared resources that subsequently and consequently alter the ecology of the system, the selective forces in operation and the evolutionary trajectories of the species involved in an ongoing fashion (Odling-Smee et al. 2003, Laland 2004, Marquet 2009; see also Bailey and Whitham 2006a,b). As such, ecological forces in tandem may ameliorate or debilitate one another (Smith et al. 2008). These complex community scenarios are supported by an increasing amount of evidence that intra-specific genetic variation of the interacting species underlies such effects (Whitham et al. 2003, 2006).

A clear example of this is that of leguminous hosts and their phytophagous insect invaders. Variability in plant hosts, such as architecture, quality and resistance to herbivory (Karban 1989, Farnsworth et al. 2002, Johnson and Agrawal 2005) can have large effects on the fitness of associated phytophagous insect communities (Whitham et al. 2006, Haloin and Strauss 2008, Hersch-Green et al. 2011, Rowntree et al. 2011). In turn, plant enemies such as sap-feeding aphids, through their exploitation, selectivity and manipulation of available resources undergo a series of co-evolutionary

interactions with their hosts and other interacting invertebrate rivals (Dixon 1977, 1987, 1998, Powell et al. 2006). In such systems, plant associated inter-players strive to maximise their own fitness and maintain a portion of the shared resource (Van Valen 1973; see also Bergstrom and Lachmann 2003). One of the most notable outcomes in this regard is a co-evolutionary arms-race scenario where each party does its best to have the upper hand (Dawkins and Krebs 1979, Berenbaum and Zangerl et al. 1998).

New insights from community genetics research have made clear the need to examine the magnitude of community genetic effects. The relationships between community genetic effects and other eco-evolutionary processes such as competition are of particular interest. Such research should focus on interacting genotypes of one or more species subject to variation in the biotic and abiotic environments that include both the interactors and their interactions (Antonovics 1992, Bonchev et al. 2005, Whitham et al. 2008, Hersch-Green et al. 2011; see also Levin 1998, Odling-Smee et al. 2003, and Juarrero 2010). Interestingly, such investigation of the magnitude of community genetic effects can be usefully achieved by devising experiments that contrast the effects of intra-specific genetic variation in host-plants with the effects of competition between plant-associated phytophagous insects (e.g. Smith et al. 2008).

Competition as an eco-evolutionary force:
Notwithstanding the debate on its magnitude as a major ecological process, competition can be considered as an influential force in nature (Hairston et al. 1960, Hutchinson 1978, Connell 1983, Schoener1983, Denno et al. 1995, Denno and Kaplan 2007; see also Kaplan and Denno 2007). Competition can be symmetric or asymmetric (e.g. -/+, +/-, 0/+), and direct or indirect via the mediation of the host

plant (Denno et al. 1995, Denno and Kaplan 2007). However, the magnitude of competition effects should be influenced by the inputs imposed by selection and the constant environment-modifying feedback of species activities (Odling-Smee et al. 2003, Bürger 2002, King 2003, Jablonka 2004, Laland 2004, Fordyce 2006, Post and Palkovacs 2009, Juarrero 2010, Ernebjerg and Kishony 2011). Nevertheless, only a handful of studies have endeavoured to examine other processes and mechanisms plausibly modulating competition effects in the light of plant genetic variation (Smith et al. 2008).

Ideal systems especially useful for the examination of such effects are the low diversity systems found in agricultural fields. Conventional agricultural systems are usually patches of homogenous genetic backgrounds, that is, mono-varietal plots where genetic diversity of the dominant species is highly restricted or entirely absent. In this context, host plant intra-specific genetic variability will significantly affect the richness, abundance, survivorship and behaviour of associated invertebrate pests (Fritz and Price 1988, Dungey et al. 2000, Whitham et al. 2003, Johnson et al. 2006, Utsumi et al. 2011, Zytynska and Preziosi 2011).

Here we present a study where we measure the relative effects of community genetics and competition. We use the presence or absence of competition, intra-specific and inter-specific competition in order to quantify the relative magnitude of the community genetic effect, the competition effect and the effect of their interaction on the fitness (reproductive success) and behaviour (on-plant distribution) of a focal pea aphid genotype. To our knowledge, this is one of very few studies to cast light on the interface of ecology and evolution from this angle, i.e. community genetics versus competition (see also Whitlock et al. 2007, Smith at al. 2008, Östman 2011]. We also examine the effects when aphids are confined to a single host plant or have multiple choices among host plants, of high economic value, in microcosms.

Understanding the constitution of such interactions between plant genotype and aphid genotype under the conditions of intra- and inter-specific competition should be of great importance to further untangle the pest-crop relationships as well as developing a better comprehension of how ecology affects evolution and *vice versa* (Odling-Smee et al. 2003, Whitham et al. 2003, Whitham et al. 2006, Johnson and Agrawal 2007, Johnson and Stinchcombe 2007, Wimp et al. 2007, Östman 2011; see also Schoener 2011).

Aphid behaviour in the light of within-plant genetic variation and freedom of choice:

For a generalist aphid, decisions about host choice and on-plant micro-site feeding positions are attributed to chemical cues resulting from plant metabolites (Chapman 2003, Powell et al. 2006, Ranger et al. 2007). The affinity to specific hosts, and to particular parts of the host plant, is based on aphid pre- and post-ingestion probing, and what follows that process, and hence determines acceptance or discrimination of the plant (Powell et al. 2006). In addition, on-plant distribution and among plant distribution (preference) of aphid is governed by the availability of suitable hosts, the varying levels of plant defence (resistance), the presence of natural enemies and the presence and density of competitors (e.g. Smith et al. 2008, Ranger et al. 2007, Schuett et al. 2011, Utsumi et al. 2011). However, abandoning a potential host plant, for example due to overcrowding effects or host deterioration, has its own fitness consequences because such behaviour increases exposure to natural enemies and aphid dehydration (Powell et al. 2006).

The literature is rich with research on the influence of the quality and variation of host plants on the performance (reproductive success and survival) of phytophagous insects (e.g. Dungey et al. 2000, Whitham et al. 2003, Hochwender and Fritz 2004, Johnson et al. 2006, Ruhnke et al. 2006, Smith et al. 2008, Ruhnke et al. 2009, Utsumi et al. 2011, Zytynska and Preziosi 2011). Moreover, host genotypes are

expected to vary in their susceptibility and resistance to invertebrate pests. Therefore, the latter will experience different rates of reproductive success (population growth rates) on distinct host genotypes (e.g. Underwood and Rausher 2000, Hughes et al. 2008). However, with regard to bottom-up and cascading effects in multi-trophic systems (Power 1992, Hambäck et al. 2007, Johnson 2008), there is a lack of studies exploring the changes in host choice and micro-site feeding preference of aphids under intra-specific and inter-specific competition, and in the light of host genetic and phenotypic variation (but see Smith et al. 2008, Utsumi et al. 2011, Zytynska and Preziosi 2011).

Ferrari et al. (2006) examined pea aphid preference for an array of host species where aphids were shown to display an affinity for the host plants they originally associated with. Furthermore, Zytynska and Preziosi (2011) proposed a genetic variation basis for aphid preference for barley. Further in their data interpretation, they implied the occurrence of 'assortative associations' between aphid and barley genotypes.

Host plants interact in various ways through roots and shoots, both physically and chemically, directly and indirectly. There is a growing body of evidence supporting the concept of plant-plant communication through "allelobiosis" i.e. inter-plant volatile signalling under pest attack (Pettersson et al. 2003, Baldwin et al. 2007). Furthermore, similar 'associational neighbourhood' effects of communication within plots between infected and healthy plants, will differentially influence susceptibility and resistance to plant enemies (Borges 2005, Barbosa et al. 2009). As such, the interaction amongst infected and relatively intact plants in a plot can have consequences for aphid fitness and host choice by aphids (Denno et al. 2000, Baldwin et al. 2007, Glinwood et al. 2009, Ninkovic and Åhman 2009). Therefore, inter-plant communication in the plot should play an important role in shaping the dynamics of

plant-pest interaction because plants will differ in their susceptibility as well as attraction to plant invertebrate consumers (Whitham 1989, Baldwin et al. 2007, Barbosa et al. 2009). This is particularly pertinent in the light of sink formation of highly attractive plant individuals (pest-attractive hotspots) that will cause aphids to colonise them in preference to adjacent plants (Whitham 1989; see also Larson and Whitham 1991, 1997, Barbosa et al. 2009 and Utsumi et al. 2011).

Logistically, in a study on aphid feeding behaviour and competition within microcosms of mono-cultivated broad bean, Salyk and Sullivan (1982) demonstrated that, in the absence of a competitor, pink pea aphid (*Acyrthosiphon pisum*) displayed a notably lower affinity to stems than leaves and preferred the undersides of leaves to the above-leaf feeding sites. However, their study showed no significant changes in the behaviour of pea aphid under competition.

To our knowledge, this is one of the first studies to examine the influence of the interaction between community genetic effect (plant genotype) and competition effect on aphids. We examined the effects on the performance (reproductive success) and behaviour (on-plant distribution and making a choice) of a focal aphid genotype:

1) With and without inter-specific competition across several host genotypes.
2) Under intra-specific competition across several host genotypes.
3) Under inter-specific competition across several host genotypes

Methodologies

Aphid performance

We compared the magnitude of community genetic effects and competition effects on a focal genotype of pea aphid (*Acyrthosiphon pisum* Harris). The focal pea aphid was the pink genotype P127 (hereafter **FP**) obtained from Imperial College (London). Intra-specific competitors were green pea aphid genotypes (G1, G116,

LLO1, BotGr; **G1**, **G2**, **G3**, **G4** respectively henceforth). Genotypes **G1**, **G2** and **G3** were also supplied by Imperial College (London) while **G4** (BotGr) was reared in the lab from a single aphid instar selected from the progeny of a gravid aphid female. The latter was collected using a bait of broad bean plant at the University of Manchester Botanical Grounds.

The inter-specific competitors we used were vetch aphid (*Megoura viciae* Buckton; clone established from a single individual collected at the University of Manchester Botanical Grounds, hereafter **Meg**), green peach aphid (*Myzus persicae* Sulzer; clone established from a single individual collected near Buxton, UK, hereafter **Myz**) and black bean aphid (*Aphis fabae* Scopoli; clone established from a single individual collected near Chorlton, Manchester, UK, hereafter **BL**). All stocks of aphids were maintained on broad bean (*Vicia faba* L. var. major Harz) in growth cabinets at [22°C and 16:8 (L:D) photoperiod]. This cultivar (major) was not used in the experimental procedures in order to avoid preconditioning effects.

Measures of fitness

Fitness is a problematic term with no definite agreement on a universal definition (Rosenberg and Bouchard 2010). However, there are several ways to estimate the fecundity and survival of parthenogenetic aphids and the factors influencing such fitness. Well documented approaches include estimation of differential reproductive success (which is equivalent to population growth rate per capita; e.g. Agrawal et. al. 2004), pest total dry weight (e.g. Petersen and Hunter 2001), and estimation of behavioural traits (i.e. consistency in behavioural differences amongst conspecifics across time and contexts e.g. Bergmüller and Taborsky 2010). In addition, plasticity traits especially polyphenism (i.e. production of extremely discretely plastic morphs such as winged individuals) can also be used as a fitness

proxy. This is useful in order to estimate the investment in reproduction (fecund apterous individuals) vs. dispersal (less fecund alate individuals); (Dixon 1977, 1987, Whitman and Agrawal 2009). Ultimately, fitness in aphids can usually be defined as the relative reproductive success of clones (genetically identical lineages); (Murray 1990, Giske et al. 1993, Agrawal et al. 2004). In this study we adopt the approach applied by Agrawal et al. (2004) in order to calculate population growth rates. The following equation was applied to obtain the population growth rates per capita; Agrawal et al. (2004):

$$GR = \frac{\left(\ln(N_2) - \ln(N_1)\right)}{t}$$

where **GR** is Population Growth rate, N_1 is the initial number of aphids N_2 is the final number of aphids and t is the amount of time the clone has been reproducing.

Further notes on fitness estimates and genetic variation

Genotypic variation regarding the response of aphid clone to environmental stimuli represents the differential response of different genotypes, belonging to one species of aphid, to suitability of a spectrum of host plants. Such response can be quantified by calculating the reproductive success (performance) of these focal genotypes. In this regard, fecundity is commonly used as an estimate of fitness for aphids (e.g. Underwood 2009, McLean et al. 2011, and Silva et al. 2012). This can be calculated over a given period of time by estimating the population growth rates/densities of a focal aphid genotype (Underwood 2009, Agrawal et al. 2004) or the intrinsic rate of increase in their numbers (Silva et al. 2012). Herein, we define plant suitability via the reproductive success of the focal aphid genotype, rather than plant traits (following

Underwood 2009). Within-cultivar differences for each of the broad bean genotypes used in this work are unknown (Underwood 2009)*.

Moreover, in this work, aphids experienced no seasonality and the conditions were mesic and hence ideal for sustained asexual reproduction. Therefore, taking into consideration the elimination of the preconditioning effects as explained above in the methods, here, the fitness of each focal aphid clone represents the fitness of its underlying genotype (*sensu* Weisser and Stadler 1994). However, the fitness of the focal genotype will be affected by the sequence of the plastic morphs proliferated in the clone in response to changes in the environment of mothers and daughters (*Idem*).

Statistical analysis

The responses of **FP** to competition and community genetic effects were based on relative performance (population growth rate). Performance of **FP** was taken as the final total number of individuals per replicate. Because the starting number of individuals and the experimental duration was the same for each replicate, analysis of final numbers is equivalent to analysis of growth rates (Agrawal et al. 2004). We included the absolute numbers of the competitor as a covariate to control for the total number of aphids on the plant. We analysed the performance data as a 2-way ANOVA with both competitor and broad bean cultivar as fixed effects. Statistical analyses were performed using JMP V9. We also analysed the data as percent **FP** on each replicate but the pattern of results was similar and so are not presented here. Data illustration was processed using MS Excel 2003 and Inkscape SVG graphic editor (ver. 0.48.01 under GPL).

---

*(See also the annex to this chapter, chapter-one methods and thesis general discussion for further discourse.)

Alate morph production

Polyphenism (here, the production of winged individuals versus the production of apterous individuals) was monitored for **FP** and its rivals for the three experiments of this work. We used polyphenism as a secondary parameter for aphid fitness i.e. the ratio 'alate:apterous' for each aphid clone indicated the level of investment in dispersing morphs against sedentary individuals (Dixon 1998, Hatano et al. 2010). This is expected to vary under the influence of plant genotype, (plant genotype x aphid) competition, and relative freedom to choose among hosts.

Aphid Behaviour

On-plant feeding site selection (on-plant distribution) was recorded for **FP** genotype in the confined enclosures and multiple choice plots, and in the presence and absence of inter-specific competition. We recorded the location of **FP** in terms of position on stem versus leaves and position on the top, middle or bottom thirds of the entire plant.

Note on aphid host preference and niche utilisation

Host choice for plants to colonise within each microcosm, under the presence and absence of the inter-specific competition with black bean aphid, was recorded for the focal pea aphid genotype **FP**. Four broad bean genotypes were used but each microcosm contained a single cultivar. Differential use of the resource (microcosm) by **FP** 'with' and 'without' competition was also estimated. All plants within each microcosm were numbered from 1 to 14 [i.e. (P1, P2, P3,…, P14)] and assigned specific positions within the microcosm. This was standardised for all microcosms across treatments of Experiment **I**. Note that we used only descriptive statistics for this part of Experiment **I** because the community genetics statistical methods for testing association under these conditions are under-developed.

For our focal aphid genotype **FP** in the presence and absence of competition, we consider the choice made for, and reproductive success achieved on, the plants which were successfully colonised in the microcosm by the end of the experiment. Any plants rejected (no colonisation established), depleted, or abandoned were recorded as zero at day 14. We used the traces of aphids' exuviae (shed skin, i.e. cast-off skins) on the plant and on the surface of the soil of a focal plant pot as a proxy to determine whether (or not) the discriminated or depleted plant had been colonised and hence abandoned before a final successful colonisation occurred elsewhere in the microcosm.

Broad bean genotypes

Aphids used in Experiment **I** were reared on each of the following broad bean (*Vicia faba*) cultivars; (Masterpiece Green Longpod, Syrian (var major), Syrian (var minor), Sutton; C1, C2, C3 and C4 respectively, henceforth). Aphids used, in both Experiment **II** and Experiment **III,** were reared on each of the following broad bean cultivars; (Masterpiece Green Longpod, Optica, Sutton; C1, C2, and C3 respectively, henceforth). All cultivars were procured via commercial seed suppliers. These selectively bred varieties (also referred to as accessions and heirloom) are synthetics (Duc 1997, Flores et al. 1998) that do not exist in the wild. These cultivars have been maintained for hundreds of generations as inbred lines that act as proprietary trademarks. Because of their long history of inbreeding we treat them here as equivalent to genotypes (Cordeiro et al. 1983, Oyedokun 1985, Underwood and Rausher 2000, Sharpe and Lydiate 2003, Emebiri et al. 2005, Hash et al. 2006, Kan et al. 2010, Rose et al. 2010). For all experiments plants were standardised in age (1 month) and trimmed constantly to a standardised height (12 cm). Broad beans were planted in John Innes no. 3 steam-sterilised compost in 3.5" pots.

*Experiment **I** – With and without competition*

We compared the performance (population growth rates) of **FP** in the presence and absence of **BL** (*Aphis fabae*) when raised on four cultivars of broad bean (C1, C2, C3, and C4). In summer 2009, broad beans were planted in mono-varietal, mesh-covered microcosms of 14 plants (8 microcosms giving a total of 112 plants). Microcosms (230 x 155 x 170mm) were placed in a growth cabinet at 22°C and 16:8 (L:D) photoperiod. Aphids were allowed to move freely among plants within each microcosm. At the start of the experiment 14 second instar aphids were added to each microcosm; 14 **FP** aphids for the 'no competition' treatments and 7 **FP** with 7 **BL** for the 'with competition' treatments. Microcosms were watered through the mesh every two days to maintain mesic conditions. All aphids on each plant were counted and their positions recorded on day 14 of the experiment.

*Experiment **II** – Inter-specific competition*

We compared the performance of **FP** in the presence of heterospecific competitor [(**Myz)** or (**Meg**)] on each of three cultivars of broad beans (C1, C2, C3). In winter 2010, broad beans were planted in 6 inch plastic pots and individually covered with 22 ounce plastic cups with two 4cm x 4cm fine mesh windows to allow ventilation. Cups were sealed to the pots with PVC tape. We had 10 replicates of each combination for a total of 60 plants (two competition treatments and three host cultivars). At the beginning of the experiment, four second instars were added to each pot; four **FP** for the 'no competition' treatment and two **FP** with two heterospecifics for the 'competition' treatments. Plant enclosures were removed from the growth cabinets and watered every two days by injecting water directly into the soil through re-sealable pores near the base of the plastic pots. Plants were re-randomised in their placement when returned to the growth cabinets. All aphids on each plant were

counted and positions recorded on day 14. Control numbers were divided by 2 to correct for the doubled starting number of instars.

*Experiment **III** – Intra-specific competition*

We compared the performance of **FP** alone and in the presence of one rival of each of the four conspecifics (**G1**, **G2**, **G3**, **G4**) on each of three cultivars of broad beans (C1, C2, C3). We had 10 replicates of each combination for a total of 120 plants (four competitor treatments and three host cultivars). Replicates were set up and maintained as in Experiment **II**. All aphids on each plant were counted and their positions recorded on day 14. Experiments **II** and **III** were run simultaneously and in the same growth cabinets and thus share the controls (no competition) described in Experiment **II** above.

Figure 1 displays the components comprising the experimental part:
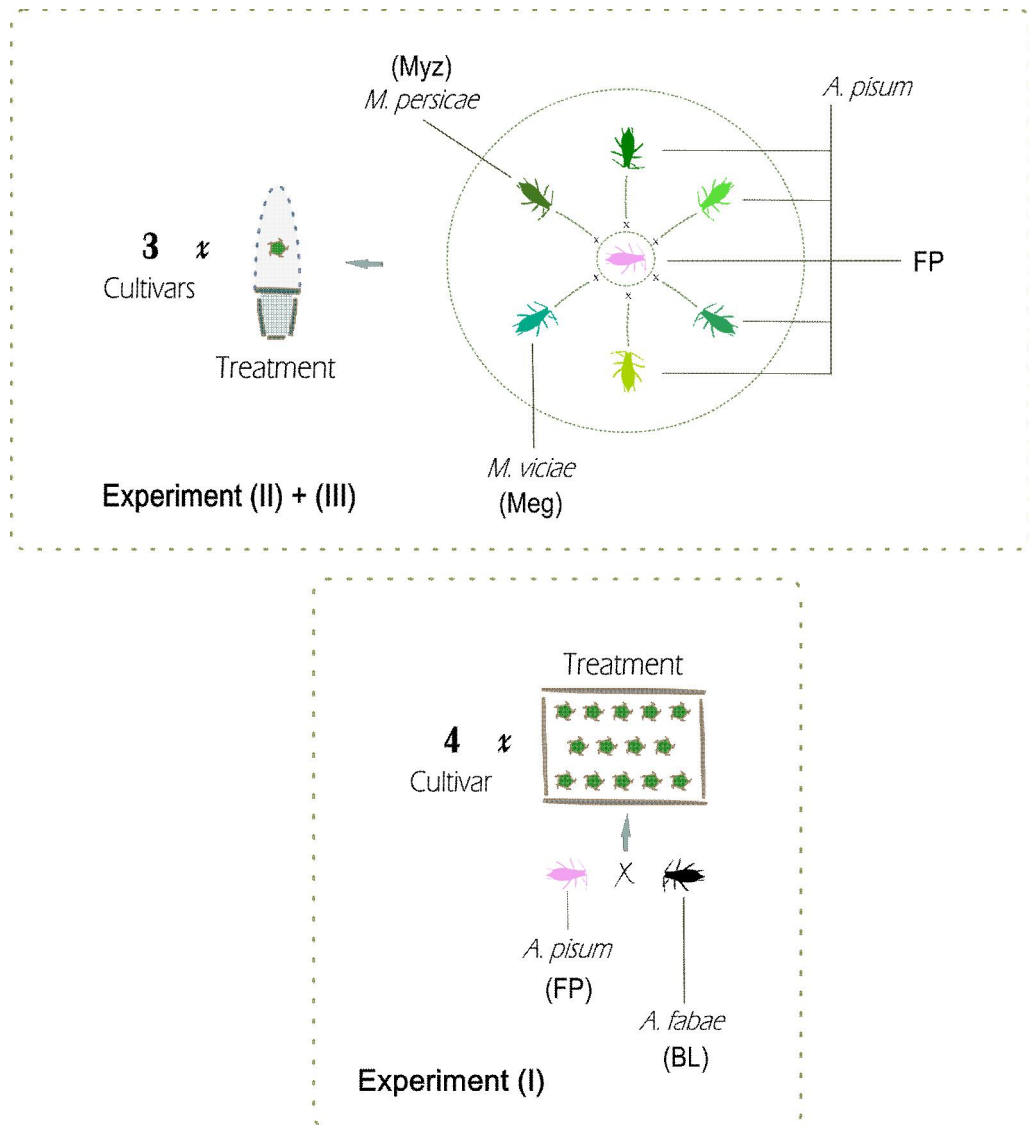
Figure 1: Experimental Design.

Experiment I: FP competed with black bean aphid. Aphids were given freedom of choice among host plants in the mono-varietal microcosms (14 plants per plot).

Experiments I + III: FP competed with each one of two heterospecifics (Experiment **II**) and each one of four green conspecifics (Experiment **III**). All aphids were confined to the focal broad bean genotype provided. Three cultivars of broad bean were used. Each host was housed in a transparent cone-shaped, 22-ounce propagator provided with meshed screens in order to maintain ventilation.

Results

Experiment I

1- FP Performance 'with' and 'without' competition:

No **FP** aphids had colonised 19 of the replicates belonging to the multiple-choice treatment at day 14 and thus these zero values were dropped from our analysis. Using the remaining 93 replicates we found a significant effect of broad bean cultivar ($F_{3,79}$=3.933, P = 0.0011), but no significant effect for the presence of competitor ($F_{1,79}$=1,2.4, P = 0.1738) or the interaction between cultivar and competitor ($F_{3,79}$=0.183, P = 0.9079, **Figure 2**).
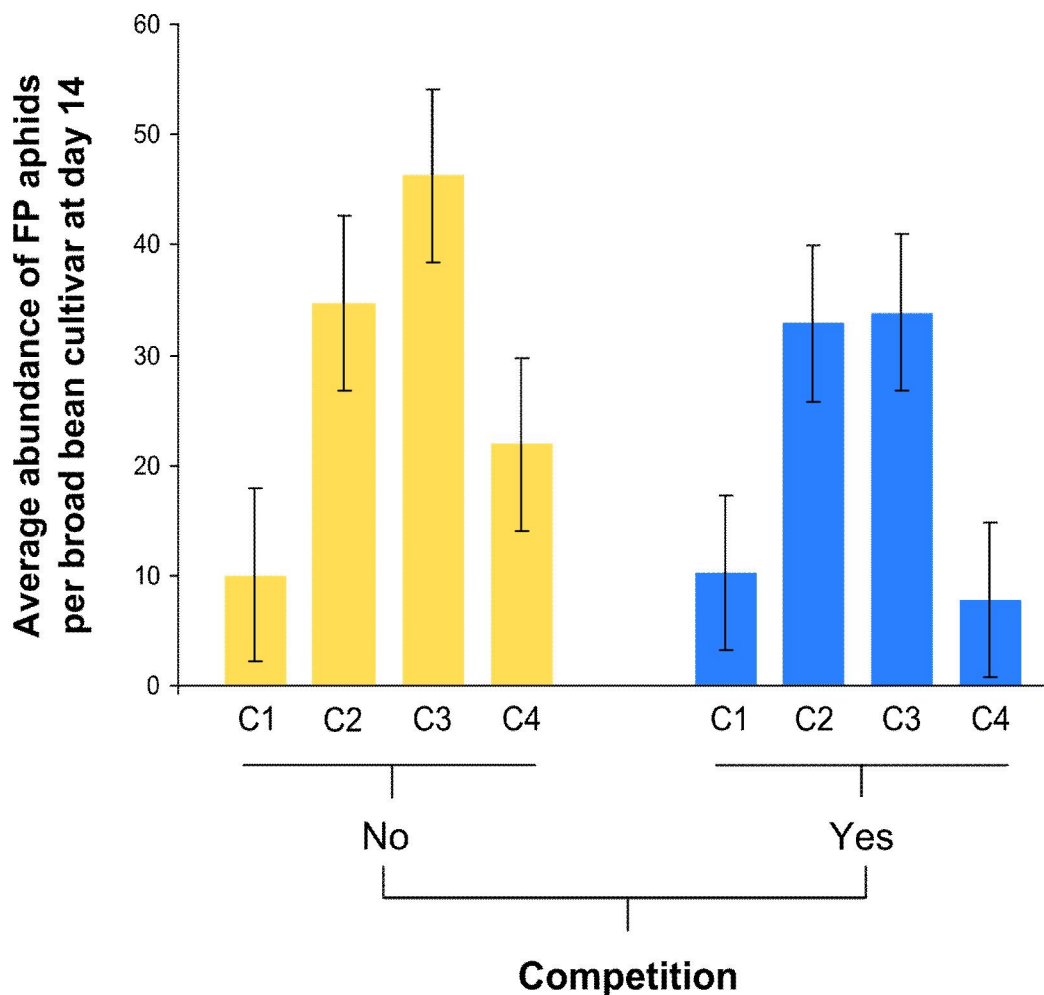
2- Production of winged FP morphs with and without Competition:

Production of alates by **FP** was extremely low at all times and was not consistently associated with the presence of competitors (**Table 1**). Only one winged morph was recorded on **C2** (no competition), and three winged morphs on **C4** (under competition).

	Alates	
Broad Bean Cultivar	**- Comp**	**+ Comp**
C1	0	0
C2	1	0
C3	0	0
C4	0	3

Table 1: Wing polyphenism. Numbers of **FP** discrete phenotypes (i.e. winged aphids a.k.a. alates) were recorded for each mono-varietal microcosm in the presence and absence of inter-specific competition. (Comp = Competition)

3- FP behavior (on-plant Distribution) 'with' and 'without' Competition:

No **FP** aphids had colonised 19 of the replicates belonging to the multiple-choice treatment at day 14 and so these replicates were dropped from our analysis.

A- Leaves vs. Stem:

We found highly significant effects of broad bean cultivar (χ^2 = 239.47, P <0.0001), but the presence of competitor had no significant effect (χ^2 = 1.90, P = 0.168). However, the interaction between cultivar and competitor was also very notable (χ^2 = 202.73, P <0.0001, **Figure 3**).

Mean abundance of FP aphids on plant shoot per cultivar at day 14

Legend: Leaves / Stem; No (solid), Yes (hatched); Comp

Competition: C1, C2, C3, C4 — each with Leaves and Stem groupings
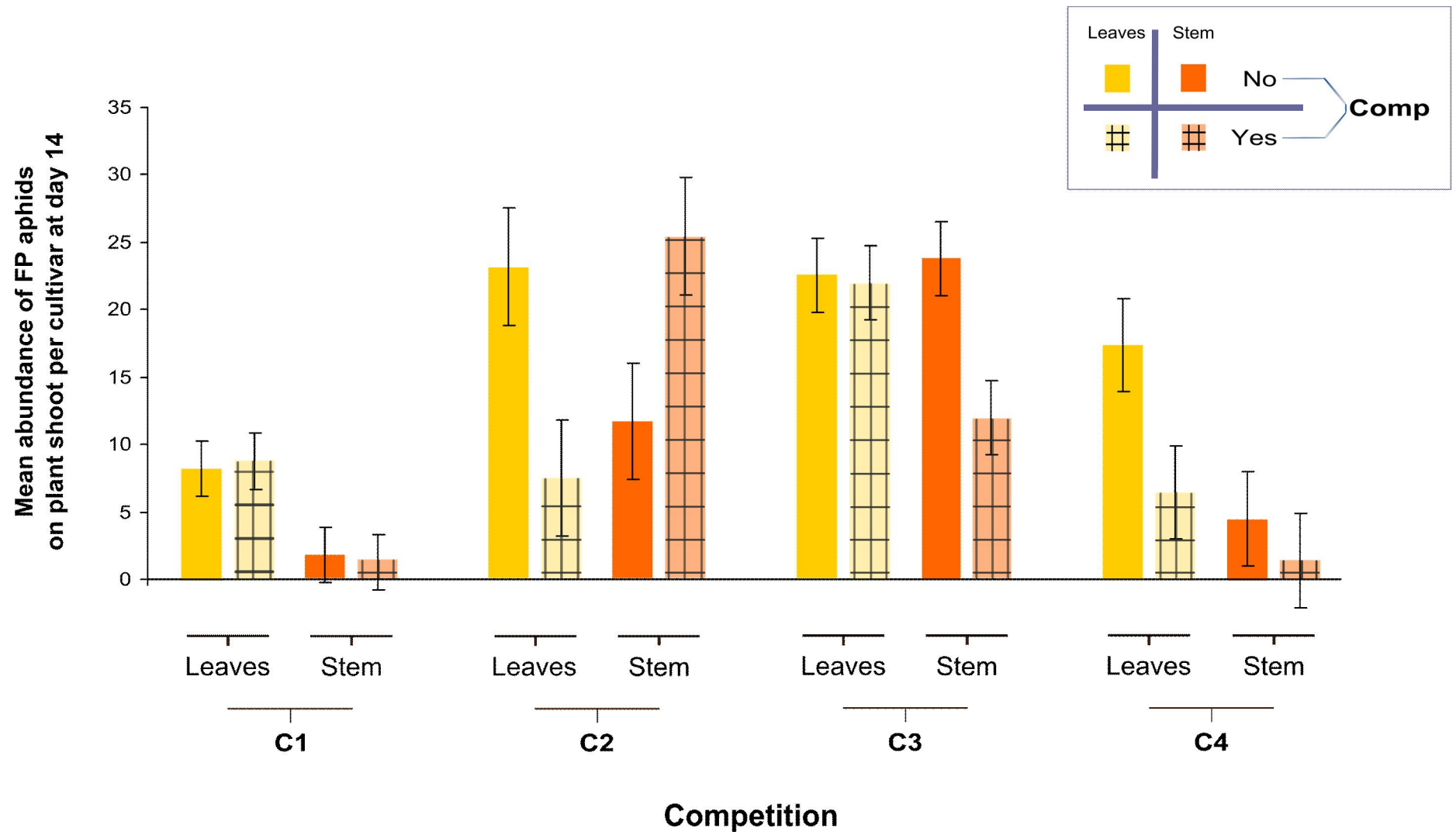
Figure 3: Aphid behavior examined as on-plant distribution; Leaves and Stem.

The patterns of affinity for leaf or stem by pea aphid focal genotype (**FP**), on four broad bean cultivars, are depicted in the presence and absence of competition. Bars represent mean abundance (+/- SE). The word 'No' refers to the case where **FP** performed without competition while 'Yes' refers to the case where **FP** performed under competition.

Further notes on FP's preference for leaves and stems in Experiment I:

❖ Proportions of **FP** on leaves were bigger than those on stems in six out eight cases; three times in the absence of the competitor on cultivars (**C1**, **C2**, **C4**), and three times under competition on cultivars (**C1**, **C3**, **C4**).

❖ Comparison of affinity for leaves 'with' and 'without' competition indicated that **FP** congregated 75% more on leaves under no competition than it did in the presence of the competitor.

❖ **FP** preferred to populate stems less under competition (3 out of 4 cases) if compared with its behaviour in the absence of competition (1 out of 4 cases).

❖ The biggest proportions on leaves was on **C2** and **C3** (no competition), and **C3** (competition). However, the least numbers of **FP** on leaves were recorded on **C1** (no competition). As for the 'under competition' lines, **FP**, in the presence of the competitor, preferred **C3** leaves the most while it showed approximate preference for the leaves of the remainder cultivars.

❖ **FP** preferred most the stem of **C3** without competition, but **C2** stem was the most preferred under competition. Conversely, the least numbers of **FP** on stems were recorded on **C1** and **C4** when the competitor was absent or present.

B- Leaf upper-sides and under-sides:

We found highly significant effects of broad bean cultivar (χ^2 = 24.17, P <0.0001), and notable effects for the presence of competitor (χ^2 = 7.84, P = 0.0051). The interaction between cultivar and competitor was highly significant (χ^2 = 50.90, P <0.0001, **Figure 4**).
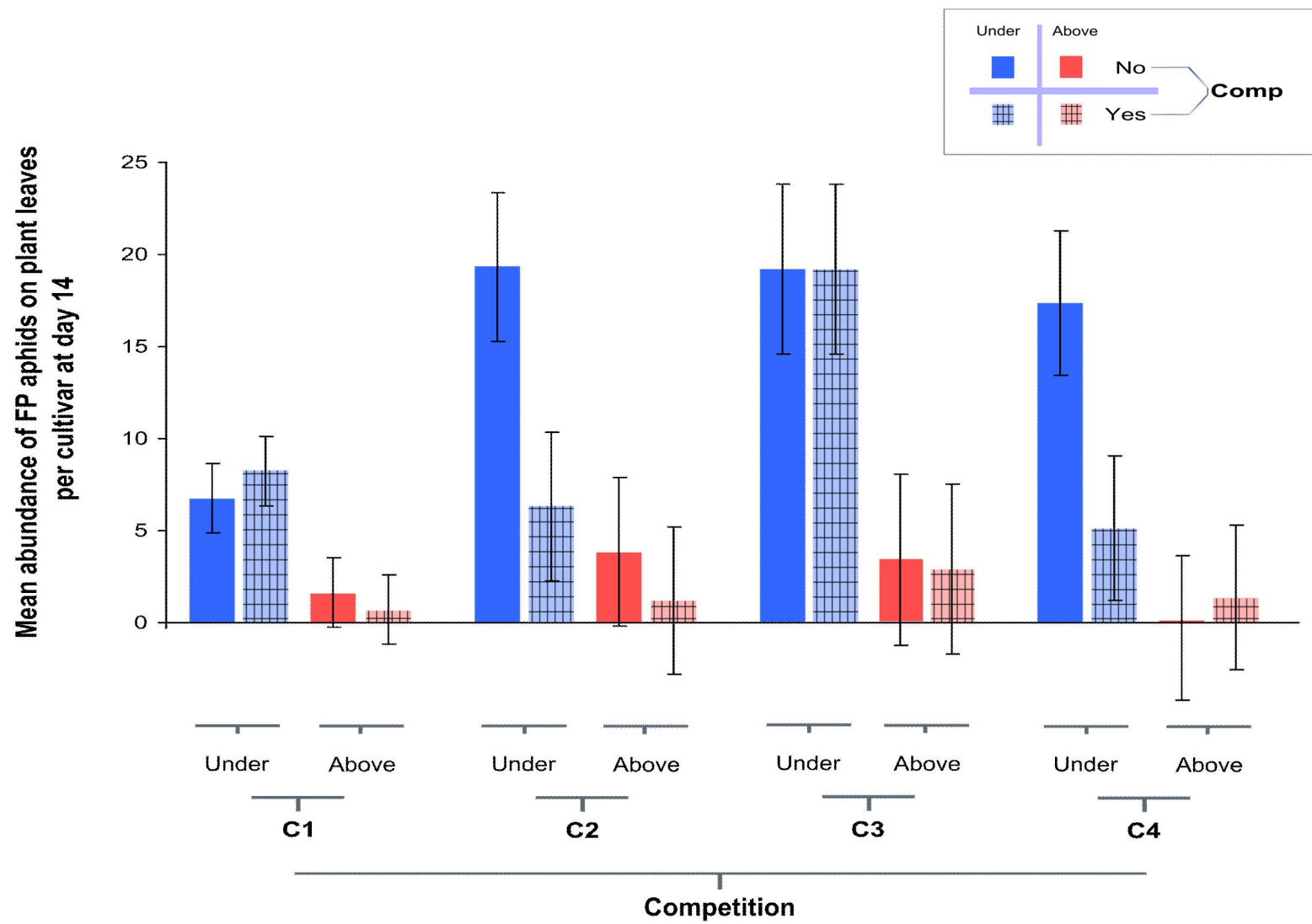
Figure 4: Aphid behaviour examined as on-plant-distribution; Upper-side and under-side of leaves.

The patterns of affinity for above-leaf surface or under-leaf surface are displayed for the pea aphid focal genotype **FP** on four broad bean cultivars, in the presence and absence of competition. Bars represent mean abundance (+/- SE). The word 'No' refers to the case where **FP** performed without competition while 'Yes' refers to the case where **FP** performed under competition.

Further notes on FP's preference for above- and under-leaf feeding sites in Experiment I:

- ❖ With 'no competition', **FP** always preferred under-sides to upper-sides of the leaves. The same pattern was observed in the presence of the competitor.

- ❖ Proportions of **FP** under leaves 'no competition' were bigger than the proportions under leaves under 'competition' in two cases out of four. However, on **C3, FP** showed similar patterns for both 'with' and 'without' competition scenarios.

- ❖ Regarding leaf upper-sides, abundances of **FP** under 'no competition' were always bigger than the abundances under competition except for the **C4** line.

- ❖ **FP** preferred to inhabit most the leaf under-sides of **C2** in the absence of competition, and it was **C3** leaf under-side that **FP** opted for when the competitor was around. However, regarding above-leaf choice, **FP** showed the most affinity for the leaf upper-sides of **C2** ('no competition' treatments), and **C3** for 'with competition' treatments.

- ❖ The least under-leaf abundance under 'no competition' was recoded on **C1**. However, under competition, **FP** occurred in minimal numbers on the leaf

123

under-sides of **C4**. However, **C4** leaf upper-sides attracted the least proportions of **FP** (no competition). In contrast, **FP** displayed the least preference for the above-leaf sites on **C1** 'under competition'.

C- More on aphid behaviour in terms of host preference and niche utilization:

The focal pea aphid genotype (**FP**) varied in its exploitation of the resource within microcosms, in the presence and absence of a heterospecific competitor (black bean aphid); **Figure 5** and **Figure 6**.

The results indicated a propensity for **FP** to spread more within the microcosm in the presence of the competitor if compared with **FP**'s host choice when the competitor was absent. The only exception was on broad bean **C3** where resource utilisation under 'no competition' was slightly bigger in percentage than the case where **FP** experienced competition; **Figure 5**. Moreover, comparatively, the most efficient utilisation of the microcosm was displayed on broad bean **C1** and **C4** where competition was in operation. However, broad bean **C4** correlated with the greatest tendency to aggregate instead of spreading throughout the microcosm. (See the annex to this chapter for further details on the depleted and discriminated plants per microcosm).
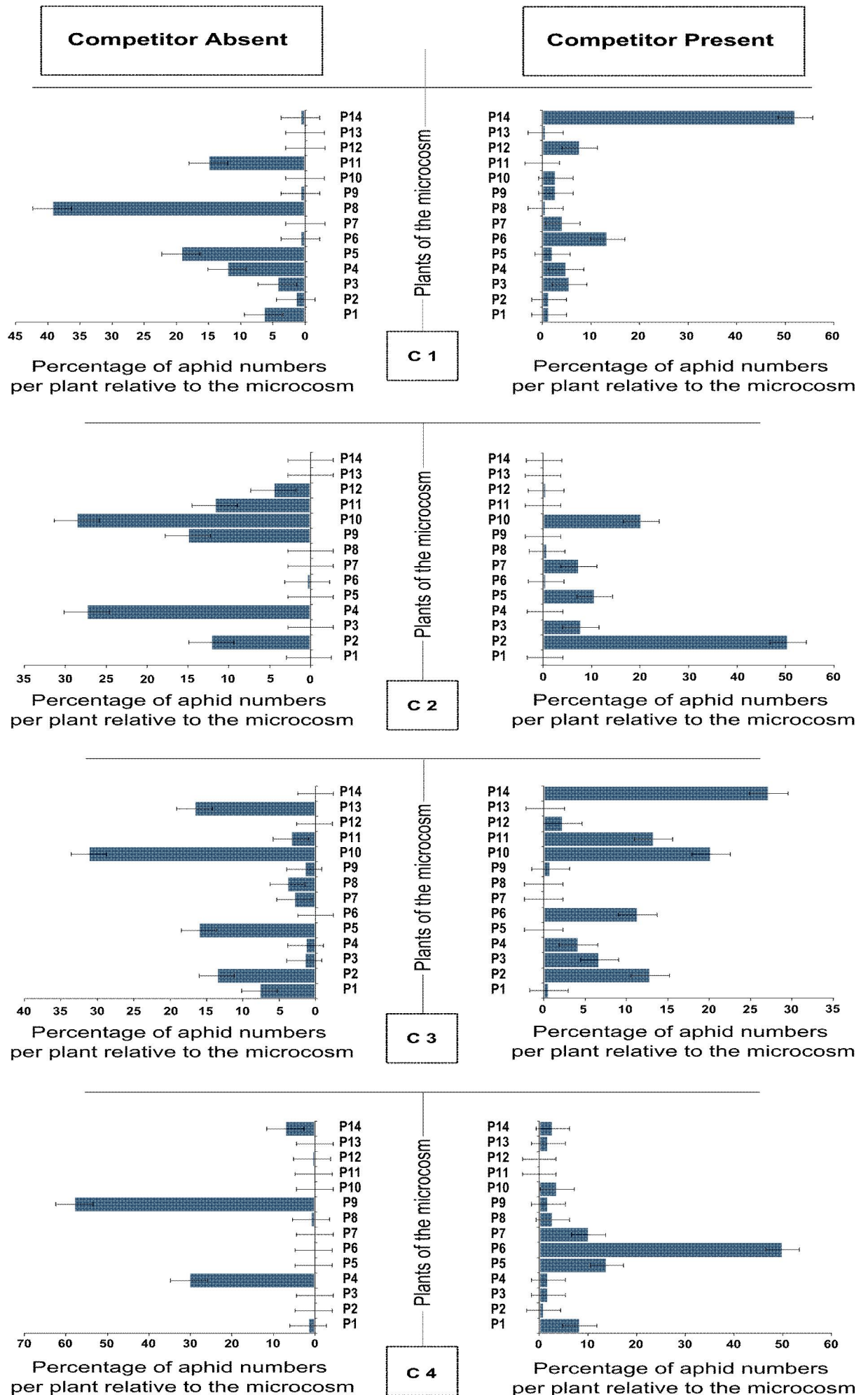
Figure 5: Illustration of aphid choice for feeding sites to colonise within mono-varietal microcosms, across four broad bean genotypes, and in the presence and absence of competitor.

Note that all the plants within a microcosm were assigned a specific location and had numerals assigned to them (1 to 14). Therefore, **P1**, **P2**, **P3**…**P14** in the assembled figure are abbreviations for the 14 numbered plants of any microcosm. All microcosms across all treatments in Experiment (I) were brought into conformity with the numbering fashion described above.

FP's choice for host plants to colonise within microcosms was contrasted in the presence and absence of the heterospecific competitor (black bean aphid) and across four broad bean genotypes. The experiment lasted for fourteen days and each microcosm included 14 plants per broad bean genotype. The percentage of **FP** numbers for each chosen and hence colonised plant within a focal microcosm is displayed relative to the total census of **FP** per focal microcosm. Percents which are reduced to nil refers to plants discriminated and thus never colonised, or plants depleted. A few plants acted as temporary stations before aphids made their final choice. Given the sedentary nature of aphids' motion and their propensity to congregate on suitable feeding sites (plants and plant parts), we note that, we inferred such choice dynamics of acceptance or discrimination of hosts and the incidence of transit-hosts by observing the exuviae of aphids (shed skin i.e. cast-off skins). For example, a healthy plant and rejected, with cast-off skins (exuviae) 'on board' or on the surface of the soil in the pot, implies that a discrimination happened after a period of plant probing. Also, note that those aphids were not given enough time to over-crowd since the experiment lasted for fourteen days. Therefore, should aphids have chosen to congregate on a specific plant in the microcosm is a matter of preference more than a consequence related to shortage of resource. [See **Figure 3**, **4** and **6** besides **Table 1**` (annex to chapter 2) for further details on the components of such dynamics].
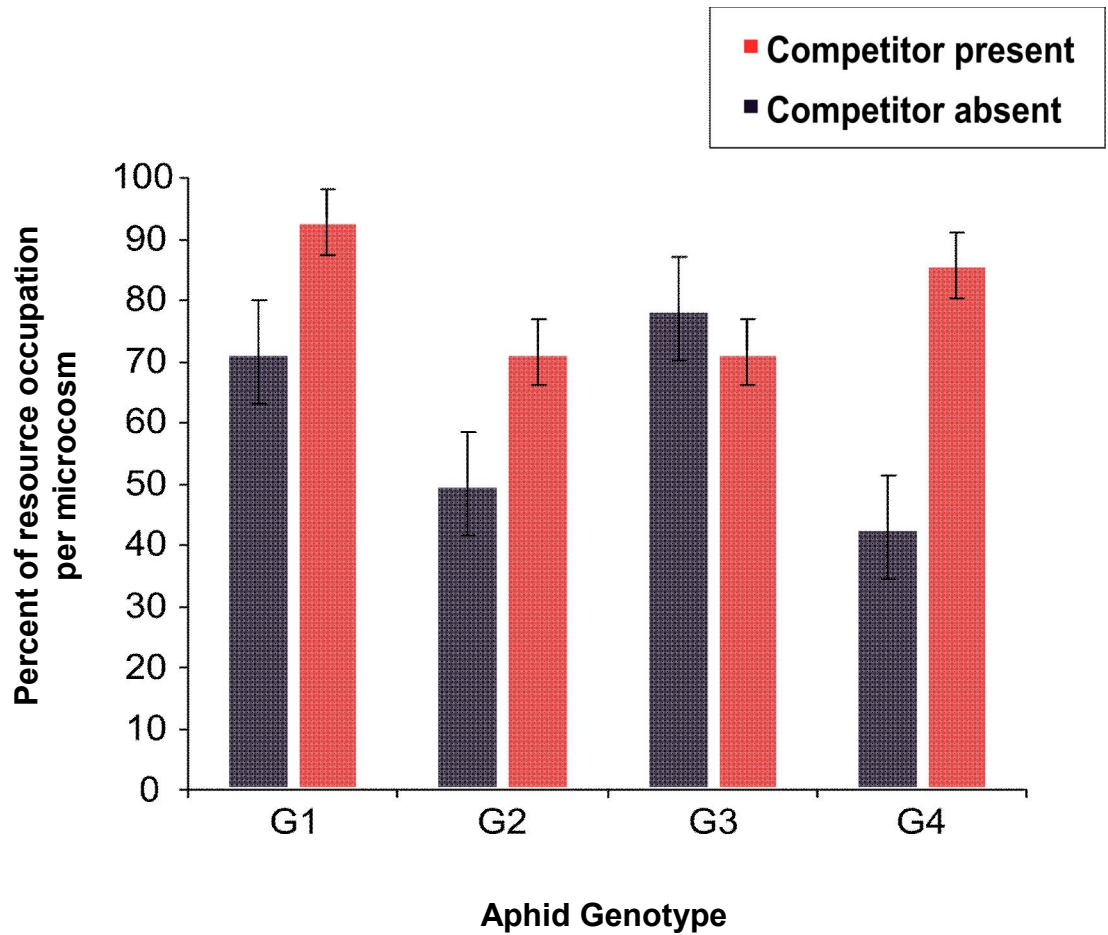
Figure 6: Percentage of resource (microcosm) usage by FP in the presence and absence of a hetero-specific competitor.

Under the presence and absence of competition, numbers of plants successfully colonised by **FP** were counted at day 14 and proportional use of the microcosm as a niche was calculated accordingly across four broad bean genotypes (cultivars). Each microcosm was mono-varietal and contained 14 broad bean standardised plants.

Experiments II + III

    1- FP Performance under intra- and inter-specific competition:

A- Inter-specific Competition (Exp. II):

FP aphids were not found on 4 replicates at day 14 and these replicates were dropped from our analysis. Using the remaining 49 plants we found no significant effect of cultivar ($F_{2,45} = 1.690$, $P = 0.196$) but the competitor showed a significant effect ($F_{1,57} =$, $P = 0.020$). However, no significant effect for the interaction between plant cultivar and competition was observed ($F_{2,22} =$, $P = 1.122$, **Figure 7**).

There was no significant effect of the number of competitor aphids as a covariate ($P = 0.130$) suggesting that it was the presence rather than number of the competitor that was important. Correcting for numbers of **FP** aphids on the control plants (i.e. $N_{competition} - N_{control}$) and using percent **FP** (i.e. N_{FP} / N_{total}) provided a very similar pattern of results, so those results are not presented here.

B- Intra-specific Competition (Exp III):

At day 14 no FP aphids were found on 9 plants and these replicates were excluded from our analysis. Using the remaining 101 replicates we found no significant effect of cultivar ($F_{2,15} =$, $P = 0.218$) or competitor genotype ($F_{3,22} = 1.034$, $P = 0.0.087$). The interaction between cultivar and competitor genotype was not significant ($F_{6,05} = 14.42$, $P = 0.774$, **Figure 7**).

There was no significant effect of the number of competitor aphids as a covariate ($P = 0.077$). As for the heterospecific experiment above, correcting for numbers of **FP** aphids on the control plants (i.e. $N_{competition} - N_{control}$) and using percent **FP** (i.e. N_{FP} / N_{total}) provided a very similar pattern of results and so those results are not presented here.
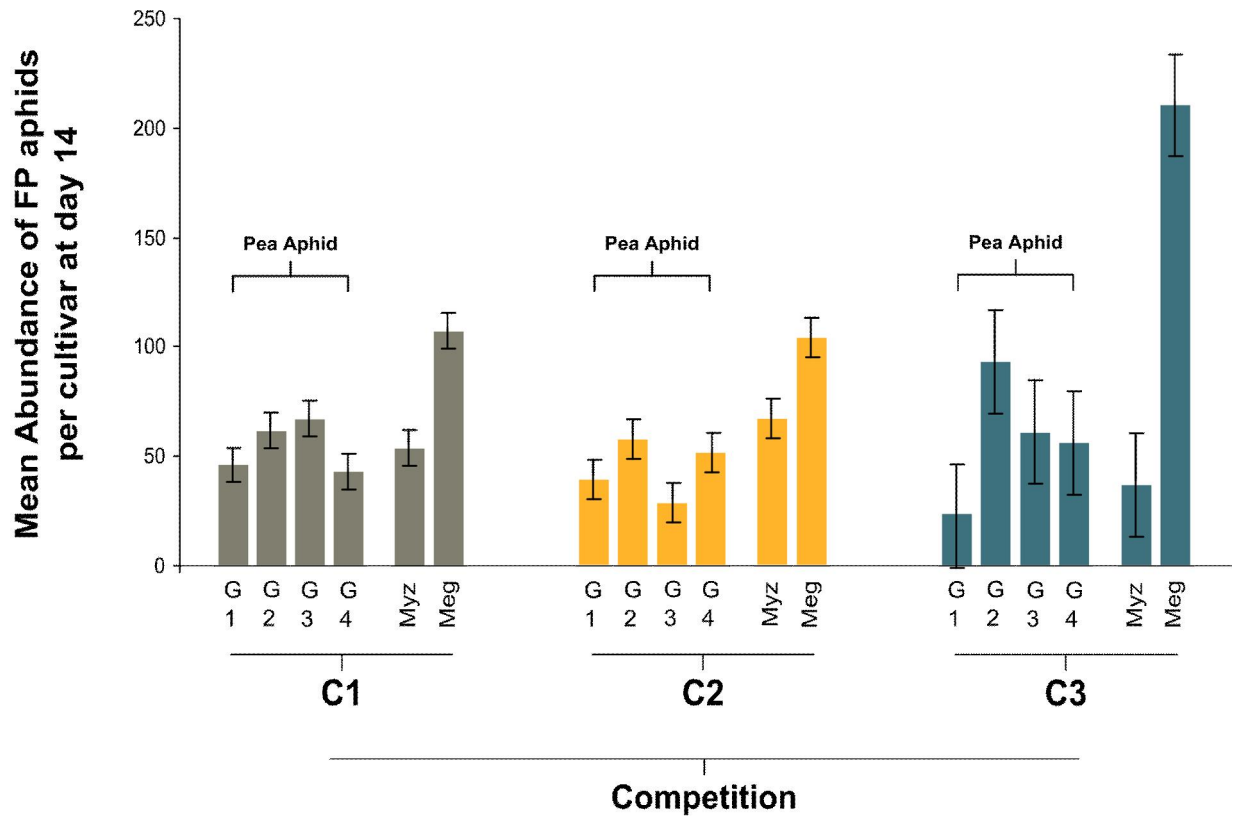
Figure 7: A plot illustrating the means and SE of FP within confined enclosures and across three broad bean cultivars.

Means of **FP** performance in front of conspecific genotypes (four) and two heterospecific genotypes were computed. The illustration displays the community genetics effects (CG) of broad bean cultivar and the interaction among rivals in enclosures. Three broad bean cultivars were used. The heterospecifics are **Meg** (vetch aphid) and **Myz** (green peach aphid). Each cultivar was given a specific colour (Gray for **C1**, Orange for **C2,** and Bluish green for **C3**).

❖ **FP** achieved the highest population growth opposite to conspecific (**G2)** on cultivar **C3**. Whilst, **FP** was most successfully reproductive on cultivar **C3** performing against the heterospecific (**Meg).**

❖ **C3** was also recorded as the cultivar hosting the least numbers of **FP** under intra-specific competition with **G1**, and against **Myz** for the inter-specific competition.

❖ The reproductive success of **FP** was less in magnitude against **Myz** than **Meg.**

❖ **FP** performed better in interaction with the heterospecific (**Meg)** on all cultivars in comparison with the performance of **FP** against its conspecifics.

2- Proportions of winged FP morphs under inter-specific competition:

Inter-specific Competition (Exp. II) and Intra-specific Competition (Exp III):
Production of winged morphs was very rare across plant genotypes and competition lines. At the intra-specific levels, alates were only noticed when **FP** competed with **G2** on **C1** (1 in average), and **C2** (0.09 in average). Conversely, inter-specifically, alates of were recorded when **FP** performed against **Myz** on **C2**, and against **Meg** on **C3**. The incidences of winged **FP** individuals were noticed on **C2** in front of con- and hetero-specific rivals (**Table 2**).

	Alates per cultivar	C1	C2	C3
Intra-sp	FP (versus **G1**)	0	0	0
	FP (versus **G2**)	1	0.09	0
	FP (versus **G3**)	0	0	0
	FP (versus **G4**)	0	0	0
Inter-sp	FP (versus **Myz)**	0	1.25	0
Inter-sp	FP (versus **Meg)**	0	0	0.11

Table 2: Averages of winged morphs.

Data showed a general notable propensity of **FP** to invest less in the production of dispersal morphs (alates) under both types of competition. **FP** slightly produced more winged individuals in the presence of heterospecifics. In total, broad bean cultivar **C2** appeared to correlate with more alate production than the other two broad bean cultivars.

3- FP behavior (On plant Distribution) with and without Competition:

A-Leaves vs. Stem:

1`- Inter-specific Competition (Exp. II):

At day 14 no **FP** aphids were found on 4 plants and these replicates were excluded from our analysis. Using the remaining 49 replicates we found very strong effect of broad bean cultivar ($\chi^2 = 177.43$, P < 0.0001) and competitor species ($\chi^2 = 318.88$, P < 0.0001). There was also highly significant effect of the interaction between cultivar and competitor species on aphid behaviour ($\chi^2 = 328.32$, P < 0.0001, **Figure 8**).

2`- Intra-specific Competition (Exp. III):

At day 14 no **FP** aphids were found on 9 plants and these replicates were excluded from our analysis. Using the remaining 101 replicates we found highly significant effect of cultivar ($\chi^2 = 157.67$, P < 0.0001) and competitor genotype ($\chi^2 = 50.04$, P < 0.0001). There was also very strong effect of the interaction between cultivar and competitor genotype ($\chi^2 = 175.02$, P < 0.0001, **Figure 8**).
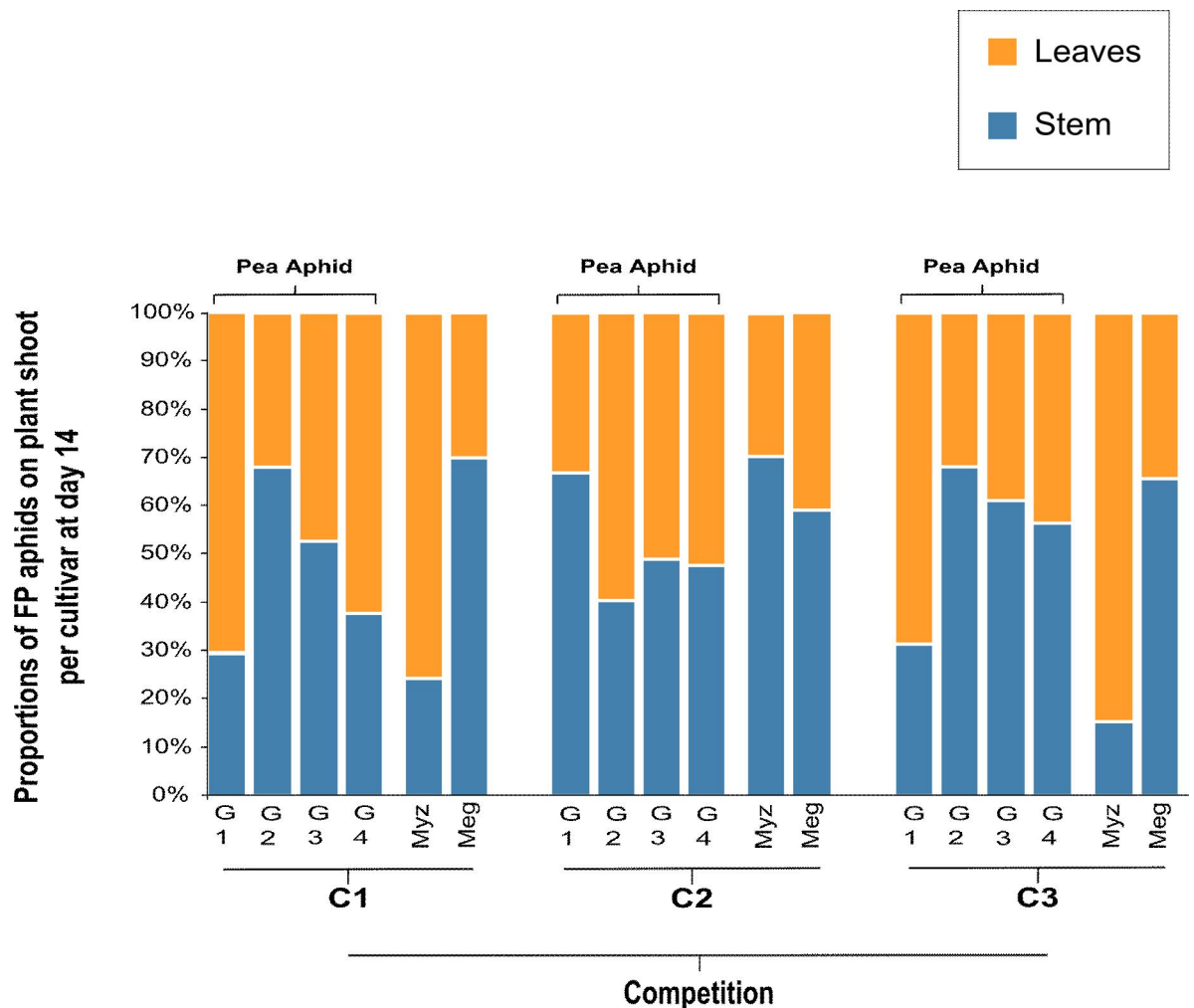
Figure 8: An illustration of on-plant feeding-site behaviour of FP (leaves or stem) under the influence of intra- and inter-specific competition and plant genotype. Proportions of **FP** pea aphid were observed on leaves versus stem for each of the six lines of interactions with other aphids (4 conspecifics and two heterospecifics) across three broad bean cultivars in individual enclosures.

Further notes on FP's preference for leaves and stems in Experiments II + III:

❖ Inter-specifically, more **FP** aphids chose to reside on stems when **FP** performed versus **Meg**. By contrast, stems hosted less **FP** aphids when **FP** competed against **Myz** (apart from the line of **C2**).

❖ **FP** under inter-specific competition preferred to congregate most on leaves against **Myz** on **C1** and **C3,** while **FP** populated leaves minimally in the presence of **Meg** on **C1, C2,** and **C3**.

❖ Against conspecifics, the highest densities on leaves of **FP** were against **G1** on **C1** and **C3**, but the lowest on leaves were against **G2** on **C1**, **G1** on **C2**, and **G2** on **C3.**

❖ Regarding preference for stem, the biggest proportions of **FP** were recorded in the presence of **Meg** on **C1** for the inter-specific competition, while the smallest proportions of **FP** were on **C3** against **Myz**.

❖ The data for intra-specific competition displayed the highest numbers of **FP** on stems across cultivars as follows: versus **G2** on **C1** and **C3**, and versus **G1** on **C2**. Whereas, **FP** showed the least affinity to stems against **G1** on **C1** and **C3**.

❖ **FP** behaviour (selectivity for micro-feeding sites on the plant) appeared to be approximately consistent when **FP** performed against **Meg** and **G3** respectively.

B- Leaf under-sides and upper-sides:

1`- Inter-specific Competition (Exp. II):

FP aphids were not found on 4 replicates at day 14 and these replicates were dropped from our analysis. Using the remaining 49 plants we found highly significant effect of cultivar (χ^2 = 23.642, P < 0.0001) but no notable effect of competitor species (χ^2 = 0.39, P = 0.531) 11767. However, there was a highly significant effect of the

interaction between cultivar and competitor genotype ($\chi^2 = 20.88$, P < 0.0001, **Figure 9**).

2`- Intra-specific Competition (Exp. III):

At day 14, no **FP** aphids were found on 9 plants and these replicates were excluded from our analysis. Using the remaining 101 replicates we found notable effect of cultivar ($\chi^2 = 14.70$, P < 0.0006) and competitor species ($\chi^2 = 156.13$, P < 0.0001). Moreover, the interaction between cultivar and competitor species had a highly significant effect ($\chi^2 = 61.62$, P < 0.0001, **Figure 9**).
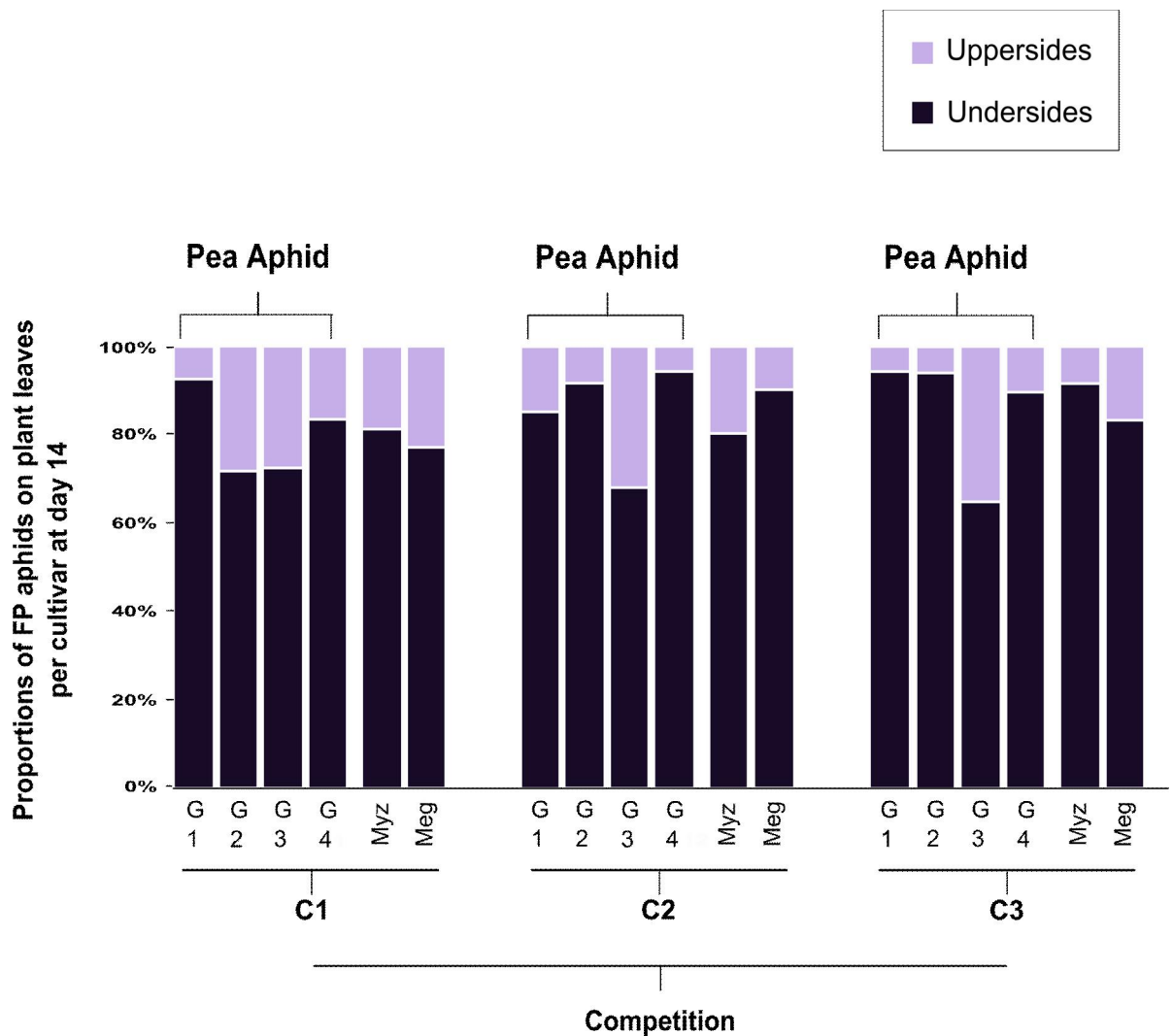
Figure 9: An illustration of FP displaying above- and under-leaf affinity (on-plant distribution) under the influence of intra- and inter-specific competition and plant genotype.

Focal genotype **FP** experienced six different competition scenarios (competition with four conspecifics and competition with two heterospecifics). The behaviour of **FP** for on-plant feeding-site preference was investigated accordingly across three broad bean cultivars.

Further notes on FP's preference for above- and under-leaf feeding sites in Experiment II + III:

- ❖ The highest readings for **FP** affinity for leaf under-sides were on **C3** against **Myz**, and on **C2** against **Meg** (inter-specific competition). **FP** showed the least abundances on leaf upper-sides for **C1** in front of the heterospecific **Meg.**

- ❖ Comparatively, against conspecifics, **FP** showed the maximal preference for under-leaf feeding sites as follows: on **C1** (vs. **G1**), on **C2** (vs. **G4** and **G2** respectively), and on **C3** (vs. **G1**, **G2**, and **G4** respectively). The minimum of **FP** proportions, under leaves, were found against **G3** on **C2** and **C3**.

Discussion

This work is one of the few studies attempting to investigate the interaction between competition and community genetic effects. In our study, competition among aphids was both intra- and inter-specific. The community genetic effect was present in both the genotypic variation in the host plant and the variation in the conspecific pea aphid competitors. This was particularly notable for the feeding-site choice of our focal aphid genotype but not for its reproductive success (performance). Our analyses

reveal a significant impact of the intra-specific genetic variation of broad bean on the response of a focal pea aphid genotype to competition with other aphids, regardless of whether the competition was heterospecific or conspecific, and regardless of whether the aphids had a choice of host plants.

Our findings support the supposition that competition as an ecological force operating at intra- and inter-specific levels can be modulated by the genotype of the host plant. Our results also directly address the question of the magnitude of community genetic effects relative to competition, and suggest that community genetic effects are of roughly equivalent influence in our study system.

Community genetics has been increasingly supported since first articulated as an experimental framework by Antonovics (1992). Genetic differences between conspecifics can result in differentiation in the structure and diversity of the plant-associated faunal communities and possibly differentially affect ecosystem processes (Whitham et al. 2003, 2006, Bailey 2011). This paper represents one of the first attempts to develop experimental work addressing the functionality of community genetic effects when other ecological processes (in this case, competition) are co-occurring (Smith et al. 2008, Hersch-Green et al. 2011).

The literature reports many examples of community genetic effects where plant hosts influence their associated invertebrate communities (e.g. Fritz and Price 1988, Karban 1989, Hunter and Price 1992, Dungey et al. 2000, Whitham et al. 2003, Johnson et al. 2006, Smith et al. 2008, Zytynska at el. 2011)..

Our results agree with Smith et al. (2008) that plant genotype by competition interaction can modulate the frequency and strength of competition. However, in their study on a system composed of three competing aphids on Milkweed (*Asclepias syriaca*), Smith et al. (2008) proposed a trade-off could occur between the intra-specific competition (influential in aphids; see Dixon 1977) and inter-specific

competition. By the same token, the balance towards any of those types of competition is also relative to the communication (i.e. interaction; probably competition) among the genetically identical copies of a single aphid clone. One governor of this intricacy is the Intra-specific Genetic Effects (IGEs) of such communication within a clone (Moore et al. 1997), whilst other stressors or motivators may take the form of the Indirect Inter-specific Genetic Effects (IIGEs); see Wolf et al. 1999 and Wolf 2000). Moreover, plant-mediated competition among aphids on a single host plant is even more likely to affect aphid survivorship (Inbar et al. 1995, Denno et al. 2000). However, there are only a few studies that examined the dynamics of the relationship between community genetic effects and competition (e.g. Fridley and Grime 2010; but see also Smith et al 2008, Östman 2011).

Phytophagous species strive through several means to maintain their existence (e.g. Van Valen 1973, Dawkins and Krebs 1979, Giordanengo et al. 2010). The roles species take within the ecological networks they comprise, and through the patterns of responses they bring about, place them into a spectrum of complex interactions (Odling-Smee et al. 2003, Kaplan et al. 2008, Ernebjerg and Kishony 2011). One type of such interactions is a co-evolutionary arms-race between pests and their host plants, where each party is constantly under natural selection to overcome the strategies of the opponent (Dawkins and Krebs 1979). Moreover, phytophagous insects are involved in a wide array of interactions with other taxa such as those cohabiting on the host plant or present within the vicinity of the host (Dixon 1998, Denno et al. 2000, Kaplan et al. 2008).

Sap-feeding aphids are extremely dependent on the diet they steal from their hosts and thus they need to be very sensitive to chemical and morphological phenotypic changes in the host plants. Their use of multiple perceptive mechanisms to detect and manipulate their hosts, make their relationship with the variably resistant

hosts intimate (Pickett et al. 1992, Powell et al. 2006). Aphids can probe, test, compare and cause biochemical changes in their hosts (Powell et al. 2006). Furthermore, they are able to discriminate among hosts and make decisions about which host to feed and reproduce on (Powell et al. 2006). Aphids also show affinity to their preferred hosts [Troncoso et al. 2005, Ferrari et al. 2006; but see (Bernays and Funk 1999) regarding the dynamics of making a choice where aphid is a specialist or a generalist]. Such characteristics are prominent attributes of parthenogenetic, polyphagous aphids regarding the selection of hosts that ensure maximal reproductive success i.e. genetic replication (*sensu* Dawkins 1976, 1982). An added complexity in aphid systems is that, under conditions that favour parthenogenetic reproduction (the conditions applied for this work), aphids reproduce clonally and thus genotypic effects occur at both individual and clonal levels. The aforementioned factors underpin the differential focal aphid's response and performance in this work to competition and plant genetic variation

Context interdependent partitioning:

This work demonstrated that on-plant distribution i.e. behaviour of the focal pea aphid genotype **FP** could be explained by their preference to populate on a specific region of the plant, and that this was very significantly influenced by plant genotype, presence of competition and the interaction between plant genotype and the presence of a competitor.

In our study, focal pink pea aphid **FP** (*Acyrthosiphon pisum*) performed in a manner opposite to **FP**'s green conspecific and heterospecific rivals (*Aphis fabae*, *Megoura viciae* and *Myzus persicae*). These other species are generalists that show sophistication in plant perception ability and consequent preference and decision making, as in **FP**. However, they (conspecifics and relatively partially heterospecifics)

share common factors regarding feeding-site habits and aggregation preferences (Pollard 1973). The 'social niche' specialisation, the interaction between plant genetics and competition, besides the trade-off between intra- and inter-competition modes are expected to underlay aphid capacity of making a choice as observed in this work (see also Smith et al. 2008, Bergmüller and Taborsky 2010).

Consequently, 'niche' specialisation of a specific aphid on its preferred host(s) is prone to change due to a set of factors e.g. changes in leaf preference by aphids according to leaf age (see Salyk and Sullivan 1982). However, in our work the broad bean plants were age-homogenised (young plants, approximately one-month-old) and maintained under standardised environmental conditions. Therefore, plant age was ruled out as a variable. (See the annex to this chapter for further insights on resource partitioning by aphids).

Zytynska and Preziosi (2011) advocated the importance of aphid choice regarding the performance-preference relationship and the concept of 'assortative association' of plant and pest genotypes. Although this may be an important aspect, the varying ability of the plants to arrest aphid movement (or resist aphid colonisation) is likely to influence aphid fitness, particularly when considering the propensity of aphids such as *A. pisum* to disperse in the patch after an initial inquiry phase (Pollard 1973, Powell et al. 2006).

Our results are concordant with the findings of Lowe and Taylor (1964) and Salyk and Sullivan (1982), in that the majority of pink pea aphids demonstrated a preference for the leaves of their hosts. However, Salyk and Sullivan (1982) recorded a marginal decrease in pea aphid propensity to reside underneath leaves when the black bean rival was present. Pea aphids prefer leaf veins (Lowe 1967, Salyk and Sullivan 1982) and their affinity to settle under leaves is strong (Muller et al. 1974, Salyk and Sullivan 1982). Salyk and Sullivan (1982) also proposed the occurrence of

inter-specific aggregation because, in their study, pea aphid showed only slight changes in preference when the heterospecific rival was present, which also in turn displayed a preference to be adjacent to pea aphids. Surprised by the oddity of the preference to colonise together, Salyk and Sullivan (1982) postulated an 'immunity' property of such aggregation that may help minimise negative impacts of standard competition. This is in contrast to the common expectation of niche separation as a result of competition among heterospecifics. However, the cited work (this study, as well) did not include a treatment where the rivals would be exposed to a threat by a natural enemy. We postulate that aggregation, whether amongst conspecifics and, more surprisingly and interestingly, amongst aphids belonging to different species, may be a logistic property of the rivals' tendency to co-exist as a guild i.e. the emergence of cooperative behaviours as extended (shared) phenotypes (Inbar and Wool 1995, Araújo et al. 2008, Yong and Miikkulainen 2009, Rawal and Rajagopalan 2010).

An additional factor regarding making a choice for a suitable host can be the presence of more chemically hospitable plants than other adjacent ones in the patch due to within-cultivar differences at the molecular level (see also Underwood 2009). After probing the available plants within the microcosm, aphids will experience spots (individuals) that are highly attractive and hence 'arrest' aphids and prompt successful colonisation. Consequently, other less attractive individuals within the microcosm will act as temporary stations or might be discriminated by aphids (see Whitham 1989).

Further explanation for the changes in preferences observed in this work can be attributed to sink effects. Foliage comprises a natural sink (source) for plants where minerals and metabolic products are assimilated. The presence of a sap-feeding enemy, particularly in large numbers, will alter the natural-sink mechanisms and dynamics. This will create artificial sinks that interfere with the normal metabolic

pathways of the host plant (Larson and Whitham 1991, Kaplan et al. 2008). However, aphids vary in the intensity of their sink effect (Inbar et al. 1995). The co-occurrence of more than one artificial sink (i.e. two or more con- or hetero-specific aphids) would be expected to burden the plant defensive system. This in turn should enhance the diversion of assimilates to profit the guild (assemblage) of sap feeders that possibly share the common goal of cooperatively maximising host exploitation under the constraint of a declining host resource [analogous to the principle of 'safe fail' or 'fail safe' as conveyed by Juarrero (2010)]. This implies that the fitness reward for guild formation and conflict avoidance, for a relative period of time, can be bigger than opting for antagonistic interactions between aphid species (e.g. Yong and Miikkulainen 2009, Rajagopalan et al. 2011). The sink effect is also expected to promote a plant-mediated interaction between aphid species or genotypes (Inbar et al. 1995). The latter can induce a trait-mediated response through the artificial aphid-sink competing with the natural one of the host plant (Kaplan et al. 2008); a response that can be an induced defence (Kaplan et al. 2008). We argue that the response of the focal aphid **FP** to competition was to modify its choices on and amongst resources in order to reduce the cost of conflict and maximise fitness gains (Dawkins 1976, 1982, Rawal and Rajagopalan 2010). Nevertheless, we note that competitors' experimental data were not presented here but were used as covariates in the analyses.

The patterns of performance and response against competitors that we observed in **FP** could be attributed to these types of plastic mechanisms. Plasticity (including polyphenism) of aphids is extensive through a spectrum of pathways, e.g. modified structures such as thrifty progeny which result in less resource consumption also through having stylets that vary in length (e.g. Inbar and Wool 1995; see also Whitman and Agrawal 2009). Such plasticity results in increased and optimised resource utilisation among aphid competitors (Inbar and Wool 1995). Such an effect

can dilute or weaken the competition effect across host cultivars [see also the factors reviewed by Fridley and Grime (2010) on the 'dilution' of the strength of interactions among species, and their ramifications on community structure and diversity]. In this sense, it is plausible to suggest that plants, despite being rather 'slow runners' (Red Queen Hypothesis; Van Valen 1973), can be resilient by recalibrating and changing their plastic defence-tactics against aphid attack in order to minimise fitness loss, and in doing so probably indirectly affect aphid propensities, choice and reproductive success (Red King Hypothesis; Bergstrom and Lachmann 2003; see also Holling 1973, 1976, Levin 1998 and Juarrero 2010). However, the inconsistency in plant response, whether it was direct, induced or both, leads to the proposition that community genetic effect can modulate the magnitude of the effects of competition.

Conclusion

We postulate that a pattern of modified competition (less antagonistic), varying according to plant vigour and resistance, aids sap feeding aphids in their manipulation of their shared resource. It is a case where exploitative competition and within-plant genetic variation moderate the effects of each other and influence aphid activities in utilising the host plant, in the presence and absence of competition. We envisage such moderation will induce a cooperative or guild-like relationship as an extended (shared) phenotype to emerge between the competing aphid species/genotypes (Dawkins and Krebs 1979, Yong and Miikkulainen 2001, Odling-Smee et al. 2003, Bergmüller and Taborsky 2010, Rajagopalan et al. 2010, Schuett et al. 2011). This empirical as well as theoretical logic diverges from the usual picture of 'antagonistic' competition into a much more cooperative type of interaction when the reduction of conflict is a requisite for a fitness pay-off (Bergmüller and Taborsky 2010,

Rajagopalan et al. 2011, Schuett et al. 2011), or probably a modified form of reciprocal altruism (Trivers 1971, Okasha 2009). Further research is needed to understand the nature of this relationship between performance and behaviour of phytophagous insects in order to increase our understanding of the influence of community genetics effects on aphid-aphid interactions on economic crops within agro-ecosystems.

References

Agrawal, A. A. (2003). Community genetics: New insights into community ecology by integrating population genetics. *Ecology*, *84*, 543-544.

Agrawal, A. A., Underwood, N., & Stinchcombe, J. R. (2004). Intraspecific variation in the strength of density dependence in aphid populations. *Ecological Entomology*, *29*, 521-526.

Antonovics, J. (1992). Toward community genetics. In R. S. Fritz, & E. L. Simms (Eds.), *Ecology and Evolution of Plant Resistance to Herbivores and*

Pathogens: Ecology, Evolution, and Genetics (pp. 426-449). USA: University Of Chicago Press.

Araújo, M. S., Guimarães, P. R., Svanbäck, R., Pinheiro, A., Guimarães, P., Dos Reis, S. F., & Bolnick, D. I. (2008). Network analysis reveals contrasting effects of intraspecific competition on individual vs. population diets. *Ecology, 89*(7), 197-213.

Bailey, J. K. (2011). From genes to ecosystems: a genetic basis to ecosystem services. *Popul Ecol 53*, 47-52.

Bailey, J. K., & Whitham, T. G. (2006a). Biodiversity is related to indirect interactions among species of large effect. In T. Ohgushi, T. Craig, & P.W. Price (Eds.), *Indirect interaction webs: Nontrophic linkages through induced plant traits* (pp. 306-328). UK: Cambridge University Press. Retrieved from http://web.utk.edu/~jbaile29/publications/Bailey%20and%20Whitham%20indi rect%20interaction%20webs.pdf

Bailey, J. K., & Whitham, T. G. (2006b). Indirect trait-mediated interactions between cottonwoods and beavers positively affect sawfly abundance. *Ecological Entomology, 31*, 294-297.

Baldwin, I. T., Halitschke, R., Paschold, A., Von Dahl, C. C., & Preston, C. (2007). Volatile signaling in plant-plant interactions: "talking trees" in the genomics era. *Science, 311*(5762), 812-815.

Barbosa, P., Hines, J., Kaplan, I., Szczepaniec, A., & Szendrei, Z. A. (2009). Associational resistance and associational susceptibility: Having right or wrong neighbors. *Annual Review of Ecology Evolution and Systematics, 40*, 1-20.

Berenbaum, M. R. & Zangerl, A. R. (1998). Chemical phenotype matching between a plant and its insect herbivore. *Proc. Natl Acad. Sci. 95*, 13743–13748. (doi:10.1073/pnas.95.23.13743)

Bergmüller, R., & Taborsky, M. (2010). Animal personality due to social niche specialisation. *Trends in Ecology & Evolution, Trends in Ecology and Evolution-1267*, 1-8. (doi:10.1016/j.tree.2010.06.012)

Bergstrom, C. T., & Lachmann, M. (2003). The red king effect: When the slowest runner wins the coevolutionary race. *Proceedings of the National Academy of Sciences, 100*(2), 593-598.

Bernays, E. A., & Funk, D. J. (1999). Specialists make faster decisions than generalists: Experiments with aphids. *Proc. R. Soc. Lond. B, 266*, 151-156.

Bonchev, D., Rouvray, D. H., & Mezey, P. G. (2005). *Complexity in chemistry, biology, and ecology.* London: Springer.

Borges, R. T. (2005). Do plants and animals differ in phenotypic plasticity? *J.Biosci, 30*(1), 41-50.

Bürger, R. (2002). Additive genetic variation under intraspecific competition and stabilizing selection: A two-locus study. *Theoretical Population Biology, 61*, 197-213.

Chapman, R. F. (2003). Contact chemoreception in feeding by phytophagous insects. *Annual Review of Entomology, 48*, 455-484.

Connell, J. H. (1983). On the prevalence and relative importance of interspecific competition: Evidence from field experiments. *Am Nat, 122*(5), 41-50.

Cordeiro, C. M. T., Maluf, W. R., & Miranda, J. E. C., (1983). Analysis of stability and genotypic similarity in a set of potato (*Solanum tuberosum* L.) cultivars. *Rev. Brazil. Genet., 2*, 279-294.

Dawkins, R. (1976). *The selfish gene.* New York City: Oxford University Press.

Dawkins, R. (1982). *The extended phenotype.* Oxford: W.H. Freeman And Company.

Dawkins, R., & J. R. Krebs. (1979). Arms races between and within species. *Proceedings of the Royal Society London. B, 205*(1161), 489-511.

Denno, R. F., & Kaplan, I. (2007). Plant-mediated interactions in herbivorous insects: Mechanisms, symmetry, and challenging the paradigms of competition past. In Ohgushi T., Craig T. P., Price P. W. (Eds.), *Ecological communities: Plant mediation in indirect interaction webs.* UK: Cambridge University Press. Retrieved from http://www.entm.purdue.edu/ecolab/pdf/07_communities.pdf

Denno, R. F., McClure, M. S., & Ott, J. R. (1995). Interspecific interactions in phytophagous insects: Competition re-examined and resurrected. *Annu. Rev. Entomol, 40,* 297-331.

Denno, R. F., Peterson, M. A., Gratton, C., Cheng, J., Langellotto, G. A., Huberty, A. F., & Finke, D. L. (2000). Feeding-induced changes in plant quality mediate interspecific competition between sap-feeding herbivores. *Ecology, 81*(7), 1814-1827.

Dixon, A. F. G. (1977). Aphid ecology: Life cycles, polymorphism, and population regulation. *Ann. Rev. Ecol. Syst., 8,* 329-353.

Dixon, A. F. G. (1987). Parthenogenetic reproduction and the rate of increase in aphids. In A. K. Minks & P. Harrewijn (Eds.), *Aphids, their biology, natural enemies and control* (pp. 269-287). Amsterdam: Elsevier.

Dixon, A. F. G. (1998). *Aphid ecology.* London: Chapman and Hall.

Duc, G. (1997). Faba bean (*Vicia faba* L.). *Field Crops Res, 53,* 99-109.

Dungey, M., Potts, H. S., Whitham, T. G., & Li, H. (2000). Plant genetics affects arthropod community richness and composition: Evidence from a synthetic eucalypt hybrid population. *Evolution, 54*(6), 1938-1946.

Emebiri, L. C., Matassa, V., & Moody, D. B. (2005). GENSTAT programs for performing Muir's alternative partitioning of genotype-by-environment interaction. *Journal of Heredity*, *96*(1), 78-79.

Ernebjerg, M., & Kishony, R. (2011). Dynamic phenotypic clustering in noisy ecosystems. *PLoS Computational Biology*, *7*(3). (doi:10.1371/journal.pcbi.1002017)

Farnsworth, K. D., Focardi, S., & Beecham, J. A. (2002). Grassland–herbivore interactions: How do grazers coexist. *American Naturalist*, *159*, 24-39.

Ferrari, J., Godfray, H. J., Faulconbridge, A., Prior, K., & Via, S. (2006). Population differentiation and genetic variation in host choice among pea aphids from eight host plant genera. *Evolution*, *60*, 1574-1584.

Flores, F., Moreno, M. T., & Cubero, J. J. (1998). A comparison of univariate and multivariate methods to analyze G x E interaction. *Field Crops Res*, *56*, 271-286.

Fordyce, J. A. (2006). The evolutionary consequences of ecological interactions mediated through phenotypic plasticity. *The Journal of Experimental Biology*, *209*, 2377-2383.

Fridley, J. D., & Grime, J. P. (2010). Community- and ecosystem-level consequences of intraspecific genetic diversity in grassland microcosms of varying species diversity. *Ecology*, *91*(8), 2272-2283.

Fritz, R. S., & Price, P. W. (1988). Genetic variation among plants and insect community structure: Willows and sawflies. *Ecology*, *69*, 845-856.

Giordanengo, P., Brunissen, L., Rusterucci, C., Vincent, C., Bel, A. V., Dinant, S., Girousse, C., Faucher, M., & Bonnemain, J. L. (2010). Compatible plant-aphid interactions: How aphids manipulate plant responses. *C R Biol.*, *333*, 516-523.

Giske, J., Aksnes, D. L., & Førland, B. (1993). Variable generation time and Darwinian fitness measures. *Evol. Ecol*, *7*, 233-239.

Glinwood, R., Ahmed, E., Qvarford, E., Ninkovic, V., & Pettersson, J. A. (2009). Airborne interactions between undamaged plants of different cultivars affect insect herbivores and natural enemies. *Arthropod–Plant Interactions*, *3*(4), 215-224.

Hairston, N., Smith, F., & Slobodkin, L. (1960). Community structure, population control and competition. *Am Nat*, *94*, 421-425.

Haloin, J. R., & Strauss, S. Y. (2008). Interplay between ecological communities and evolution: A review of feedbacks from microevolutionary to macroevolutionary scales the year in evolutionary biology. *Annals of the New York Academy of Science*, *1133*, 87-125.

Hambäck, P. A., Vogt, M., Tscharntke, T., Thies, C., & Englund, G. (2007). Top-down and bottom-up effects on the on the spatiotemporal dynamics of cereal aphids: Testing scaling theory for local density. *Oikos*, *116*, 1995-2006.

Hash, C. T., Blummel, M., & Bidinger, F. R. (2006). Genotype × environment interactions in food-feed traits in pearl millet cultivars. *International Sorghum and Millets Newsletter*, *47*, 153-157.

Hatano, E., Kunert, G., Weisser, W. W., & Sunnucks, P. (2010). Aphid wing induction and ecological costs of alarm pheromone emission under field conditions. *PLoS ONE*, *5*(6): e11188. (doi:10.1371/journal.pone.0011188)

Hersch-Green, E. I., Turley, N. E., & Johnson, M. T. J. (2011). Community genetics: What have we accomplished and where should we be going? *Phil. Trans. R. Soc. B*, *366*(1569), 1453-1460.

Hochwender, C. G., & Fritz, R. S. (2004). Plant genetic differences influence herbivore community structure: Evidence from a hybrid willow system. *Oecologia*, *138*, 547-557.

Holling, C. S. (1973). Resilience and stability of ecological systems. *Annual Review of Ecology and Systematics*, *4*(1), 421-973.

Holling, C. S. (1976). Resilience and stability of ecosystems. In: E. Jantsch & C. H. Waddington (Eds.), *Evolution and Consciousness: Human Systems in Transition*. Addison Wesley, Reading, Mass., 73-92.

Hughes, A. R., Inouye, B. D., Johnson, D., T, M. T. J., Underwood, N., & Vellend, M. (2008). Ecological consequences of genetic diversity. *Ecology Letters*, *11*, 609-623.

Hunter, M. D., & Price, P. W. (1992). Playing chutes and ladders: Heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology*, *73*(1), 724-732.

Hutchinson, G. E. (1978). *An introduction to population ecology.* New Haven: Yale University Press.

Inbar, M., & Wool, D. (1995). Phloem-feeding specialists sharing a host tree: Resource partitioning minimizes interference competition among galling aphid species. *Oikos*, *73*, 109-119.

Inbar, M., Eshel, A., & Wool, D. (1995). Interspecific competition among phloem - feeding insects mediated by induced host plant sinks. *Ecology*, *76*(5), 1506-1515.

Jablonka, E. (2004). From replicators to heritably varying phenotypic traits. the extended phenotype revisited. *Biology & Philosophy*, *19*, 353-375.

Johnson, M. T. J. (2008). Bottom-up effects of plant genotype on aphids, ants and predators. *Ecology, 89,* 145-154.

Johnson, M. T. J., & Agrawal, A. A. (2005). Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (*Oenothera biennis*). *Ecology*, *86*, 874-885.

Johnson, M. T. J., & Agrawal, A. A. (2007). Covariation and composition of arthropod species across plant genotypes of evening primrose (*Oenothera biennis*). *Oikos*, *116*, 941-956.

Johnson, M. T. J., & Stinchcombe, J. R. (2007). An emerging synthesis between community ecology and evolutionary biology. *Trends in Ecology and Evolution*, *22*, 250-257.

Johnson, M. T. J., Lajeunesse, A. A., & Agrawal, A. A. (2006). Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. *Ecology Letters*, *9*, 24-34.

Juarrero, A. (2010). Complex dynamical systems theory. "Cognitive Edge Network". Retrieved from http://cognitive-edge.com/uploads/articles/100608%20Complex_Dynamical_Systems_Theory.pdf

Kan, A., Kaya, M., Gürbüz, A., Sanli, A., Özcan, K., & Çiftçi, C. Y. (2010). A study on genotype x environment interaction in chickpea cultivars (*Cicer arietinum* L.) grown in arid and semi-arid conditions. *Scientific Research and Essays*, *5*(10), 1164-1171.

Kaplan, I., & Denno, R. F. (2007). Interspecific interactions in phytophagous insects revisited: A quantitative assessment of competition theory. *Ecol Lett*, *10*(1), 977-994.

Kaplan, I., Halitschke, R., Kessler, A., Sardanelli, A., & Denno, R. F. (2008). Constitutive and induced defenses to herbivory in above- and belowground plant tissues. *Ecology*, *89*(2), 392-406.

Karban, A. (1989). Fine-scale adaptation of herbivorous thrips to individual host plants. *Nature*, *340*, 60-61.

King, C. C. (2003). Biocosmology 2: Evolutionary epoch: Complexity, chaos and complementarity. *340*. Retrieved from http://www.dhushara.com/pdf/biocos2.pdf

Laland, K. N. (2004). Extending the extended phenotype. *Biology and Philosophy*, *19*, 313-325.

Larson, K. C., & Whitham, T. G. (1991). Manipulation of food resources by a gall-inducing aphid: The physiology of sink–source interactions. *Oecologia*, *88*, 15-21.

Larson, K. C., & Whitham, T. G. (1997). Competition between gall aphids and natural plant sinks: Plant architecture affects resistance to galling. *Oecologia*, *109*, 575-582.

Levin, S. A. (1998). Ecosystems and the biosphere as complex adaptive systems. *Ecosystems*, *1*, 431-436.

Lowe, H. J. B. (1967). Interspecific differences in the biology of aphids (Homoptera) (Aphididae) on leaves of *Vicia faba*. *Entomol. Exp. Appl.*, *10*, 347-357.

Lowe, H. J. B., & Taylor, R. (1964). Population parameters, wing production and behavior in red and green *Acyrthosiphon pisum* (Harris) (Hom: Aphididae). *Entomol. Exp. Appl.*, *7*, 287-295.

Marquet, P. A. (2009). Beyond Darwin: On the role of niche construction and self-organization in evolution. *Revista Chilena De Historia Natural*, *82*, 493-496.

McLean, A. H. C., van Asch, M., Ferrari, J., & Godfray, H. C. J. (2011). Effects of bacterial secondary symbionts on host plant use in pea aphids. *Proc. R. Soc. B*, *278*(1706), 760-766.

Moore, A. J., Brodie, E. D., & Wolf, J. B. (1997). Interacting phenotypes and the evolutionary process: I. Direct and indirect genetic effects of social interactions. *Evolution*, *51*(5), 1352-1362.

Muller, F. P., Berger, L., & Herrman, O. (1974). Experiments on the settling behavior of two races of *Acyrthosiphon pisum* (Harris) on *Vicia faba* L. (Leguminosae) (Hom: Aphididae). *Biol. Zentralbl*, *93*, 675-686.

Murray, B. G. (1990). Population dynamics, genetic change, and the measurement of fitness. *Oikos*, *59*(2), 189-199.

Ninkovic, V., & Åhman, I. (2009). Aphids acceptance of Hordeum genotypes is affected by volatile exposure and is correlated with aphid growth. *Euphytica*, *169*, 177-185.

Odling-Smee, F. J., Laland, K. N., & Feldman, M. W. (2003). *Niche construction: The neglected process in evolution*. Princeton, NJ: Princeton University Press.

Okasha, S. (2009). Biological Altruism. In *The Stanford Encyclopedia of Philosophy*. Zalta, E. N. (Ed.). Retrieved from http://plato.stanford.edu/archives/win2009/entries/altruism-biological/

Östman, Ö., (2011). Interspecific competition affects genetic structure but not genetic diversity of daphnia magna. *Ecosphere*, *2*(3). (doi: 10.1890/ES11-00039.1)

Oyedokun, J. B. (1985). Genotype environment interactions and the identification of adaptable cultivars of upland rice (*Oryza sativa*) in South-Western Nigeria. *Exp. Agric*, *21*, 53-58.

Pelletier, F., Garant, D., & Hendry, A. P. (2009). Eco-evolutionary dynamics. *Philos Trans R Soc Lond B Biol Sci*, *364*(1523), 1483-1489.

Petersen, M., & Hunter, M. (2001). Variation in the outcome of competition between two aphid species on pecan: Plants matter more than predators. *Oikos*, *92*, 107-118.

Pettersson, J., Ninkovic, V., & Glinwood, R. (2003). Plant activation of barley by intercropped conspecifics and weeds: Allelobiosis. *BCPC Crop Science and Technology*, *2003*, 1135-1144.

Pickett, J. A., Wadhams, L. J., Woodcock, C. M., & Hardie, J. (1992). The chemical ecology of aphids. *Ann. Rev. Entomol*, *6*, 349-360.

Pollard, D. G. (1973). Plant penetration by feeding aphids (Hemiptera, Aphidoidea): A review. *Bull. Ent. Res.*, *62*, 631-714. Retrieved from http://journals.cambridge.org/download.php?file=%2FBER%2FBER62_04%2FS0007485300005526a.pdf&code=2d67f0d7794c2017454f2789ffa4ab4d

Post, D. M., & Palkovacs, E. P. (2009). Eco-evolutionary feedbacks in community and ecosystem ecology: Interactions between the ecological theatre and the evolutionary play. *Phil. Trans. R. Soc. B*, *364*, 1629-1640.

Powell, G., Tosh, C. R., & Hardie, J. (2006). Host plant selection by aphids: Behavioral, evolutionary, and applied perspectives. *Annu. Rev. Entomol.*, *51*, 309-330.

Power, M. E. (1992). Top-down and bottom-up forces in food webs: Do plants have primacy? *Ecology*, *73*, 733-746.

Rajagopalan, P., Rawal, A., & Miikkulainen, R. (2010). Emergence of competitive and cooperative behavior using coevolution. *Proceedings of the 12th Annual Conference on Genetic and Evolutionary Computation*. Retrieved from http://dl.acm.org/citation.cfm?id=1830681&bnc=1

Rajagopalan, P., Rawal, A., Miikkulainen, R., Wiseman, M. A., & Holekamp, K. E. (2011). The role of reward structure, coordination mechanism and net return in the evolution of cooperation. *Proceedings of the IEEE Conference on Computational Intelligence and Games (CIG 2011), Seoul, South Korea*. Retrieved from http://bit.ly/rPXszL

Ranger, C. M., Singh, A. P., Johnson-Cicalese, J., Polavarapu, S., & Vorsa, N. (2007). Intraspecific variation in aphid resistance and constitutive phenolics exhibited by the wild blueberry *Vaccinium darrowi*. *J Chem Ecol*, *33*, 711-729.

Rawal, A., & Rajagopalan, P. (2010). Emergence of competitive and cooperative behavior and arms race through coevolution. *Neural Networks Research Group*. Retrieved from http://nn.cs.utexas.edu/?armsrace

Rose, T. J., Damon, P., & Rengel, Z. (2010). Phosphorus-efficient faba bean (*Vicia faba* L.) genotypes enhance subsequent wheat crop growth in an acid and an alkaline soil. *Crop & Pasture Science*, *61*(2), 1009-1016.

Rosenberg, A., & Bouchard, F. (2010). Fitness. In *The Stanford Encyclopedia of Philosophy*. Zalta, E. N. (Ed.). Retrieved from http://plato.stanford.edu/entries/fitness/

Rowntree, J. K., McVennon, A., & Preziosi, R. F. (2010). Plant genotype mediates the effects of nutrients on aphids. *Oecologia*, *163*(3), 675-679.

Rowntree, J. K., Shuker, D. M., & Preziosi, R. F. (2011). Forward from the crossroads of ecology and evolution. *Phil. Trans. R. Soc. B*, *366*(1569), 1322-1328.

Ruhnke, H., Schädler, M., Klotz, S., Matthies, D., & Brandl, R. (2009). Variability in leaf traits, insect herbivory and herbivore performance within and among individuals of four broad-leaved tree species. *Basic and Applied Ecology*, *10*, 726-736.

Ruhnke, H., Schädler, M., Matthies, D., Klotz, S., & Brandl, R. (2006). Are sawflies adapted to individual host trees? A test of the adaptive deme formation hypothesis. *Evolutionary Ecology Research 8*, *8*, 1039-1048.

Salyk, R., & Sullivan, D. J. (1982). Comparative feeding behavior of two aphid species: Bean aphid (*Aphis fabae* Scopoli) amd pea aphid (*Acyrthosiphon*

pisum [Harris]) (Homoptera: Aphididae). *Journal of the New York Entomological Society, 90*(2), 87-93.

Schoener, T. W. (1983). Field experiments on interspecific competition. *Am Nat, 122,* 240-285.,

Schoener, T. W. (2011). The newest synthesis: Understanding the interplay of evolutionary and ecological dynamics. *Science, 331,* 426-429.

Schuett, W., Dall, S. R. X, Baeumer, J., Kloesener, M. H., Nakagawa, S., Beinlich, F. , & Eggers, T. (2011). 'Personality' variation in a clonal insect: The pea aphid, *Acyrthosiphon pisum. Developmental Psychobiology, 53,* 631-640.

Sharpe, A. G., & Lydiate, D. J. (2003). Mapping the mosaic of ancestral genotypes in a cultivar of oilseed rape (*Brassica napus*) selected via pedigree breeding. *Genome, 46,* 461-468.

Silva, A. X., Bacigalupe, L. D., Luna-Rudloff M., Figueroa, C. C. (2012). Insecticide resistance mechanisms in the green peach aphid *Myzus persicae* (Hemiptera: Aphididae) II: Costs and Benefits. *PLoS ONE, 7*(6): e36810. (doi:10.1371/journal.pone.0036810)

Smith, R. A., Mooney, K. A., & Agrawal, A. A. (2008). Coexistence of three specialist aphids on common milkweed, *Asclepias syriaca. Ecology, 89*(8), 2187-2196.

Thompson, J. N. (1994). *The coevolutionary process.* Chicago: University Of Chicago Press.

Trivers, R. L. (1971). The evolution of reciprocal altruism. *The Quarterly Review of Biology, 46*(1), 35-57.

Troncoso, A. J., Vargas, R. R., Tapia, D. H., Olivares-Donoso, R., & Niemeyer, H. M. (2005). Host selection by the generalist aphid *Myzus persicae* (Hemiptera:

Aphididae) and its subspecies specialized on tobacco, after being reared on the same host. *Bulletin of Entomological Research*, *95*, 23-28.

Underwood, N. (2009). Effect of genetic variance in plant quality on the population dynamics of a herbivorous insect. *Journal of Animal Ecology*, *78*, 839–847.

Underwood, N., & Rausher, M. D. (2000). The effects of host–plant genotype on herbivore population dynamics. *Ecology*, *81*, 1565-1576.

Utsumi, S., Ando, L., Craig, T. P., & Ohgushi, T. (2011). Plant genotypic diversity increases population size of a herbivorous insect. *Proc. R. Soc. B.* (In Press). (doi: 10.1098/rspb.2011.0239)

Van Valen, L. (1973). A new evolutionary law. *Evolutionary Theory*, *1*, 1-30.

Weisser, W. W., & Stadler, B. (1994). Phenotypic plasticity and fitness in aphids. *Eur.J.Entomol*, *91*, 71-78.

Whitham, T. G. (1989). Plant hybrid zones as sinks for pests. *Science*, *244*, 1490-1493.

Whitham, T. G., Bailey, J. K., Schweitzer, J. A., Shuster, S. M., Bangert, R. K., LeRoy, C. J., Lonsdorf, E. V., Allan, G. J., DiFazio, S. P., Potts, B. M. Fischer, D. G., Gehring, C. A., Lindroth, R. L., Marks, J. C., Hart, S. C., Wimp, G. M., & Wooley, S. C. (2006). A framework for community and ecosystem genetics: From genes to ecosystems. *Nat Rev Genet*, *7*, 510-523.

Whitham, T. G., DiFazio, S. P., Schweitzer, J. A., Shuster, S. M., Allan, G. J., Bailey, J. K., & Woolbright, S. A. (2008). Extending genomics to natural communities and ecosystems. *Science*, *320*, 492-495.

Whitham, T. G., Young, W. P., Martinsen, G. D., Gehring, C. A., Schweitzer, J. A., Shuster, S. M Wimp, G. M., Fischer, D. G., Bailey, J. K., Lindroth, R. L., Woolbright, S. , & Kuske, C. R. (2003). Community and ecosystem genetics: A consequence of the extended phenotype. *Ecology*, *84*, 559-573.

Whitlock, R. J., Grime, J. P., Booth, R. D., & Burke, T. (2007). The role of genotypic diversity in determining grassland community structure under constant environmental conditions. *Journal of Ecology*, *95*, 895-907.

Whitman, D. W., & Agrawal, A. A. (2009). What is phenotypic plasticity and why is it important? In D. W. Whitman & T. N. Ananthakrishnan (Eds), *phenotypic plasticity of insects: Mechanisms and consequences* (pp. 1-54). Moscow, USA: Science.

Wimp, G. M., Wooley, S., Bangert, R. K., Young, W. P., Martinsen, G. D., Keim, P., Rehill, B., Lindroth, R. L., & Whitham, T. G. (2007). Plant genetics predicts intra-annual variation in phytochemistry and arthropod community structure. *Mol Ecol*, *16*, 5057-5069.

Wolf, B. J. (2000). Indirect genetic effects and gene interactions. In B. J. Wolf, E. D. Brodie, & M. J. Wade (Eds.), *Epistasis and the Evolutionary Process* (pp. 158-176). New York: Oxford University Press.

Wolf, J. B., Brodie, E. D., & Moore, A. J. (1999). Interacting phenotypes and the evolutionary process II: Selection resulting from social interactions. *Am Nat*, *153*, 254-266.

Yong, C. H., & Miikkulainen, R. (2001). Cooperative coevolution of multi-agent systems. *Technical Report "AI07-338"*, Department of Computer Sciences, the University of Texas at Austin.

Yong, C. H., & Miikkulainen, R. (2009). Coevolution of role-based cooperation in multiagent systems. *IEEE Transactions on Autonomous Mental Development*, *1*(30), 170-186.

Zytynska, S. E., & Preziosi, R. F. (2011). Genetic interactions influence host preference and performance in a plant-insect system. *Evol Ecol*, *25*, 1321-1333.

Zytynska, S. E., M. F. Fay, D. Penney, & Preziosi, R. F. (2011). Genetic variation in a tropical tree species influences the associated epiphytic plant and invertebrate communities in a complex forest ecosystem. *Phil Trans R Soc B, 366*, 1329-1336.

Annex

Additional analyses

Further notes on resource utilisation by aphids in Experiment I:

In **Table 1`**, for each microcosm, we provide details on the plants that were

discriminated (no colonisation occurred), or depleted due to over-exploitation.

Microcosm Genotype		Not colonised		Total
Plant		**Rejected (No successful colonisation occurred)**	**Depleted due to over-exploitation**	
C1	**Competitor Absent**	3	1	4
	Competitor present	0	1	1
C2	**Competitor Absent**	1	6	7
	Competitor present	3	3	6
C3	**Competitor Absent**	3	0	3
	Competitor present	4	0	4
C4	**Competitor Absent**	8	0	8
	Competitor present	1	1	2

Table 1`. Plants not colonised within each microcosm; discrimination and overexploitation of the plants within each microcosm. Further details are provided here across four broad bean genotypes on the plants illustrated with nil percent in **Figure 5** (in the paper). Plants are categorised as:

1) Rejected (No successful colonisation occurred): Plants were discriminated but with signs of aphids' exuviae (shed skin, i.e. cast-off skins) on the plant or the surface of the soil in the plant pot. [Possibly rejected by aphids for non-suitability after palpation (probing)]. **2) Depleted due to over-exploitation:** Plants dead with signs of aphids' exuviae (shed skin, i.e. cast-off skins).

FP behavior (On plant Distribution) under inter-specific competition:

Plant bottom, middle, top:

1`- Inter-specific competition in the presence and absence of competitor; Experiment I:

No **FP** aphids had colonised 19 of the replicates belonging to the multiple-choice treatment at day 14 and so these zero values were dropped from our analysis. Using the remaining 93 replicates we found on 9 plants and these replicates were excluded from our analysis. We found very notable effect of cultivar ($\chi^2 = 67.36$, P < 0.0001) and competitor species ($\chi^2 = 36.59$, P < 0.0001). The interaction between cultivar and competitor's species had highly significant effect on the distribution of aphid on plant ($\chi^2 = 131.10$, P < 0.0001, **Figure 1`**).
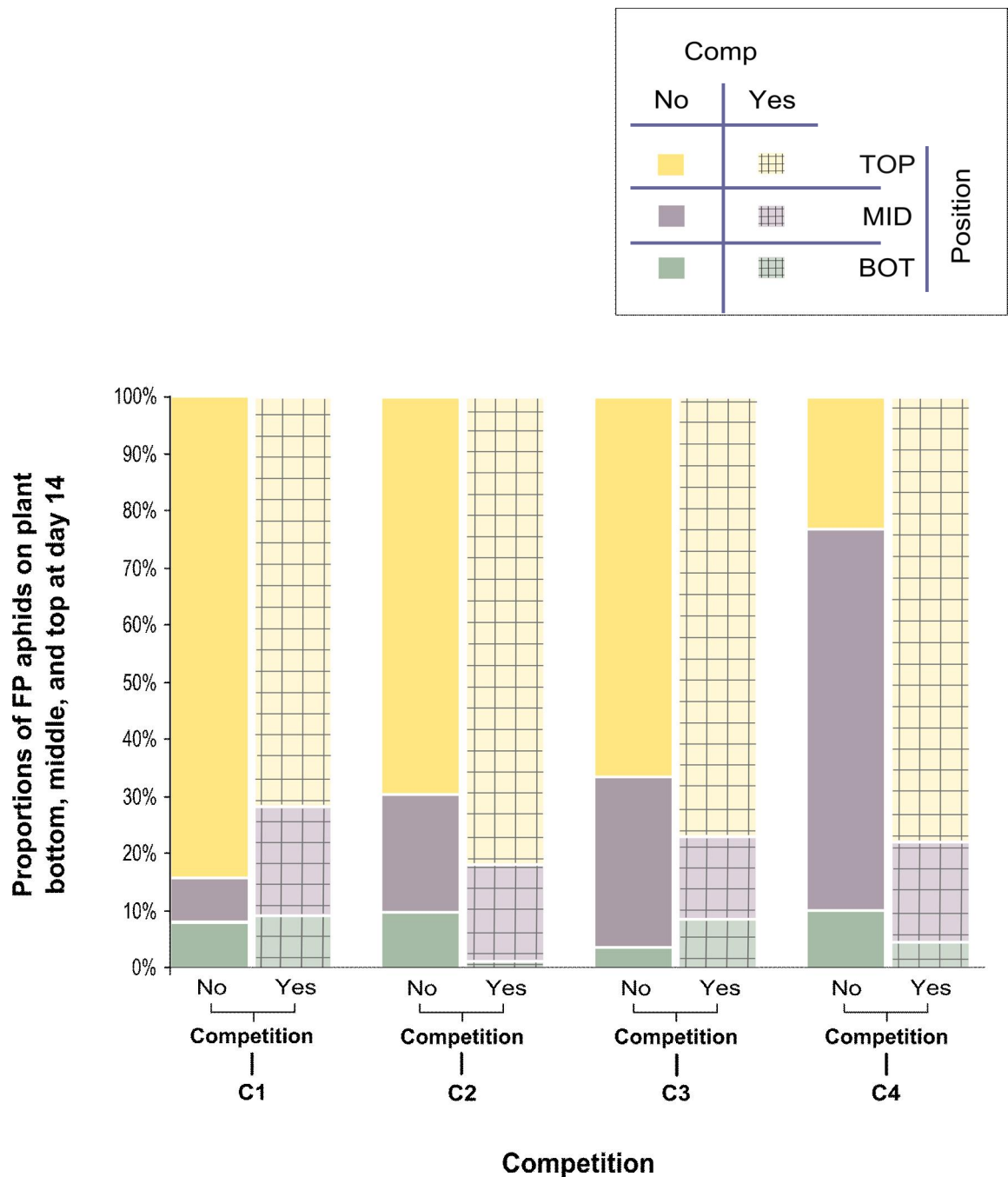
Figure 1`: Aphid behavior explained by on-shoot distribution; Bottom, Middle, Top.

FP preference for on-plant feeding-sites was observed and explained by the proportions of the focal aphid FP residing each third of the host plant. Such on-plant distributional patterns displayed by FP were surveyed across four broad bean cultivars in mono-cultivated microcosms and under the presence and absence of inter-specific competition with black bean aphids.

2`- Inter-specific Competition (Exp. II):

FP aphids were not found on 4 replicates at day 14 and these replicates were dropped from our analysis. Using the remaining 49 plants we found highly significant effect of cultivar ($\chi^2 = 143.27$, P < 0.0001) and competitor's species ($\chi^2 = 157.43$, P < 0.0001). There was also a highly significant effect of the interaction between cultivar and competitor's species on aphid behavior ($\chi^2 = 117.69$, P < 0.0001, **Figure 2`**).

1`- Intra-specific Competition (Exp. III):

At day 14, no **FP** aphids were found on 9 plants and these replicates were excluded from our analysis. Using the remaining 101 replicates, we found highly significant effect of cultivar ($\chi^2 = 52.28$, P < 0.0001) and competitor's genotype ($\chi^2 = 596.33$, P < 0.0001). There was also a highly significant effect of the interaction between cultivar and competitor genotype on aphid behaviour ($\chi^2 = 412.99$, P < 0.0001, **Figure 2`**)
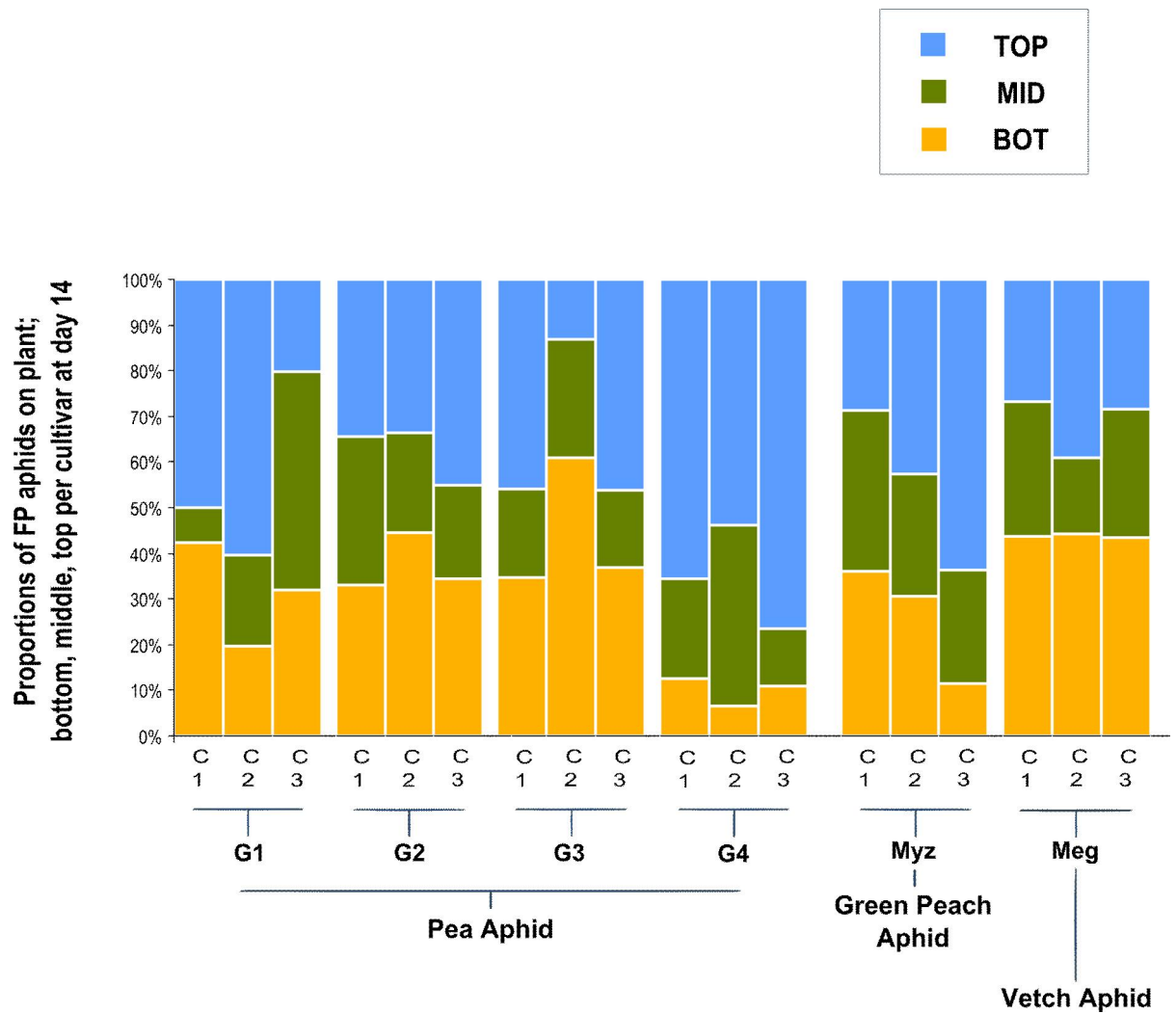
Figure 2`: An illustration of FP behaviour explained by on-shoot distribution; bottom, middle, and top.

The plot exhibits the proportions of **FP** on each plant third under the influence of plant genotype (three cultivars) and the interaction between **FP** and other rival genotypes (at the conspecific and the heterospecific levels). Treatment replicates were confined in individual propagators.

More on the differential response of generalist aphids to environmental cues

Plant host genotypes, even within species, differ in their uptake of nutrients. Rowntree et al. (2010) demonstrated that different nutrient levels in the soil had different effects on aphid reproductive success dependent on host genotype. Plant metabolites also vary among individual plants (Chapman 2003, Powell et al. 2006) and this individual variation is usually associated with genetic differences (Whitham et al. 2003, 2006). Therefore, the variation in host plants alongside the differential feeding habits of aphids will, in combination, affect the nutritional quality available to aphids and thus their choice, ingestion and reproductive success in both the short and long terms (Dungey et al. 2000, Underwood and Rausher 2000, McIntyre and Whitham 2003, Powell et al. 2006, Pfennig and McGee 2010, Tariq et al. 2010). In this regard, generalist aphids may behave so as to minimise niche over-lap in order to decrease the negative aspects of inter-specific interference type of competition (Yong and Miikkulainen 2001, Rajagopalan et al. 2010, 2011; but see Pianka 1974). This argument should be regarded in the light of the 'cost-sensitive' nature of decision making by aphids, as proposed by McAllister et al. (1990). The 'cost-sensitive' characteristic can be considered as a bet-hedging evolutionary game tactics [(Olofsson et al. 2009); see also the insight below)].

May and MacArthur (1972), depicted niche-overlap as a function of environmental variability (but see Inbar and Wool 1995). In our experiments, niche space, as defined by Litvak and Hansell (1990), was more extensive in Experiment **I** than in Experiment **II** and Experiment **III**. Niche differentiation can account for the facilitation and apparent coexistence between competitors (Inbar and Wool 1995, Inbar et al. 1995). In Experiment **I**, although the broad bean genotypes used were consistent and agronomically stable, we argue that aphid infestation was expected to induce in-plant physiological variation in resistance. Thus, aphids experienced an

environment with relatively variable host resistance (Karban and Myers 1989, Karban and Baldwin 1997), even though host plants were of the same genotype for each treatment line. This is despite the fact that only two generations of the focal aphid genotype (**FP**) were allowed to occur during the length of the experiment. Therefore, direct competition (interference) was reduced. This would likely have influenced the patterns of aphid distribution due to the interaction between the community genetic effect and the competition effect.

However, it is important to take into account how aphids, through their activities and interactions on/with their hosts, modify their shared resources and thus affect themselves and the biotic environment where they interact. This in turn can produce differential feedback on selective forces (natural selection) and alter them (Odling-Smee et al. 2003); a process that has been advocated as being constant and cyclic (Laland 2004). In this context, a reduction of conflict towards more synergistic interplay, amongst aphid species/genotypes, promotes the emergence of extended and shared phenotypes beyond the genetic constitution of each interactor (Dawkins 1976, 1982, Whitham et al. 2003, Jablonka 2004, Laland 2004; see also Araújo et al. 2008, Rawal and Rajagopalan 2010).

Further insight: Aphid mothers hedge the bet of the clone*

"Bet hedging occurs when a single genotype shows a variety of phenotypes in the same environment, and each phenotype is successful only when the particular circumstances to which it is adapted occur."; Hopper (2003).

---

*This phrase is adapted from the work proposed by Philippi and Seger (1989); 'Hedging one's evolutionary bets, revisited.'

Parthenogenetic aphids form colonies of genetically identical individuals (clones) under favourable conditions. Therefore, according to such criteria, kin selection (*sensu* Hamilton 1963,1964,1972; see also Dawkins 1979 and Alonso 1998) should operate at the level of the clone. Although pea aphids are not eusocial insects, they do display remarkable division of tasks (ecological roles) amongst the all-female population, with explicit ability to produce adaptable morphs through telescoping of generations (Dixon 1998, Wu et al. 2010). One of the best examples in this regard is the altruistic behaviour shown by aphids under parasitoid attack, in order to reduce the risk of subsequent attacks and thus increase the fitness of their kin (the clonal copy), along with decreasing the fitness of the opponent (McAllister et al. 1990, Wu et al. 2010).

I consider the optimisation of the clonal fitness as 'within-generation' shaping (determination) of the offspring's phenotype (Hopper 2003; see also Plaistow et al. 2006, 2007, and Underwood 2009), as well as 'risk spreading' (*sensu* Hopper 1999). This bet hedging or risk spreading is expected to be a varying evolutionary-game 'manoeuvre' (Hopper 1999, Olofsson et al. 2009) within the focal clone of aphid, prone to both environmental and genetic cues (Hanski and Saccheri 2006, Underwood 2009). Logically, Leimar (2009) suggested the hypothetical collation of the genetic and environmental facets of phenotype determination into one category in order to further understand the evolution of phenotypic polymorphism. Concomitantly, a focal produced phenotype, through within-generation bet hedging, will experience uneven selection pressures (Hopper 2003). Here, I maintain that maternal effects in parthenogenetic aphids are adaptive as well as dynamic with benefits for both mothers and daughters (*sensu* Marshall and Uller 2007). The maternal effects within the clone will be contingent on the variation of the host plant, intra- and inter-clonal interactions, and the past and present states of their 'kin' (i.e. a trans-generational effect; Podjasek et al. 2005, Plaistow et al. 2006, 2007, Zehnder et al. 2007).

Moreover, the maternal effects in one focal clone can extend to affect the offspring of other aphid clones sharing the same resource (i.e. an inter-generational effect; Plaistow et al. 2006). In other words, aphid genotypes are expected to vary in their performance and behaviour especially when they often become "wedged between bottom-up processes (variation in plant quality) and top-down processes (differential impacts of natural enemies)" as adapted from Stadler (2004). However, interestingly, the differential fitness resulting from the differential performance of the genetically identical members of the clone can be due to variation at the molecular level, and is expected to differentially affect the dynamics of the clone [analogous to what is proposed by Hanski and Saccheri (2006); see also Dombrovsky et al. 2009). It is also useful to consider the phenotype determination in aphids as 'density-dependent phase polyphenism', similar to the density-dependent 'gregarious' and 'non-gregarious' phenotypes/phases expressed by locusts (Simpson et al. 2011). However, in the case of aphids, the replacement of 'gregarious' is 'apterous', and the substitute to 'non-gregarious' is 'alate' and 'thrifty'. An epigenetic basis for this polyphenism in asexually reproductive aphid genotypes is receiving increasing support (Alex et al. 2003, Simpson et al. 2011, Srinivasan and Brisson 2012). This is a peculiar epigenetic phenomenon, i.e. non genetic external factors possibly via DNA methylation akin to the vertebrate methylation system (Simpson et al. 2011; see also Alex et al. 2003 and Srinivasan and Brisson 2012).

References

Alex, C., Wilson, C., Sunnucks, P., & Hales, D. F. (2003). Heritable genetic variation and potential for adaptive evolution in asexual aphids (Aphidoidea). *Biological Journal of the Linnean Society*, *79*(1), 115–135.

Alonso, W. J. (1998). The role of kin selection theory on the explanation of biological altruism: A critical review. *J. Comp. Biol.*, *3*(1), 1-14. Retrieved from http://www.origem.info/KS/Alonso_1998_Kin_selection_&_altruism.pdf

Araújo, M. S., Guimarães, P. R., Svanbäck, R., Pinheiro, A., Guimarães, P., Dos Reis, S. F., & Bolnick, D. I. (2008). Network analysis reveals contrasting effects of intraspecific competition on individual vs. population diets. *Ecology, 89*(7), 197-213.

Chapman, R. F. (2003). Contact chemoreception in feeding by phytophagous insects. *Annual Review of Entomology, 48*, 455-484.

Dawkins, R. (1976). *The selfish gene.* New York City: Oxford University Press.

Dawkins, R. (1979). Twelve Misunderstandings of Kin Selection. *Zeitschrift für Tierpsychologie, 51*(2), 184–200. (doi: 10.1111/j.1439-0310.1979.tb00682.x)

Dawkins, R. (1982). *The extended phenotype.* Oxford: W.H. Freeman And Company.

Dixon, A. F. G. (1998). *Aphid ecology.* London: Chapman and Hall.

Dombrovsky, A. A., Arthaud, L. L., Ledger, T. N., Tares, S., & Robichon, A. (2009). Profiling the repertoire of phenotypes influenced by environmental cues that occur during asexual reproduction. *Genome Research, 19*, 2052-2063. (doi: 10.1101/gr.091611.109)

Dungey, M., Potts, H. S., Whitham, T. G., & Li, H. (2000). Plant genetics affects arthropod community richness and composition: Evidence from a synthetic eucalypt hybrid population. *Evolution, 54*(6), 1938-1946.

Hamilton, W. D. 1972. Altruism and related phenomena, mainly in social insects. *Ann. Rev. Ecol. Syst., 3*, 193-232.

Hamilton, W.D. 1963. The evolution of altruistic behavior. *Am. Nat., 97*, 354-6.

Hamilton, W.D. 1964. The genetical theory of social behaviour I, II. *J. Theor. Biol., 7*, 1-52.

Hanski, I., & Saccheri, I. (2006). Molecular-level variation affects population growth in a butterfly metapopulation. *PLoS Biology*, *4*, 0719-0726.

Hopper, K. (2003). Within-generation bet hedging: A seductive explanation? *Oikos*, *101*(1), 219-222.

Hopper, K. R. (1999). Risk-spreading and bet-hedging in insect population biology. *Annu. Rev. Entomol.*, *44*, 535-560.

Inbar, M., & Wool, D. (1995). Phloem-feeding specialists sharing a host tree: Resource partitioning minimizes interference competition among galling aphid species. *Oikos*, *73*, 109-119.

Inbar, M., Eshel, A., & Wool, D. (1995). Interspecific competition among phloem - feeding insects mediated by induced host plant sinks. *Ecology*, *76*(5), 1506-1515.

Jablonka, E. (2004). From replicators to heritably varying phenotypic traits. the extended phenotype revisited. *Biology & Philosophy*, *19*, 353-375.

Karban, R., & Baldwin, I. T. (1997). *Induced responses to herbivory.* Chicago and London: University Of Chicago Press.

Karban, R., & Myers, J. H. (1989). Induced plant responses to herbivory. *Annu Rev Ecol Syst*, *20*, 331-348.

Laland, K. N. (2004). Extending the extended phenotype. *Biology and Philosophy*, *19*, 313-325.

Leimar, O. 2009. Environmental and genetic cues in the evolution of phenotypic polymorphism. *Evol. Ecol., 23*, 125-135

Litvak, M. K., & Hansell, R. H. C. (1990). A community perspective on the multidimensional niche. *Journal of Animal Ecology*, *59*(3), 931-940.

Marshall, D. J., & Uller, T. (2007). When is a maternal effect adaptive? *Oikos, 116*, 1957-1963.

May, R. M., & MacArthur, R. H. (1972). Niche overlap as a function of environmental variability. *Proc. Nat. Acad. Sci*, *69,* 1109-1113.

McAllister, M. K., B. D. Roitberg, & K. L. Weldon. (1990). Adaptive suicide in pea aphids: Decisions are cost sensitive. *Animal Behaviour*, *40*(1), 167-175.

McIntyre, P. J., & Whitham, T. G. (2003). Plant genotype affects long-term herbivore population dynamics and extinction: Conservation implications. *Ecology*, *84*(2), 311-322.

Odling-Smee, F. J., Laland, K. N., & Feldman, M. W. (2003). *Niche construction: The neglected process in evolution.* Princeton, NJ: Princeton University Press.

Olofsson, H., Ripa, J., & Jonzén, N. (2009). Bet-hedging as an evolutionary game: the trade-off between egg size and number. *Proc. R. Soc. B*, *276* (1669), 2963-2969.

Pfennig, D. W., & McGee, M. (2009). Resource polyphenism increases species richness: A test of the hypothesis. *Phil. Trans. R. Soc. B*, *365*(1540), 577-591.

Philippi, T., & Seger, J. (1989). Hedging one's evolutionary bets, revisited. *Trends Ecol Evol*, *4*(2), 41-44.

Pianka, E. R. (1974). Niche overlap and diffuse competition. *Proc. Nat. Acad. Sci*, *71*(5), 2141-2145.

Plaistow, S., Lapsley, C. T., & Benton, T. G. (2006). Context-dependent intergenerational effects: the interaction between past and present environments and its effect on population dynamics. *Am. Nat.*, *167*, 206-215.

Plaistow, S., St Clair, J. J., Grant, J., & Benton, T. G. (2007). How to put all your eggs in one basket: Empirical patterns of offspring provisioning through out a mother's lifetime. *Am. Nat.*, *170*(4), 520-529.

Podjasek, J. O., Bosnjak, L. M., Brooker, D. J., & Mondor, E. B. (2005). Alarm pheromone induces a transgenerational wing ployphenism in the pea aphid, *Acyrthosiphon pisum. Can. J. Zool, 83*, 1138-1141.

Powell, G., Tosh, C. R., & Hardie, J. (2006). Host plant selection by aphids: Behavioral, evolutionary, and applied perspectives. *Annu. Rev. Entomol., 51*, 309-330.

Rajagopalan, P., Rawal, A., & Miikkulainen, R. (2010). Emergence of competitive and cooperative behavior using coevolution. *Proceedings of the 12th Annual Conference on Genetic and Evolutionary Computation*. Retrieved from http://dl.acm.org/citation.cfm?id=1830681&bnc=1

Rajagopalan, P., Rawal, A., Miikkulainen, R., Wiseman, M. A., & Holekamp, K. E. (2011). The role of reward structure, coordination mechanism and net return in the evolution of cooperation. *Proceedings of the IEEE Conference on Computational Intelligence and Games (CIG 2011), Seoul, South Korea*. Retrieved from http://bit.ly/rPXszL

Rawal, A., & Rajagopalan, P. (2010). Emergence of competitive and cooperative behavior and arms race through coevolution. *Neural Networks Research Group*. Retrieved from http://nn.cs.utexas.edu/?armsrace

Rowntree, J. K., McVennon, A., & Preziosi, R. F. (2010). Plant genotype mediates the effects of nutrients on aphids. *Oecologia, 163*(3), 675-679.

Simpson, S. J., Sword, G. A. & Lo, N. (2011). Polyphenism in insects. *Current Biology, 21*(18), R738-749. (doi: 0.1016/j.cub.2011.06.006)

Srinivasan, D. G., & Brisson, J. A. (2012). Aphids: A model for polyphenism and epigenetics. *Genet Res Int. 2012:* 431531. Epub. (doi:10.1155/2012/431531)

Stadler, B. (2004). Wedged between bottom-up and top-down processes: Aphids on tansy. *Ecological Entomology, 29*, 106–116.

Tariq, M., Wright, D. J., & Staley, J. T. (2010). Maternal host plant effects on aphid performance: Contrasts between a generalist and a specialist species on Brussels sprout cultivars. *Agricultural and Forest Entomology*, *12*, 107-112.

Underwood, N. (2009). Effect of genetic variance in plant quality on the population dynamics of a herbivorous insect. *Journal of Animal Ecology*, *78*, 839–847.

Underwood, N., & Rausher, M. D. (2000). The effects of host–plant genotype on herbivore population dynamics. *Ecology*, *81*, 1565-1576.

Whitham, T. G., Bailey, J. K., Schweitzer, J. A., Shuster, S. M., Bangert, R. K., LeRoy, C. J., Lonsdorf, E. V., Allan, G. J., DiFazio, S. P., Potts, B. M. Fischer, D. G., Gehring, C. A., Lindroth, R. L., Marks, J. C., Hart, S. C., Wimp, G. M., & Wooley, S. C. (2006). A framework for community and ecosystem genetics: From genes to ecosystems. *Nat Rev Genet*, *7*, 510-523.

Whitham, T. G., Young, W. P., Martinsen, G. D., Gehring, C. A., Schweitzer, J. A., Shuster, S. M Wimp, G. M., Fischer, D. G., Bailey, J. K., Lindroth, R. L., Woolbright, S. , & Kuske, C. R. (2003). Community and ecosystem genetics: A consequence of the extended phenotype. *Ecology*, *84*, 559-573.

Wu, G. M., Boivin, G., Brodeur, J., Giraldeau, L. A., & Outreman, Y. (2010). Altruistic defence behaviours in aphids. *BMC Evolutionary Biology*, *10*(19), 1-10. Retrieved from: http://www.biomedcentral.com/content/pdf/1471-2148-10-19.pdf

Yong, C. H., & Miikkulainen, R. (2001). Cooperative coevolution of multi-agent systems. *Technical Report "AI07-338"*, Department of Computer Sciences, the University of Texas at Austin.

Zehnder, C. B., Parris, M. A., & Hunter, M. D. (2007). Effects of maternal age and environment on offspring vital rates in the oleander aphid (Hemiptera: Aphididae). *Environ Entomol, 36*, 910-917.

Chapter 3: Parasitoid wasps influence where aphids die via an inter-specific indirect genetic effect.

*Statement of the nature and extent of my own contribution:

The work was based on a project conducted by Johan Oldekop. I expanded the work, revised the experimental design and addressed further critical questions. Dr. Richard Preziosi and Dr. David Shuker helped in the development of the formulae for the statistical analysis and conducted those analyses. I conducted the literature review, achieved the experimental work, collected and handled data, ran part of the analyses, and wrote the manuscript in collaboration with my co-authors.

Article for *Biology Letters*

Parasitoid wasps influence where aphids die via an inter-specific indirect genetic effect.

Mouhammad Shadi Khudr[1], Johan Oldekop[1], David M. Shuker[2], Richard F. Preziosi[1]

[1]Faculty of Life Sciences, The University of Manchester, Michael Smith Building, M13 9PT

[2]School of Biology, The University of St. Andrews, Harold Mitchell Building, St Andrews, Fife, KY16 9TH, U.K.

Abstract

Host-parasite interactions are a key paradigm for understanding the process of coevolution. Central to all co-evolutionary change is how genetic variation across various traits in the interacting species allows hosts and parasites to evolve new strategies against one another. Importantly, genetic variation in the parasite may be associated with changes in the phenotype of the host, thereby changing the pattern of selection on both host and parasite. This process is encapsulated by inter-specific indirect genetic effects theory. For instance, parasites often induce changes in the morphology, ontogeny and behaviour of their host to maximise their own transmission and therefore fitness as part of an evolutionary arms-race. However, the genetic basis for the behavioural manipulation of a host by a parasite has not yet been fully demonstrated. Here we show that the genotype of the parasitoid wasp *Aphidius ervi* has a significant effect on where their aphid hosts *Acyrthosiphon pisum* move to die

following parasitism, including the likelihood that the aphids abandon the host plant entirely. These results provide the first clear example of an inter-specific indirect genetic effect whereby the genetics of one species influences the expression of a specific behavioural trait in another.

Introduction

When two or more individuals interact, phenotypes arise that are the products of the genotypes of all the interactors: this view of interacting phenotypes changes how we consider the genotype-phenotype map and may have important evolutionary consequences, as encapsulated in indirect genetic effects (IGE) theory. Crucially, both selection and the genetic component of the phenotypes that selection acts on become products of the interactors' genotypes [1,2]. Interacting individuals may either be from the same species (such as parents and offspring) or members of different species (such as hosts and parasites). Despite the ongoing development of IGE theory, there remain rather few examples of IGEs influencing phenotypes [3]. This is particularly true for inter-specific IGEs. Here then we consider the potential for indirect genetic effects in a host-parasitoid wasp interaction.

Because survival and development of parasites depend on the survival of the host during parasite development, parasites often do attempt to maximise fitness by host manipulation [4]. Parasites can induce various changes in their host traits [5,6] making the latter more attractive to predators [6,7]. For instance, parasites that require more than one host to complete the life cycle may increase their intermediate host's exposure to predators, which heightens the chance of transmission to the definitive host [6,8].

In contrast, parasite-induced phenotypic alterations can involve predator evasion responses in their hosts [9] to avoid either premature transmission or the joint death of host and parasite [10]. Hosts will, in turn, be selected to avoid manipulation by parasites or change their behaviour to reduce either the costs or success of parasitism (i.e. become more tolerant of, and/or resistant to, parasitism). An important consideration is that genetic variation in the pertinent traits in both host and parasite are necessary for any (co)evolutionary response to occur [11-15], also see [8]. In terms of parasite manipulation of a host, genetic variation associated with this manipulation will alter the pattern of selection on the parasite population as well as providing the variation for an evolutionary response to that selection, as envisaged by inter-specific indirect genetic effects (IIGE) theory [16]; see also [2].

Parasitoid wasps attack a vast array of invertebrate hosts, consuming and destroying the host from the outside in, or the inside out [17]. They show metabolic dependence on their hosts [17], and tend to develop numerous tactics throughout their life cycle [11,17]. An example, in this regard, is the evasion of intra-guild-predators (i.e. competitive threat of other guild members) via ontogenetic modification of the parasitised hosts [11.12]. Furthermore, parasitoids can stimulate the aphids they attack to produce winged offspring that are dispersive [18]. Moreover, alteration of aphid response (locomotion and reaction to environmental change) after parasitisation has been recorded recently [19]. Host stimulation by parasitoid may also entail manipulation of parasitised aphids to reduce abandonment of the colony before mummification completes [20]. Manipulation of host is also probably beneficial to the parasitoid as a reduction of the risk of hyperparasitism [20]; but see [21]. However, more inactive aphid species show a reduced tendency to mummify away from the rest of their clone whereby there may also be effects of factors such as ant attendance and varying aphid resistance to the parasitoid attack [21]. Another counter example is ante

mortem selection for safer or more micro-climatically suitable habitats by aphids away from the mother colony [22,23]. See also [23,24], and [2,21] for further reviews on host alteration by parasitoids in order to maintain the survival of the parasitoids' offspring).

In the case of *Aphidius ervi*, the parasitoid life cycle begins with the female injecting eggs into the body cavity of its host (including the pea aphid *Acyrthosiphon pisum*). The egg hatches and then the wasp larva devours the host from the inside, ultimately leaving only the exoskeleton which acts as a puparium for the larva (often referred to as a "mummy"). The host aphids can display a range of resistance mechanisms, such as physical repulsion of the ovipositing wasp, or by immunological attacks on the parasitoid eggs in the haemocoel [4,17,20,21]. An additional possibility is the potentially altruistic act of promoting predation before the parasitoid completes development [4]; see also [27,28]. However, little is known about the mechanisms of host behaviour alteration by parasitoids. An example, in this regard, is the findings on the modified behaviour of caterpillars by parasitoids [25]. Parasitoids induce and manipulate an aggressive tactile movement in caterpillars that is useful to resist predators [25]. The aggressive induced movement of the caterpillars will deter the intra-guild predation and thus protect the parasitoid juveniles in the parasitised caterpillars [25]. See also see [17,20] for reviews on parasitoid-aphid interactions. It can be argued, nevertheless that the relationship between parasitoids and their hosts (such as aphids) take the form of co-evolutionary arms-race where each party strives to acquire the upper hand in a continuous fashion [26].

Aphid colonies often comprise clonal lineages. Promoting the destruction of parasitoid larvae by self-sacrifice may be favoured if by doing so other members in the colony of genetically identical aphids are less likely to be attacked next generation of parasitoids [4,13,14,27,28]. This could be facilitated by changing positions on the

plant so that parasitised individuals are more exposed to predators [4,28]. It has already been shown that aphids parasitised by an endoparasitoid wasp differ in their mummification positions on the host plant depending on the developmental stage of the parasitising wasp larvae [18,22]. Aphids with dormant larvae tend to abandon the plant and mummify in obscured sites [18, 22], whilst individuals containing non-diapausing larvae tend to leave the aphid colony to mummify on the upper-leaf surfaces [22,18].

Materials and methods

We used a parasitoid wasp-aphid host system. *Aphidius ervi* is an endoparasitic parasitoid of a range of aphids, including the pea aphid *Acyrthosiphon pisum*. Under laboratory conditions [20ºC, 16:8 (L:D) cycle], the development of *Aphidius ervi* from an egg to an adult takes approximately 10 days. As in all Hymenoptera, *A. ervi* has a haplodiploid genetic system (fertilised eggs give rise to diploid females while unfertilised ones produce haploid males). The pea aphid *Acyrthosiphon pisum* is a common host of *A. ervi*. These aphids feed on herbaceous legumes and can reproduce asexually by parthenogenesis for repeated generations under stable moderate laboratory conditions as well as during spring and summer months in temperate regions, before reproducing sexually as day-length shortens at the end of the season [29]. Thus, it is easy to maintain clonal groups of aphids in the laboratory. We used a half-sib breeding design [30] for a haplodiploid system to estimate the genetic variance components of the parasitoid's influence on host distribution on the plant after parasitisation. This design allows separation of additive (heritable) genetic effects from non-additive (non-heritable) and maternal effects. We used one genotype clone of *A. pisum*. We note that this experimental approach does not allow a

distinction between the immediate effects of the parasitoid female on the aphids prior to parasitisation and the effects of the developing larvae post parasitisation.

Sampling, Rearing & Mating:

A single genotype of pea aphid (*Acyrthosiphon pisum)* was obtained from Imperial College (London). Aphids were maintained on a continually replaced stock of broad bean plants (*Vicia faba* L. var major Harz; Green Windsor breed) at low to medium densities to maintain plant health and to avoid production of alate and sexual morphs. Broad beans were germinated in Jiffy pots (no. 7) and then potted in John Innes no. 1 compost in 3-inch plastic pots. Plants and aphids were kept at 22C° and a 16:8 (L: D) photoperiod cycle.

Aphidius ervi was obtained from Koppert Systems (NL). Two hundred and fifty wasps were used to establish a colony which was maintained on pea aphids under the conditions described above. Emerging adult wasps were fed using cotton dental rolls drenched in 10% sucrose solution. To control for maternal effects, the colony was maintained in the laboratory for at least one generation before individuals were sampled for the breeding experiments.

Experimental Breeding Design.

Aphid pupae ("mummies") were collected over several days and isolated individually in Petri dishes and kept in the dark at 4ºC in order to synchronise the eclosion of a large number of individuals. We randomly selected freshly-eclosed male and female parents for the breeding experiment. Each mating pair was placed together in a '00' pharmaceutical gelatine capsule. Thirteen sires were each mated to 3 randomly selected dams to generate a total of 39 full-sib families. Note that females are

receptive to mating for a very short period following eclosion (ca. 2 hours) after which they may reproduce parthenogenetically [31]. Mated females were individually introduced into plastic cages with an aphid-proof mesh tops. Each cage contained a standardised broad bean plant (height ca. 25cm and age ca. 5 weeks) infested with 30 juvenile aphids (1st and 2nd instars balanced in numbers across all replicates and treatments). Cages were maintained at 22°C and a 16:8 (L:D) photoperiod. Mummies (containing wasp pupae) were collected after 9 to 10 days and isolated individually in Petri dishes and maintained under the same growth conditions. Four unmated daughters were selected from each family and placed individually into plastic cages with a height and age standardised broad bean plants infested with 30 1st and 2nd instar aphids as before. Because of the logistic constraints on mating wasps (i.e. the limited time of receptivity of females and the short lifespan of males), sire families were set up as they became available. Cages were initially grouped by family but were then haphazardly rearranged within the growth chamber every other day during watering and checking each cage. There was no significant effect of position within incubator ($P \geq 0.117$) or date of setup ($P \geq 0.274$) on any of the response variables.

The positions of mummies were recorded on and off the plant. For each mummy on the plant, we recorded whether they were in the top, middle or bottom third of the plant. In some cases when plants were height standardised by cutting the growing tip, new shoots were produced from the base of the plant.

Data Analysis.

Data were analysed in JMP (v.9). For comparisons of proportions of aphids on and off the plant, data were analysed using likelihood ratio tests. For the comparisons of aphid distributions among locations on the plant, a nominal logistic regression model was used. For the genetic analysis, where there were more than two categories, data with

regard to the comparisons of proportions of aphids were analysed using a nominal logistic regression model (JMP v.9). Furthermore, for such analyses the positions of aphids on the plant were the dependent categories populated by the frequency of aphids in each position. Because of the unbalanced design, significance testing was performed using maximum likelihood and likelihood ratio chi-square tests. The quantitative genetic model used was a nested half-sib design where;

Position = Sire + Dam (Sire) + Daughter [Dam (Sire)] + error

Results

Here we consider genetic variation in the interaction between the wasp *Aphidius ervi* and the pea aphid *Acyrthosiphon pisum*. First, in laboratory experiments we showed that parasitism causes behavioural modification in the aphids in terms of where they move. More aphids were found to leave their experimental plants when exposed to a parasitoid (73%) than when unexposed (9%: Likelihood ratio test, $\chi^2_1 = 561.5$, P < 0.0001). Moreover, of the aphids that remained on the plant prior to mummification, parasitism influenced where on the plants the mummies were found, with more parasitised mummies found lower down on the plants (Likelihood ratio test: $\chi^2_2 = 54.9$, P<0.0001: **Fig. 1**). Using a standard half-sib breeding design, we also showed significant additive genetic variation for the level of parasitism (proportion/number of aphids parasitised: significant sire effect; **Table 1**).

The quantitative genetic model used was a nested half-sib design where;

Position = Sire + Dam (Sire) + Daughter [Dam (Sire)] + error

There were also significant dam effects (i.e. differential maternal effects within-sib lines, and within-half-sib lines. We note that three dams were mated to a single sire of the thirteen male wasps used). This suggests non-additive and/or maternal effects influencing parasitism rate (**Table 1**). Parasitoid wasp genotype was highly significantly associated with aphid behavioural modification. In terms of aphid mummification on or off the plant, there was again both a sire (**Fig. 2**, **Table 1**) and dam effect (**Table 1**). The same was also true for the distribution of parasitised mummies that remained on the plant (**Fig. 3**, **Table 1**).

Discussion

These data therefore suggest both additive and non-additive sources of genetic variation for the wasp's effect on the aphid's behaviour. Our results confirm that parasitism by a parasitoid wasp can lead to behavioural modifications in an aphid host [22,23]. Such modifications can be favoured to protect parasitoid offspring from intra-guild competition [4,13,14,28], see also [11]. On the contrary, these alterations could be beneficial for the parthenogenetic aphid to disperse towards more suitable microclimatic conditions [18,22,23]. Such alterations also trigger specific plastic phenotypic responses in aphids, transmittable through generations, to escape (disperse) [32], and resist or evade subsequent parasitoid attacks [4,28]. Although genetic variation in parasitoid virulence and aphid resistance has been found before [13,14,27], we have shown here for the first time that variation in host modification is associated with genetic variation in the parasitoid, providing an example of an inter-specific indirect genetic effect. Whether or not the overall change in location of aphid pupation following parasitism is to the benefit of the host or the parasitoid remains unclear. Such change, in any case, may well be context-dependent (concordant with

findings on potato aphid-natural enemy system [22,23]). Nonetheless, the extent of that change in behaviour is influenced by genetic variation in the wasps. Indeed, this behaviour of a parasitised aphid is best thought-of as a shared phenotype of both the wasp's genome and the aphid's genome, as envisaged by IGE theory [1,33]. Interestingly, our results are concordant with the observations made recently providing a genetic basis for the influence of the genetics of a parasite, functioning beyond the individual level of the parasite, on its host phenotype [34]. Whilst the phenotype may be shared, there is an important asymmetry for understanding the evolutionary consequences though: wasps have greater potential to choose their hosts than the aphids do to choose their parasites [35] (exemplified by the 'life vs. lunch' dilemma where preys strive to save their life whereas the attacker forages for provisions [36], but see [37]. It could be argued that aphid resistance will potentially select on more manipulative parasitoids. Therefore, forming a mechanism of aphid choice will be a non-random success of parasitoid genotypes since selective pressure operates on both parties [13,14,27,38,39]

Our work highlights the implications of understanding the genetics of the interactions between natural enemies, including in terms of untangling the manipulative impact of the natural enemies on their hosts [2], for enhanced biological control and ecosystem conservation strategies.

Glen Powell at Imperial College (London) for providing the initial sample of green pea aphid genotype (JF01/29).

References

1. Moore, A. J., E. D. Brodie, & J. D. Wolf. 1997. Interacting phenotypes and the evolutionary process: I. direct and indirect genetic effects of social interactions. *Evolution* 51: 1352-1362.

2. Wolf, B. J. 2000. Indirect genetic effects and gene interactions. In B. J. Wolf, E. D. Brodie, and M. J. Wade (Eds.), *Epistasis and the Evolutionary Process* (158–176). New York: Oxford University Press.

3. Chenoweth, S. F., H. D. Rundle, & M. W. Blows. 2010. Experimental evidence for the evolution of indirect genetic effects: Changes in the interaction effect coefficient, psi (Ψ), due to sexual selection. *Evolution*, 64(4): 1849-1856.

4. McAllister, M. K., B. D. Roitberg, & K. L. Weldon. 1990. Adaptive suicide in pea aphids: Decisions are cost sensitive. *Animal Behaviour* 40 (1): 167-175.

5. Poulin, R. 2006. *Evolutionary Ecology of Parasites.* New Jersey, USA: Princeton University Press.

6. Thomas, F., Brodeur, J., Maure, F., Franceschi, N., Blanchet, S. & T. Rigaud, 2011. Intraspecific variability in host manipulation by parasites. *Infection, Genetics and Evolution* 11: 262-269.

7. Ebert, D. 2005. Ecology, Epidemiology, and Evolution of Parasitism in Daphnia [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology. Retrieved from http://www.ncbi.nlm.nih.gov/books/NBK2040/

8. Moore, J. 2002. *Parasites and the Behavior of Animals.* New York, USA: Oxford University Press.

9. Hammerschmidt, K., K. Koch, M. Milinski, G. A. Parker, & J. C. Chub. 2009. When to Go: Optimization of Host Switching in Parasites with Complex Life Cycles. *Evolution* 63 (8): 1976-1986.

10. Levri, E. P. 1998. The influence of non-host predators on parasite-induced behavioral changes in a freshwater snail. *Oikos* 81 (3): 531-537.

11. Brodeur, J., & J. A. Rosenheim. 2000. Intraguild interactions in aphid parasitoids. *Entomologia Experimentalis et Applicata* 97 (1): 93-108.

12. Everard, A., C. T. Griffin, & A. B. Dillon. 2009. Competition and intraguild predation between the braconid parasitoid *Bracon hylobii* and the entomopathogenic nematode *Heterorhabditis downesi*, natural enemies of the large pine weevil, *Hylobius abietis*. *Bulletin of Entomological Research* 99 (02): 151-161.

13. Henter, H. J. 1995. The potential for coevolution in a host-parasitoid system: II. Genetic variation within a population of wasps in the ability to parasitize an aphid host. *Evolution* 49 (3): 439-445.

14. Henter, H. J., & S. Via. 1995. The potential for coevolution in a host-parasitoid system: I. Genetic variation within an aphid population in susceptibility to a parasitic wasp. *Evolution* 49 (3): 427-438.

15. Thompson, J. N. 1994. *The Coevolutionary Process*. Chicago, Illinois, USA: University of Chicago Press.

16. Shuster, S. M., E. Lonsdorf, G. M. Wimp, T.G. Whitham & J. K. Bailey. 2006. Community heritability measures the evolutionary consequences of indirect genetic effects on community structure. *Evolution* 60: 991-1003.

17. Godfray, H.C. J. 1994. *Parasitoids: Behavioral and Evolutionary Ecology.* Princeton, New Jersey, USA: Princeton University Press.

18. Brodeur, J., & J. N. McNeil. 1990. Overwintering microhabitat selection by an endoparasitoid (Hymenoptera: Aphidiidae): Induced phototactic and thigmokinetic responses in dying hosts. *Journal of Insect Behavior* 3 (6): 751-763.

19. Lagos, N. A., E. Fuentes-Contreras, F. Bozinovic, & H. M. Niemeyer. 2001. Behavioural thermoregulation in *Acyrthosiphon pisum* (Homoptera: Aphididae): the effect of parasitism by *Aphidius ervi* (Hymenoptera: Araconidae). *J Therm Biol* 26(2): 133-137.

20. Wajnberg, É., C. Bernstein, & Van Alphen, J. 2008. *Behavioral ecology of insect parasitoids: From theoretical approaches to field applications.* Retrieved from http://bit.ly/A0yfNu

21. Müller, C. B., Völkl, V., & Godfray, H. C. J. 1997. Are behavioural changes in parasitised aphids a protection against hyperparasitism? *European Journal of Entomology* 94(2): 221-234.

22. Brodeur, J., & J. N. McNeil. 1989. Seasonal microhabitat selection by an endoparasitoid through adaptive modification of host behavior. *Science* 244 (4901): 226-228.

23. Brodeur, J., & J. N. McNeil. 1992. Host behaviour modification by the endoparasitoid *Aphidius nigripes*: A strategy to reduce hyperparasitism. *Ecological Entomology* 17(2): 97-104.

24. Pennacchio, F. M., & M. R. Strand. 2006. Evolution of developmental strategies in parasitic Hymenoptera. *Annual Review of Entomology* 51: 233-258.

25. Grosman, A.H., A. Janssen, E.F. de Brito, E.G. Cordeiro, F. Colares, et al. 2008. Parasitoid increases survival of its pupae by inducing hosts to fight predators. *PLoS ONE* 3(6): e2276. (doi:10.1371/journal.pone.0002276)

26. Dawkins, R., and J. R. Krebs. 1979. Arms races between and within species. *Proceedings of the Royal Society London. B* 205 (1161): 489-511.

27. Ferrari, J., C. B. Müller, A. R. Kraaijeveld, and H.C J. Godfray. 2001. Clonal variation and covariation in aphid resistance to parasitoids and a pathogen. *Evolution* 55 (9): 1805-1814.

28. Smith Trail, D. R. 1980. Behavioral interactions between parasites and hosts: host suicide and the evolution of complex life cycles. *Am. Nat* 116: 77-91.

29. Moran, N. A. 1992. The evolution of aphid life cycles. *Annual Review of Entomology* 37: 321-348.

30. Becker, W. A. 1984. *Manual of Procedures in Quantitative Genetics.* Pullman, WA, USA: Academic Enterprises.

31. Gilchrist, G. W. 1996. Quantitative Genetic Analysis of Thermal Sensitivity in the Locomotor Performance Curve of *Aphidius ervi. Evolution* 50 (4): 1560-1572.

32. Sloggett, J. J., and W. W. Weisser. 2002. Parasitoids induce production of the dispersal morph of the pea aphid, *Acyrthosiphon pisum. Oikos 98*(2): 323-333.

33. Dawkins, Richard. 1982. *The Extended Phenotype: The Gene as the Unit of Selection.* Oxford, USA: W.H. Freeman & Company.

34. Hoover, K., M. Grove, M. J. Gardner, D. P. Hughes, J. McNei, and J. Slavicek, 2011. A gene for an extended phenotype. *Science* 333: 1401.

35. Lapchin, L., and T. Guillemaud. 2005. Asymmetry in host and parasitoid diffuse coevolution: When the red queen has to keep a finger in more than one pie. *Frontiers in Zoology* 2 (4): 1-5. (doi:10.1186/1742-9994-2-4)

36. Krebs, J. R., and N. B. Davies. 1998. *An Introduction to Behavioural Ecology.* Oxford, England: Blackwell Science Ltd.

37. Van Valen, L. 1973. A new evolutionary law. *Evolutionary Theory* 1: 1-30.

38. Carius, H. J., T. J. Little, and T. D. Ebert. 2001. Genetic variation in a host-parasite association: Potential for coevolution and frequency-dependent selection. *Evolution* 55 (6): 1136-1145.

39. Fritz, R. S. 1982. Selection for host modification by insect parasitoids. *Evolution* 36 (2): 283-288.

Figures

Fig 1.
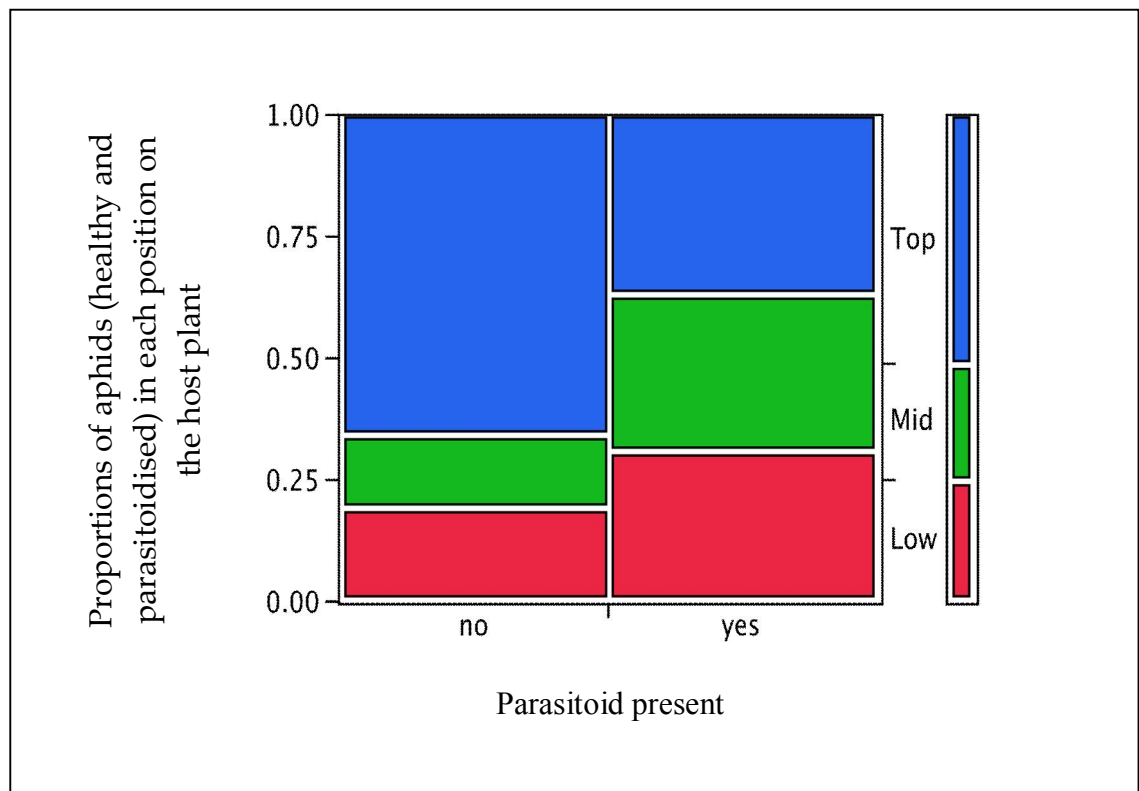
Fig 2.
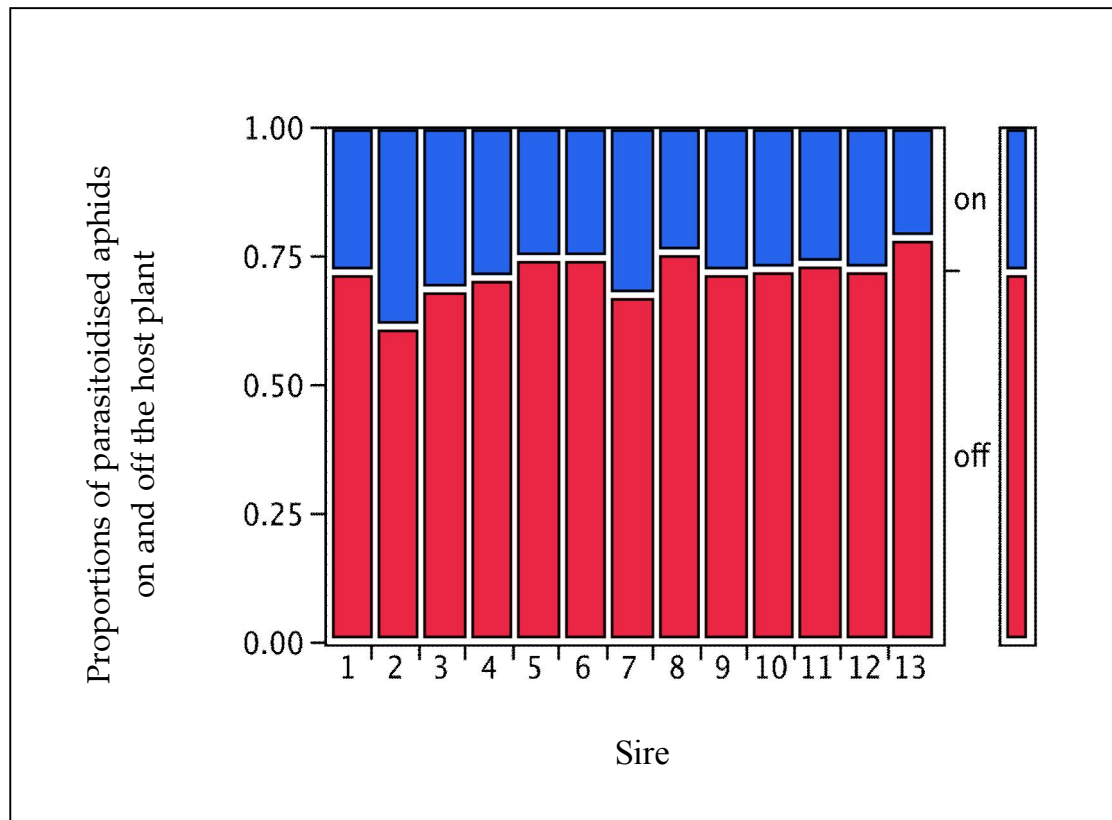
Fig 3.
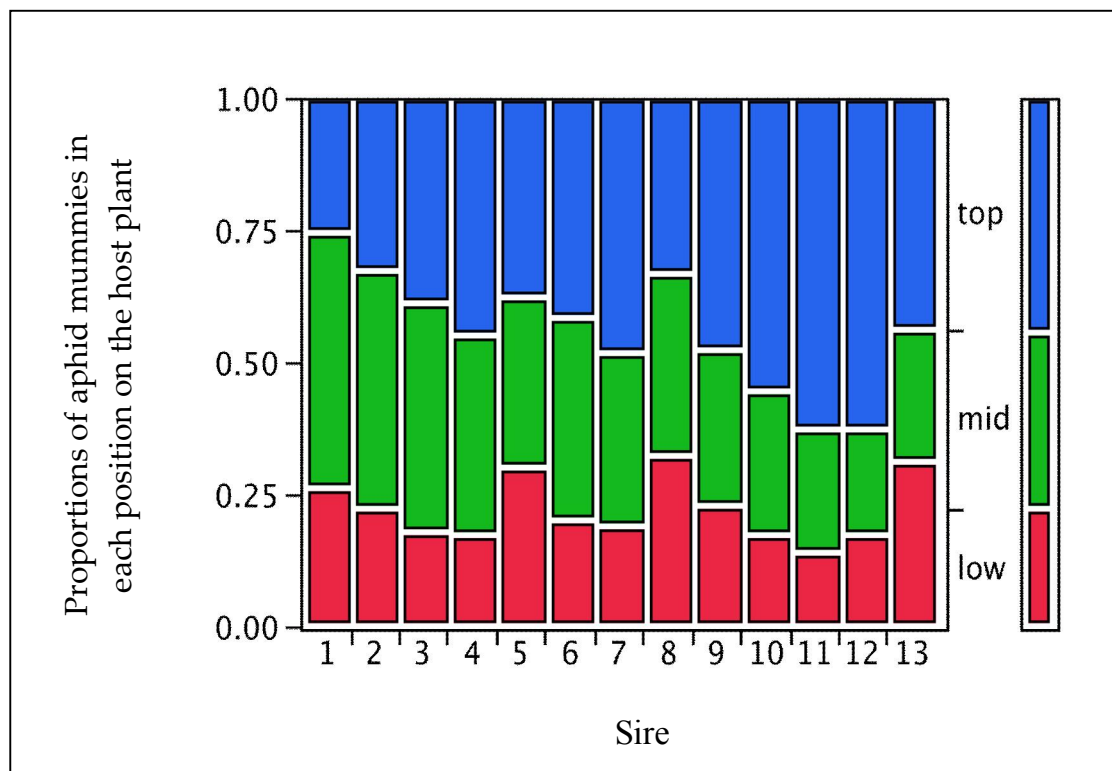
Figure Legends

Figure 1. Aphid position on the plant was significantly influenced by parasitism. Different colours indicated position (top third, middle third, and lowest third) on the experimental plant.

Figure 2. The proportion of aphids forming mummies on and off the plant for each sire family.

Figure 3. The proportion of aphids forming mummies on each of the thirds of the plant.

Tables

Table 1. Effects of Sire, Dam and Daughter on aphid position and likelihood of parasitism. Aphid counts for each category were analysed using a multi-nomial logistic regression analysis (JMP v9). Significance testing was via Likelihood Ratio tests and presented as chi-square values. There were a total of 13 sires, 33 dams and 125 daughters. For classes where the frequency of observations was zero that class was dropped from the model.

Source	On vs. Off host plant		Top, middle or bottom of host plant		Parasitised vs. non-parasitised	
	χ^2 (df)	P	χ^2 (df)	P	χ^2 (df)	P
Sire	28.85(12)	0.0042	41.06(24)	0.0164	58.21 (12)	<0.0001
Dam	47.89 (13)	<0.0001	75.68(40)	0.0006	42.51 (12)	<0.0001
Daughter	183.89 (74)	<0.0001	328.58(182)	0.00107	291.51 (41)	<0.0001

Parameter device + Additional analyses
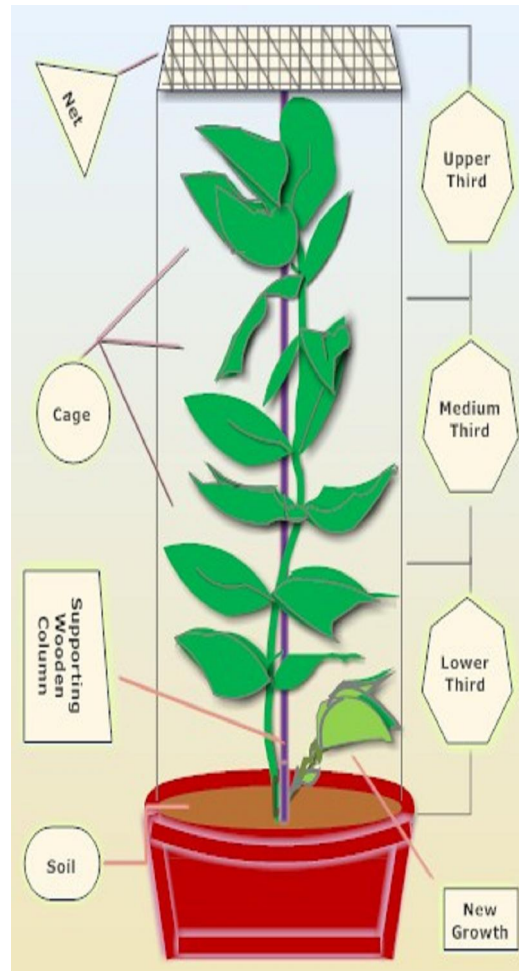
Figure 1`: Illustration of experimental units.

Each individually caged plant was divided into three parts; Bottom, Middle and Top using a ruler. Once the aphids had mummified, the location of each mummy was recorded according to its position on the caged plant or on other locations within the cage. A wooden stick was used to support the plant.
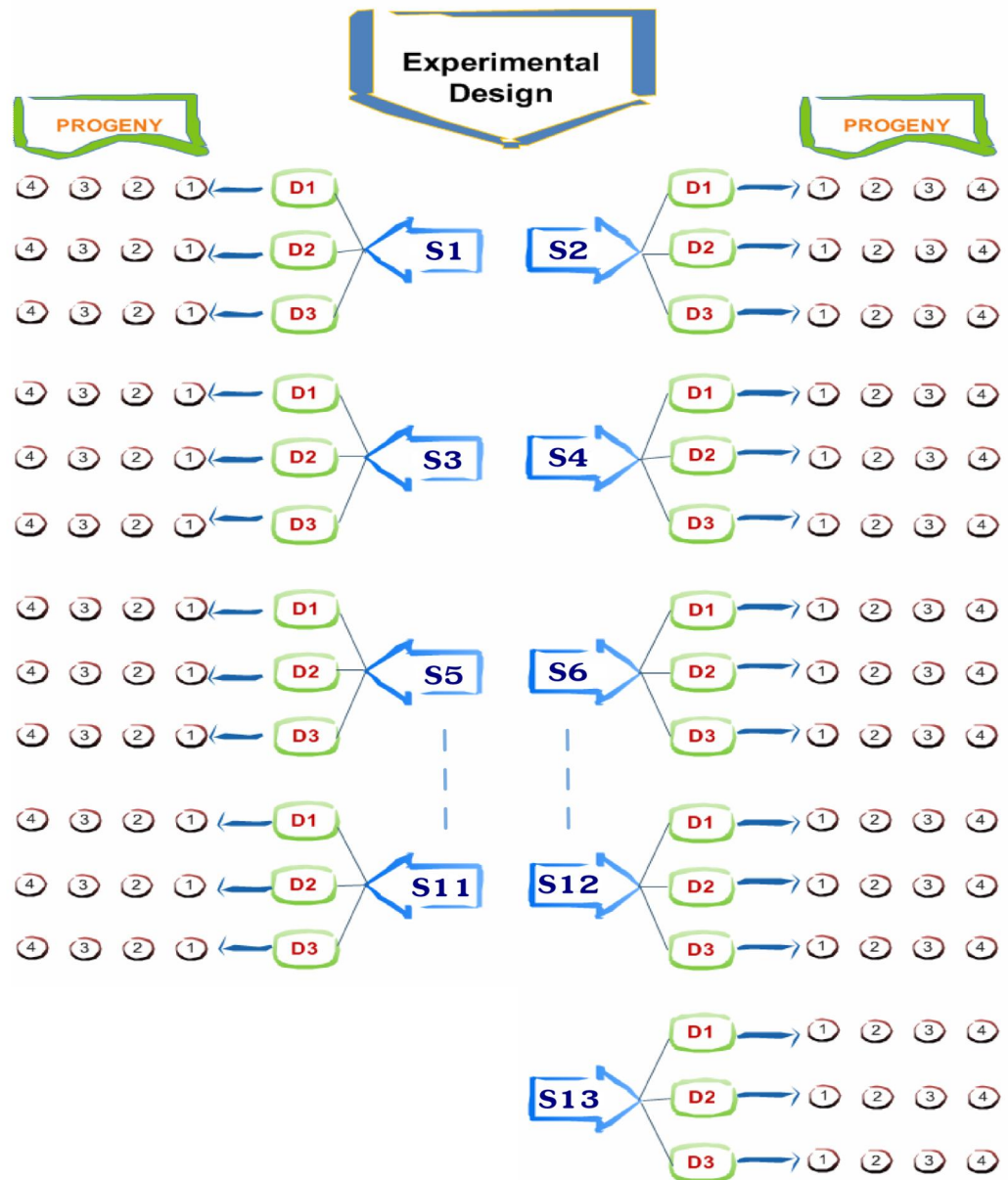
Figure 2`: Experimental Design. To obtain wasps for the bioassay, each of the 13 sires was mated to 3 randomly selected females through which a quantitative genetic half-sib design was created. Four offspring of each dam family (n = 39) were introduced into a new cage containing an aphid-infested broad bean plant.

Did parasitoidisation (as a process) induce aphids to leave their host plant?

YES!
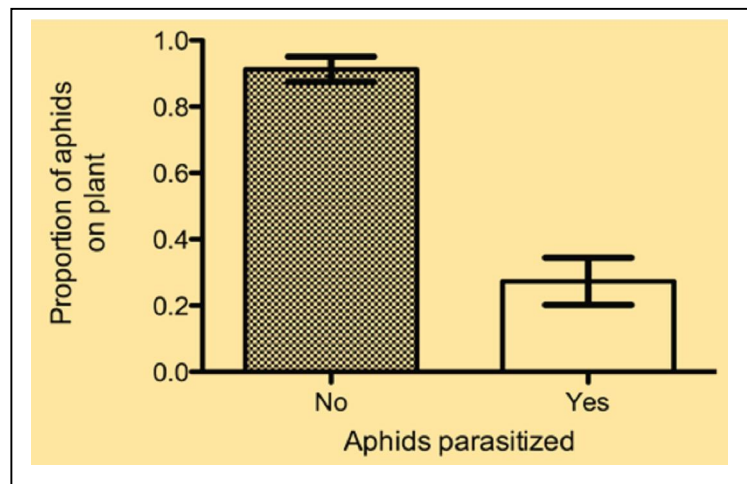
Figure 3`: Proportion of aphids on and off the plant due to parasitoidism.
91% of unparasitised aphids were found on the host plant whereas only 27% of parasitised aphids were found on the host plant (t=15.85, df =21, P<0.0001).

Did parasitoidisation (as a process) influence the distribution of aphids on their host plant?

YES!
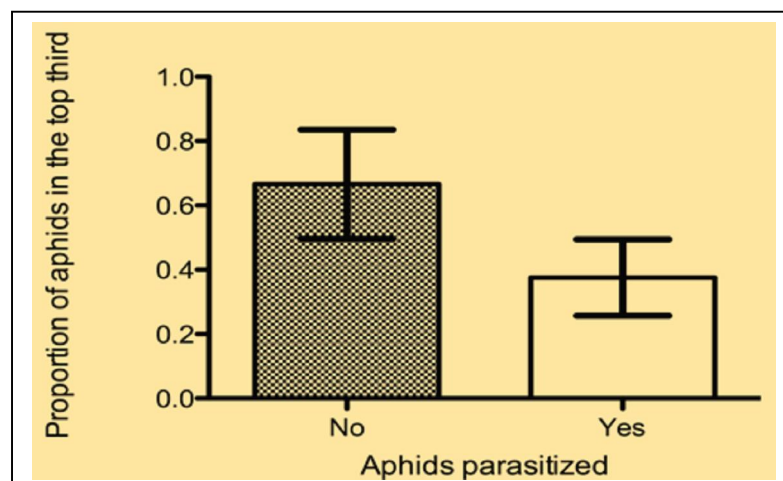
Figure 4`: On-plant distribution for aphids subject to parasitoidism.

Notable proportion (67%) of non-mummified aphids were found on the top third of the plant, while only 38% of parasitised aphids (mummies) were found on the top third of the host plant (t=3.210, df =21, P=0.004).

Was the proportion of aphids that get parasitised influenced by parasitoid genotype?

 YES!

Source	DF	L-R Chi-Square	Prob>ChiSq
Sire	12	42.8425116	<.0001
Dam[Sire]	13	54.9324932	<.0001
Daughter[Dam]	12	44.7571128	<.0001

Table 1`: Parasitoid virulence measured as differential mummification rates.

There was a significant additive genotype (sire) effect on the distribution of parasitised and non-parasitised aphids. There was also a significant effect of dams indicating a maternal influence on the distribution of parasitised vs. non-parasitised aphids. This could reflect a fitness-difference between sire families.

Parasitoid Manipulative Gambits

[24,39,44,49]

Aphid neonates are more preferable

[3,49]

Preferably
no super-parasitism occurs.
This is in order to ensure the well-being of
the developing parasitoid larva

[17,30]

Start

Oligophagous Solitary Parasitoid

[15,21]

Host Detection

[33,38]

Probing, palpation

Rejection of already
parasitised individuals   [4,5]

Host discrimination ———[34,46]

Random **search** and
systematic **scan** ———[40,50]
of plant

[27,30]——— Associative Learning

Behavioural plasticity

Host acceptance

Preference is
influenced by genotype ——— [38,39]

[49]

[5,39]

The younger the aphid, ———
the easier the parasitoid's task

[1,19,36,38]

Plant volatiles
,under aphid infestation,
are emitted and thus may attract
parasitoids;
"semiochemicals; synomones"

[3,21,49]

Single egg per **oviposition**;
with high female
parasitoid's fecundity

Quarrel

Ongoing counter-response to aphid's resistance and countermeasures

Increased Virulence

Escalated Resistance

[14,32,49] **Co-evolutionary Arms-race**

[40,50]——— Chemical cues

Direct and indirect **manipulation** of host———[35,49]

Genetic variation basis
for
parasitoid virulence
and
aphid resistance

'The enemy of my enemy is my friend '

Varying parasitoid virulence; contingent on
aphids' response + environment

[28,29,32]

[20,25,26]

[36]

'Contact chemicals'

[29]

Production of overlapping generations ———[9,21]

[10,11,49]———Avoidance of amid-haemocoel oviposition

[7,8,22,49]

Alteration of host
behaviour and physiology

[37,49]———

Mechanistic and morphological changes
in the egg in order to deceive
host's defences

[6,18]

Host castration or
halting gonad-function

[2,11,12,13,23,42]———

Elusive egg coating to evade
host
physiological resistance

Oppressing and altering
host immune system

[29,48]

[31,42,45]———

Avoidance of encapsulation of larva

[18,37,47,48]

Host Regulation

[37,45]———

Relative time lag between oviposition
and successful parasitoidism

Hindering the production of
wings

GOAL >>> To increase the Likelihood of juveniles' survival

Resuming activity >>> subsequent attacks

[16]

Host regulation varies according
to changes in the
age, size and quality
of hosts

Continuity

End

Continuum of
development, fecundity
and virulence

Emergence of new
parasitoid adult
=
Host is dead

Mummification of host

[29,35,37,49]

[21,29,41,43,48,49]

[29]

[21,29,49]——— *Predation* facet of Parasitoidism

Symbiotic bacteria, and viruses are thought to
play role in shaping the manipulation tactics

Parasitisation facet of Parasitoidism

Aphids are used as puparia

Figure 5`: An overview on parasitoidism and major parasitoid tactics regarding host manipulation.

This illustration casts light on the several tactics applied by solitary parasitoids, such as the braconid wasp *Aphidius ervi* Haliday, in order to manipulate their hosts.

Parasitoidism is an eco-evolutionary process which starts by laying eggs (via an ovipositor) into suitable hosts. *Aphidius ervi* females prefer to inject a single egg per host. Preferable hosts are fruit flies (*Drosophila sp.*) and aphids [pea aphid (*Acyrthosiphon pisum*), in particular]. The process ends by the predation of the host by the developing parasitoid juvenile which emerges, upon completion of the life cycle, from the remainder of the host (exoskeleton i.e. mummy).

Early instars are the most favourable age stage for oviposition for a variety of reasons. For example, early instars display less mechanical resistance i.e. kicking, besides the fact that their cornicles/siphunculi are smaller in size than the adults' cornicles and thus less harmful during the quarrel between the female parasitoid and the detected potential host. The female parasitoid examines (palpates) the detected host and will mostly discriminate it if the host is already parasitised.

The interaction between parasitoid wasps and aphids is considered to follow a co-evolutionary arms-race scenario where each one of these two natural enemies strives to cap each others' fitness. This is observed through varying virulence and host-regulation strategies applied by the parasitoid in order to subdue its host, and the ongoing resistance shown by hosts as counter-response to manipulation by parasitoids. The interaction is also subject to the indirect effects of the biotic resource (host plant), and may vary according to environmental change. *Aphidius ervi* shows a notable ability to scan plants in search for hosts. There is a good body of evidence supporting a capacity for 'associative learning' maintained by this forager.

References

Note that the references in this annex are alphabetically ordered then numbered, unlike the references in the relevant mother chapter-paper. This is due to the fact that this annex is an illustrative addendum and makes no part of the manuscript. The latter complies with the guidelines of the *Biology Letters*.

1. Agrawal, A. A. (2000). Mechanisms, ecological consequences and agricultural implications of tri-trophic interactions. *Curr Opin Plant Biol., 62*(2), 329-335.

2. Asgari, S., Theopold, U., Wellby, C., & Schmidt, O. (1998). A protein with protective properties against the cellular defense reactions in insects. *Proc. Natl. Acad Sci, 95*, 3690-3695.

3. Bai, B. (1991). *Conspecific and heterospecific interactions in two species of aphid parasitoids, Aphidius ervi and Aphelinus asychis (Hymenoptera: Aphidiidae, aphelinidae).* Retrieved from http://summit.sfu.ca/system/files/iritems1/4794/b14511447.pdf

4. Bai, B., & Mackauer, M. (1990). Host discrimination by the aphid parasitoid *Aphelinus asychis* (Hymenoptera: Aphelinidae): When superparasitism is not adaptive. *Can. Entomol. 122*, 363-372.

5. Bai, B., Luck, R. F., Forster, L., Stephens, B., & Janssen, J. J. M. (1992). The effect of host size on quality attributes of the egg parasitoid, *Trichogramma pretiosum. Entomologia Experimentalis et Applicata. 64*, 37-48.

6. Beckage, N. E., & Drezen, J. M. (2012). *Parasitoid viruses: Symbionts and pathogens.* Retrieved from http://bit.ly/N7inL4

7. Brodeur, J., & McNeil, J. N. (1989). Seasonal microhabitat selection by an endoparasitoid through adaptive modification of host behavior. *Science. 244* (4901), 226-228.

8. Brodeur, J., & McNeil, J. N. (1992). Host behaviour modification by the endoparasitoid *Aphidius nigripes*: A strategy to reduce hyperparasitism. *Ecological Entomology*. *17*(2), 97-104.

9. Campbell, A. (1973). *Seasonal changes in abundance of the pea aphid and its associated parasites in the southern interior of British Columbia.* (Doctoral Dissertation). Retrieved from

   http://summit.sfu.ca/system/files/iritems1/4328/b13651171.pdf

10. Carton, Y. & Nappi, A. J. (1997). *Drosophila* cellular immunity against parasitoids. *Parasitol. Today*. *13*, 218-227.

11. Carton, Y., & Nappi, A. J. (1991). The *Drosophila* immune reaction and the parasitoid capacity to evade it: Genetic and coevolutionary aspects. *Acta Oecologica*, *12*, 89-104.

12. Corley, L., & Strand, M. R. (2003). Evasion of encapsulation by the polyembryonic parasitoid *Copidosoma floridanum* is mediated by a polar body-derived extraembryonic membrane. *J. Invert. Pathol*, *83*, 86-89.

13. Davies, D. H., & Vinson, S. B. (1986). Passive evasion by eggs of braconid parasitoid *Cardiochiles nigriceps* of encapsulation in vitro by haemocytes of host *Heliothis virescens*. Possible role for fibrous layer in immunity. *Journal of Insect Physiology*, *32*, 1003-1010.

14. Dawkins, R., & Krebs, J. R. (1979). Arms races between and within species. *Proceedings of the Royal Society London. B. 205*(1161), 489-511.

15. Daza-Bustamante, P., Fuentes-Contreras, E., & Niemeyer, H. M. (2003). Acceptance and suitability of *Acyrthosiphon pisum* and *Sitobion avenae* as hosts of the aphid parasitoid *Aphidius ervi* (Hymenoptera: Braconidae). *Eur. J. Entomol.*, *100*(1), 49-53.

16. Demmon, A. S., Nelson, H. J., Ryan, P. J., Ives, A. R., & Snyder, W. E. (2004). *Aphidius ervi* increases its adult size by disrupting host wing development. *Physiological Ecology*, *33*, 1523-1527.

17. Desneux, N., Barta, R. J., Hoelmer, K. A., Hopper, K. R., & Heimpel, G. E. (2009). Multifaceted determinants of host specificity in an aphid parasitoid. *Oecologia*, *160*, 387-398.

18. Digilio, M. C., Isidoro, M. C., Tremblay, N. E., & Pennacchio, F. (2000). Host castration by *Aphidius ervi* venom proteins. *Journal of Insect Physiology*, *46*, 1041-1050.

19. Du, Y. A., Poppy, G. M., Powell, W., & Wadhams, L. J. (1997). Chemically mediated associative learning in the host foraging behavior of the aphid parasitoid *Aphidius ervi* (Hymenoptera: Braconidae). *Journal of Insect Behavior*, *10*(4), 509-522.

20. Ferrari, J., Müller, C. B., Kraaijeveld, A. R., & Godfray, H.C J. (2001). Clonal variation and covariation in aphid resistance to parasitoids and a pathogen. *Evolution*. *55*(9), 1805-1814.

21. Godfray, H. C. J. (1994). *Parasitoids: Behavioral and Evolutionary Ecology*. Princeton, New Jersey, USA: Princeton University Press.

22. Harvey, J. A. (1996*). Venturia canescens* parasitizing *Galleria mellonella and Anagasta kuehniella*: Is the parasitoid a conformer or regulator? *Journal of Insect Physiology*, *42*, 1017-1025.

23. Hayakawa, Y., & Yazaki, K. (1997). Envelope protein of parasitic wasp symbiont virus, polydnavirus, protects the wasp eggs from cellular immune reactions by the host insect. *Eur J Biochem*, *246*(3), 820-826.

24. He, X. Z., & Wang, Q. (2006). Host age preference in *Aphidius ervi* (Hymenoptera: Aphidiidae). *New Zealand Plant Protection*, *59*, 195-201.

25. Henter, H. J. (1995). The potential for coevolution in a host-parasitoid system: II. Genetic variation within a population of wasps in the ability to parasitize an aphid host. *Evolution*. *49*(3), 439-445.

26. Henter, H. J., & Via, S. (1995). The potential for coevolution in a host-parasitoid system: I. Genetic variation within an aphid population in susceptibility to a parasitic wasp. *Evolution*. *49* (3), 427-438.

27. Hollis, K. L., College, M. H., & Guillette, L. M. (2011). Associative learning in insects: Evolutionary models, mushroom bodies, and a neuroscientific conundrum. *Comparative Cognition & Behavior Reviews*, *6*, 24-45. (doi: 10.3819/ccbr.2011.6004)

28. Hufbauer, R. A. (2001). Pea aphid–parasitoid interactions: Have parasitoids adapted to differential resistance? *Ecology*, *82*(3), 717-725.

29. Jervis, M. A. (2005). *Insects as Natural Enemies: A Practical Perspective*. Kluwer Academic Publishers.

30. Langley, S. A., Tilmon, K. J., Cardinale, B. J., & Ives, A. R. (2006). Learning by the parasitoid wasp, *Aphidius ervi* (Hymenoptera: Braconidae), alters individual fixed preferences for pea aphid color morphs. *Oecologia*, *150*(1), 172-179.

31. Lavine, M. D., & Beckage, N. E. (1995). Polydnaviruses: Potent mediators of host insect immune dysfunction. *Parasitology Today*, *11*, 368-378.

32. Li, S., Falabella, P., Giannantonio, S., Fanti, P., Battaglia, D., Digilio, M. C., Volkl, W., Sloggett, J. J., Weisser, W. & Pennacchio, F. (2002). Pea aphid clonal resistance to the endophagous parasitoid *Aphidius ervi*. *Journal of Insect Physiology*, *48*, 971-980.

33. Libbrecht, R., Gwynn, D. M., & Fellowes, M. D. E. (2007). *Aphidius ervi* preferentially attacks the green morph of the pea aphid, *Acyrthosiphon pisum*. *Journal of Insect Behavior*, *20*(1), 25-32.

34. Micha, S. G., Wellings, P. W., & Morton, R. (1992). Time-related rejection of parasitised hosts in the aphid parasitoid, *Aphidius ervi*. *Entomologia Experimentalis et Applicata*, *62*(2), 155-161. (doi: 10.1111/j.1570-7458.1992.tb00655.x)

35. Oliver, K. M., Noge, K., Huang, E. M., Campos, J. M., Becerra, J., & Hunter, M. S. (2012). Parasitic wasp responses to symbiont-based defense in aphids. *BMC Biology*, *10*(11), 1-10. (doi:10.1186/1741-7007-10-11)

36. Paré, P. W., & Farag, M. A. (2008). Natural enemy attraction to plant volatiles. In *Encyclopedia of entomology*. Springer Science+Business Media B.V. Retrieved from http://courses.ttu.edu/ppare/Research/PDF%20Articles/Encyclopedia%20of%2 0Entomology%202008.pdf

37. Pennacchio, F., & Strand, M. R. (2006). Evolution of developmental strategies in parasitic hymenoptera. *Annual Review of Entomology*, *51*, 233-258.

38. Powell, W., & Wright, A. F. (1988). The abilities of the aphid parasitoids *Aphidius ervi* Haliday and *A. rhopalosiphi* De Stefani Perez (Hymenoptera: Braconidae) to transfer between different known host species and the implications for the use of alternative hosts in pest control strategies. *Bull. Entomol. Res*, *78*, 683-693.

39. Rehman, A., & Powell, W. (2010). Host selection behaviour of aphid parasitoids (Aphidiidae: Hymenoptera). *Journal of Plant Breeding and Crop Science*, *2*(10), 299-311.

40. Roitberg, C., Li, B. D., & MacKauer, M. (1992). The search pattern of a parasitoid wasp, *Aphelinus asychis*, for its host. *Oikos*, *65*(2), 207-212.

41. Sampaio, M. V., Bueno, V. H. P., & De Conti, B. F. (2008). The effect of the quality and size of host aphid species on the biological characteristics of *Aphidius colemani* (Hymenoptera: Braconidae: Bphidiinae). *Eur. J. Entomol*, *105*, 489-494.

42. Schmidt, O., Theopold, U., & Strand, M. (2001). Innate immunity and its evasion and suppression by hymenopteran endoparasitoids. *BioEssay*, *23*, 344-351.

43. Sequeira, & Mackauer, M. (1993). The nutritional ecology of a parasitoid wasp, *Ephedrus californicus* Baker (Hymenoptera: Aphidiidae). *Can. Entomol*, *125*, 423-430.

44. Soler, R., Pineda, A., Li, Y., Ponzio, C., Van Loon, J. J. A., Weldegergis, B. T., & Dicke, M. (2012). Neonates know better than their mothers when selecting a host plant. EV-1-EV-12. (In press). (doi:10.1111/j.1600-0706.2012.20415.x)

45. Strand, M. R., & Pech, L. L. (1995). Immunological compatibility in parasitoid-host relationships. *Annu. Rev. Entomol*, *40*, 31-56.

46. Verheggen, F., Vandermoten, S. M., Frère, I., Genin, V. J., Haubruge, E., & Hance, T. (2010). *Involvement of odorant cues in the process of superparasitism avoidance*. Poster session presented at the IXth European Congress of Entomology, Budapest, Hungary. Retrieved from http://orbi.ulg.ac.be/bitstream/2268/68468/1/Poster.pdf

47. Vinson, S. B. (1990). How parasitoids deal with the immune-system of their host -an overview. *Arch Insect Biochem Physiol*, *13*, 3-27.

48. Vinson, S. B., & Iwantsch, G. F. (1980). Host regulation by insect parasitoids. *The Quarterly Review of Biology*, *55*(2), 143-165.

49. Wajnberg, É., C. Bernstein, & Van Alphen, J. 2008. *Behavioral ecology of insect parasitoids: From theoretical approaches to field applications.* Retrieved from http://bit.ly/A0yfNu

50. Wellings, P. W. (1993). Foraging behaviour in aphid parasitoids: Spatial scale and resource assessment. *European Journal of Entomology*, *90*(4), 377-382.

Chapter 4: Plant community genetic effect mediates spatially separated competitors

Case Study

*Statement of the nature and extent of my own contribution:

I designed, performed and developed the experimental work, collected, entered and visualised the data and wrote the manuscript under Dr. Richard Preziosi's supervision.

I and Dr. Preziosi produced the paper in collaboration.

Dr. Nouri Baker participated in the maintenance of the root-knot system and helped in the nematode census.

Article for *Oikos*:

Plant Community Genetic Effect Mediates Spatially Separated Competitors

Case Study

Mouhammad Shadi Khudr[1], Nouri Baker[1], Richard Preziosi[1]

[1]Faculty of Life Sciences, The University of Manchester

Abstract

The role of indirect ecological effects (IEEs) between below-ground and above-ground communities associated with host plants is now well established. However, little is known about how such IEEs might influence competition within these communities and less is known about how plant genotype might influence these relationships. In this study we used a system comprising of tomato host plants, root-galling nematodes and sap-feeding aphids. We used multiple tomato and aphids genotypes, and examined the fitness consequences of herbivory and competition for each of the three organisms. First, we investigated the effect of multiple herbivores on plant biomass across several tomato cultivars (genotypes). Second, we observed the influence of intra-plant variation on the numbers of root-knot nematodes (RKN) in the absence of aphids. We also investigated the influence of two parthenogenetic aphid species when competing with RKN. Third, we quantified the impact of RKN on reproductive success and behaviour (on plant distribution) of a focal aphid species mediated by the variation among tomato cultivars (a community genetic effect). Our

data demonstrate that the biomass of the host plant depended not only on the host genotype but also on its interaction with the type of herbivory. We found that the fitness of each species in our model system was influenced by interactions between community genetic effects, competition, and IEE (i.e. the differential host modification by root or shoot herbivores). The performance of RKN was influenced by the interaction of competing aphids and host cultivar. The performance and behaviour of the focal species of aphid were influenced by the interaction of competition and host plant cultivar. This clearly demonstrates that community genetic effects can significantly moderate above-ground – below-ground IEEs. These findings have significant implications for the field of evolutionary ecology in terms of exploring the complexity of the interaction among IEEs, intra-specific genetic variation of the host plant, and inter-specific competition. Our results increase our understanding of the impact of spatially separated competition on crop productivity and on ecosystem properties and services. In addition, this work also highlights the direct applicability of community genetics in unravelling the dynamics of the ecology and evolution of economically important crop-pest relationships within agro-ecosystems.

Keywords:

Indirect ecological effects (IEE), community genetics, above- and below-ground interaction, tomato cultivars, green peach aphid, root-knot nematode, spatially separated competition.

Introduction

Community genetic effects (CGs) are the differential impacts of focal specific genotypes i.e. within-species (intra-specific) genetic variation on associated communities of species (Antonovics 1992, Whitham et al. 2003, 2006; see also thesis chapters 1 to 3). Intra-specific genetic variation effects of fundamental taxa (CGs) are now clearly demonstrated in empirical studies in more than one model system (e.g. Johnson and Agrawal 2005, Whitham et al. 2006, Tétard-Jones et al. 2007, Rowntree et al. 2011a, Zytynska et al. 2010, 2011). Furthermore, community genetic effects of ecologically important species, e.g. keystone species, have the potential to influence ecosystem properties and services (Luck et al. 2003, Whitham et al. 2003, 2006, Smith et al. 2008, Bailey 2011). However, the extent to which these effects reciprocally alter, and are altered by, other well-defined ecological processes, such as indirect ecological effects (IEEs: Wootton 1994, 2002, Astles et al. 2005) and competition (Smith et al. 2008; see also Östman 2011), has been the subject of much fewer empirical studies [see also (Hersch-Green et al. 2011)].

Despite the dominance of agricultural systems in the landscape (nearly 72% of the UK land surface is used for agricultural purposes; DEFRA 2011), there is a limited number of studies focusing on annual crop systems, and fewer still that examine the effects of genetic variation within crop species on natural pests, pathogens or associated communities. This is surprising given that community genetic effects are expected to be strong in agro-ecosystems (Neuhauser et al. 2003) because of the simplified nature of their communities (due to the conventional agricultural practices of maintaining mono-varietal plots). Consequently, the significantly reduced genetic variation in most cropping systems has increased vulnerability to serious pathogen and pest outbreaks (e.g. Larson and Whitham 1991, 1997, Revilla-Molina 2009).

However, the status quo of agro-ecosystems provides an opportunity for research on the interactions between host plant genotype, pest species/genotypes and IEEs, in order to untangle a portion of the complexity of plant – pest relationships and perhaps suggest more efficacious biological control strategies.

Below-ground herbivores:

Meloidogyne hapla (hereinafter referred to as RKN), is a polyphagous parthenogenetic nematode (Bird et al. 1996, 2009). This nematode is characterised as a serious root-knot phytoparasite of a wide range of economic plants including the family Solanaceae. It modifies host plant root cells causing their transformation into giant feeding cells through hyperplasia and hypertrophy (Caillaud et al. 2008, Moens et al. 2009). The gigantic cell effect (i.e. the formation of knots or galls) is a result of the alteration of plant gene expression by the pest. Plant hosts such as tomato (*Lycopersicon esculentum*) differ in their susceptibility to the root-knot nematodes (e.g. Singh and Khurma 2007; see also Morriën et al. 2011). Alteration by RKN changes the rate of uptake and use of water and nutrients by the host plant, but these changes should depend on the varying degree of host resistance to nematodes and aphids (Goggin et al. 2004, Cooper and Goggin 2005, Cooper et al. 2005, Goggin et al. 2006, Singh and Khurma 2007). Root-knot nematodes are expected to exert a selective pressure on hosts where such pressure varies in correlation with different host genotypes (Wurst and Van der Putten 2007, Moens et al. 2009).

Above-ground herbivores:

Generalist aphids such as *Myzus persicae* Sulzer (green peach aphid) and *Aphis fabae* Scopoli (black bean aphid*)* are serious pests on crops of economic importance (Blackman and Eastop 2000). Both species are polyphagous and infest a wide range of

host plants, although they show relative discrimination among plant species (Blackman and Eastop 2000, Powell et al. 2006). Trans-generational developmental effects have been demonstrated in aphids (Agrawal et al. 1999, Podjasek et al. 2005). Such effects are explained by viviparity, telescoping of generations, and maternal effects (Dixon 1987, 1998, Agrawal et al. 1999, Podjasek et al. 2005). This also applies to the accelerated production of winged morphs or a range of functional morphs, having different ecological roles upon alert, over-crowding and resource depletion (Dixon 1977, 1987, 1998, Dombrovsky et al. 2009). This means that mothers can influence their daughters' performance (reproductive success) and behaviour (host choice and distribution); (Zehnder et al. 2007). Such influence is interdependent with the environment experienced by both mothers and their offspring (Zehnder et al. 2007). Furthermore, the trade-off occurring between intra- and inter-clonal competition among aphid lineages (Smith et al. 2008), and mediated by plant genotype (Smith et al. 2008), may hinder or enhance host discrimination and hence reproductive success in generalist aphids. Moreover, because these aphid species display sophistication in their perception of host suitability their response is expected to be strong (Fenemore 1988, Powell et al. 2006).

Bottom up effects mediated via plant genetics:

Genetic variation in fundamental host plant species has important effects on the community composition of host-associated herbivores (Whitham et al. 2003, 2006). The within-species genetic variation of host plants has also been demonstrated to affect the performance (reproductive success and population growth rates), preference (selection for more suitable host plants) and behaviour (affinity for specific feeding locations on the host plant) of host-associated phytophagous insects (Karban 1992,

Cronin and Abrahamson 1999, Awmack and Leather 2002, Gonzáles et al. 2002, Powell et al. 2006, Ruhnke et al. 2006, 2009).

The influence of plant hosts can extend and magnify over many levels through multi-trophic systems via direct and indirect routes (Miller and Travis 1996, Astles et al. 2005, Johnson and Agrawal 2005, Bailey and Whitham 2006b, Schädler et al. 2010). Plant phenotypic variation responsible for these influences is partially underlain by plant genetics and partly by herbivore-induced changes in the host (biotic environment). This occurs, within ecological networks, either as induced plant defences against herbivores or as result of herbivore manipulation of the host plant. The induced changes alter plant traits influencing quality, physiology, and architecture of host plants (Ohgushi 2005, Kessler and Halitschke 2007, Ohgushi 2008). These changes can, in turn, directly or indirectly influence both the herbivores that induced the original change and others members of the community with a potential to affect the entire ecosystem (Wootton 1994, Moore et al. 1997, Wolf et al. 1998, Wolf 2000, Wootton 2002, Shuster et al. 2006, Bailey et al. 2009, Kaplan et al. 2009). Furthermore, Morriën et al. (2011) proposed a linear 'additive effect' to occur between the spatially separated, yet host plant associated, communities such as shoot-feeders and root-dwellers. However, Morriën et al. (2011) also argued that such effects would be governed by the feedback between the host-associated herbivores and the differences in host plant reaction (sensitivity) to its enemies, which, in turn, is partly due to the plant's genotype.

Spatially separated competition and plant genetics interactions:

The genotype of host plants and multiple plant associated herbivores are all expected to interact in a complex fashion to determine the fitness of all elements in the system. Concomitantly, ecological interactions are expected to vary in influence

according to scarcity of food, density, change in resource availability (Dixon 1998), the influence of competitors and enemies (e.g. Dolman 1995, Sorvari and Hakkarainen 2004), and mediation effects of other species such as parasites (Price et al. 1986).

Inter-specific competition (i.e. interference and exploitative) plays a prominent role in shaping communities of phytophagous insects (Connell 1983, Denno et al. 1995; but see Bengtsson 1993). Furthermore, inter-specific competition will be context dependent (e.g. Juliano 2010), and subject to reciprocal moderation by host plant quality and genotype (Karban and Mayer 1989, Inbar et al. 1995, Smith et al. 2008; see also Power 1992 for a discussion of the magnitude of host plant influence). Moreover, there is evidence that the 'competitive dynamics' of the interactors have an impact on the fitness of their host plants (Lee et al. 2011). As suggested above, the fitness of two species infecting a single plant will depend not only on each organism's genotype, but also on that of the competitor.

Additional complexity occurs when spatially separated herbivores are co-habiting the same plant host. Sap-feeding aphids and root-knot nematodes exert physiological, morphological and ontogenetic changes in their host plant and on each other. Very few studies have investigated the interactions between these types of plant enemies (Williamson and Gleason 2003, Goggin et al. 2006, Bhattarai et al. 2007, Kaplan et al. 2009; see also Morriën et al. 2011), and even fewer have sought to determine if host plant genetics play a part in the mediation of these interactions. Using a system of tobacco plant, root nematodes, and aphids, Kaplan et al. (2009) attributed the competition between the shoot and root feeders to be influenced by, or occur through, induced changes in the shared host plant. In another system on soybean, Hong et al. (2010) demonstrated an impact of a cyst nematode to affect aphid response (behaviour) rather than performance.

In addition, host plant genotype will also play a part by influencing the interaction between the parasites. The influence of each individual type of herbivorous pest should be different and contingent on various factors such as density, intra- and inter-generational communication (e.g. Podjasek et al. 2005, Zehnder et al. 2007), host plant resistance and susceptibility (Goggin et al. 2006, Singh and Khurma 2007), as well as the interaction between plant genotype and competition (e.g. Smith et al. 2008).

Aphids and RKN, like their host plants, are highly adaptive and phenotypically plastic (Blackman and Eastop 2000, Borges 2005, Castagnone-Sereno 2006, Tagu et al. 2008, Whitman and Agrawal 2009, Tariq et al. 2010). This creates a cycle of reciprocity and co-evolutionary response (Dawkins and Krebs 1979, Janzen 1980, Odling-Smee et al. 2003, Strauss et al. 2005) in an environment that is largely determined by the expressed genomes of the other closely interacting members of the community (Antonovics 1992, Whitham et al. 2003, 2006, 2008, Rowntree et al. 2011b). Exposed to pressure on both aerial and subterranean parts, host plants respond to the induced effects by herbivores with a change, the herbivores react to such change with a change and so forth. As such, a pattern of cyclical change emerges due to the plant and herbivore interactions [Odling-Smee et al. 2003, Laland 2004, Marquet 2009, Pelletier et al. 2009, Schoener 2011; see also (Levin 1998 and Juarrero 2010) for helpful insights on the dynamics of complex adaptive systems].

Although stimulating different physiological pathways of defence in the plant (Cooper and Goggin 2005, Cooper et al. 2005, Bhattarai et al. 2007), both aphids and root-knot nematodes are considered sink-forming herbivores in that they create abnormal assimilate-sinks. These artificial sinks function in contrast to the normal sinks naturally constructed by the plant and dependent on its own genotype and degree of resistance to the nemesis of the host plant (Larson and Witham 1991, 1997).

Therefore, the competing species sharing a host are expected to variably influence each other's performance and behaviour through the host they share (Levine 1976, Lawler 1979, Kaplan et al. 2009). Interestingly, it can be suggested that the sink itself may act as a mediating structure for competition between aphid species (Inbar and Wool 1995, Inbar et al. 1995); a concept that may be extended to account for the case of spatially separated competitors (see also Kaplan and Denno 2007 and Kaplan et al 2009). These trait-mediated emergent interactions among herbivores (Utsumi et al. 2010) may be dramatic and may oscillate between cooperation and antagonism (for example Kaplan et al. 2008, 2009, Morriën et al. 2011; but see Masters et al. 1993, 2001, Rawal and Rajagopalan 2010).

Finally, the activities of the interactors infesting a shared plant will affect the fitness of the host plant through the modification of plant health and the ongoing defensive and induced response of the host plant (Bird 1996, Agrawal and Sherriffs 2001, Odling-Smee et al. 2003, Williamson and Gleason 2003, Laland 2004, Powell et al. 2006). Such complex interaction is expected to follow a co-evolutionary arms-race scenario (Dawkins and Krebs 1979, Rawal and Rajagopalan 2010).

Here we use a system of tomato cultivars as host plants that may cause community genetic effects in the sense that the plants' genotypes directly influence the species they interact with as well as moderate indirect interactions between above- and below-ground communities of herbivores. We use aphids as an important plant herbivore that commonly experience varying degrees of competition in natural and agricultural systems (Smith et al. 2008). We use root knot nematodes (RKN) as a plant root pest that has a significant impact on plant health. RKN is predicted to cause an IEE on the above-ground community of herbivores, mediated by the host plant (Wootton 1994, 2002, Kaplan et al. 2009). Tomatoes also represent an agriculturally important species and both aphids and RKN are economically important pests of this crop.

We address the relative importance of community genetic effects as a factor that mediates the influence of an IEE (RKN impact) on competition among aphid species/genotypes in addition to the effects of above-ground and below-ground competition on plant fitness (measured as plant dry weight and shoot-root indices).

We also examine the relative reproductive success of the two spatially separated competitors across six different genotypes of tomato. Because aphids are known to use behavioural means to respond to competition, resource limitation and natural enemies, we measured the behavioural responses of our focal aphid genotype as on-plant micro-site feeding preference (on-plant distribution). Our specific hypotheses are:

1a- Plant genotypes will respond differentially to herbivory by aphids and RKN.

1b- Both plant genotype effects (community genetic effects) and spatially separated competition effects (between RKN and aphids) interact to influence plant biomass.

2- RKN fitness will be influenced by above-ground herbivory mediated by host plant genotype.

3a- IEEs between the focal aphid species and plant genotype that are generated by RKN will alter the rate of reproductive success of the focal aphid species.

3b- Behaviour (on-plant micro-site feeding affinity) of the focal aphid genotype will vary, mediated by the interaction between host plant genetics and RKN.

3c- IEEs between the focal aphid species and plant genotype that is generated by RKN will modify the level of competition between shoot and root consumers. This effect will be dependent on plant genotype.

Methods

Root-knot nematodes:

Meloidogyne hapla was used to induce a change in the root growth and the physiology of the host plant that was expected to result in a clear indirect ecological effect (IEE) in the form of a stress effect on above-ground herbivores (aphid species). The nematodes were originally obtained from an agricultural laboratory at Reading University, maintained in culture at The University of Manchester, and kept on the 'Tiny Tim' tomato cultivar within growth cabinets at 22°C and a 16:8 (L:D) photoperiod. Tomatoes were planted in 3.5" pots (John Innes No. 3 compost). In order to collect nematodes for our experiments we isolated galled tomato roots infested with *M. hapla* through dissecting the roots using a dissecting-microscope kit. Galled roots showing signs of egg masses were washed clear of soil, fragmented, left soaked in diluted commercial sodium hypochlorite (0.5%) for few minutes, then dissected under a fine-grid microscope to obtain free nematodes. The suspension of isolated nematodes was filtered through a cotton-wool filter paper and incubated at 28° C for two to three days in order to ensure any remaining eggs had hatched. Second stage juveniles (J2) were collected daily and used to inoculate experimental plants within 48h.

Tomato cultivars:

We used six cultivars of tomatoes (*Lycopersicon esculentum*) that vary in a set of traits including green colour intensity, architecture (e.g. indeterminacy), growth rate, trichome density or type, and size of the yield (including truss size and number). Four cultivars (genotypes), (San Marzano 2: **G2**, Tigerella**: G3**, Costoluto fiorentino: **G5**, and Orange Berry**: G6;** hereafter respectively) were procured from Johnson's Seed

Company and two cultivars (Tiny Tim: **G1**, and Sweet melon: **G4**; hereafter respectively) were provided by Reading University Agricultural Research Station. The seventh cultivar (Moneymaker) was purchased from Barters Company and was used exclusively to maintain and rear aphids prior to experimental work. This cultivar was not used in the experiments in order to eliminate any preconditioning effects. All cultivars are inbred lineages maintained for quality and uniformity through cultivation as synthetics and thus not they do not exist naturally in the wild (see for example: Duc 1997, Hannan et al 2007). All cultivars used in this research were selected from different inbreeding backgrounds. Therefore, they are likely to vary in their susceptibility and resistance to aphids and the plant parasitic root-knot nematode *Meloidogyne hapla.*

Aphids:

Green peach aphid: A clone of green peach aphid (*Myzus persicae* Sulzer) was established from one individual collected from Glossop, Derbyshire, UK in summer 2010.

Black bean aphid: A clone of black bean aphids (*Aphis fabae* Scopoli) were established from a single individual collected in Chorlton, Manchester, UK in spring 2009

In order to avoid any preconditioning effects, all aphids used in this experiment were descendents of lines reared on a different tomato cultivar (Moneymaker) than those cultivars used in our experiments. All aphids used in the experiment were first or second instars in order to reduce maternal effects. Numbers of first and second instars were balanced across treatments.

Plant preparation and conditioning: The six tomato cultivars were germinated from seeds in 3.5" pots containing perlite. Seedlings were transferred after fifteen days to vapour sterilised loam-based compost (50g John Innes No. 3 in 3.5" pots). Plants that were exposed to RKN were inoculated by multiple pipette injections into the compost near the roots. A total of 1ml of solution (containing 50 larvae/ml) was added to each pot. All the nematode-infested plants were then left for 45 days to ensure the establishment of nematode population on the host plants prior to aphid infestation. Nematode infested plants were then split into two sets. The first six plants of each cultivar were exclusively root-knot nematode infested plants (i.e. a no-aphid control) while the remainder (10 plants) were infected with aphids according to the treatments listed below. At this stage, all tomato plants were placed into plastic propagators with fine mesh-covered openings for ventilation. Propagators were made from clear and clean re-usable two-litre water bottles. Plants were maintained in growth cabinets at 22°C and a 16:8 (L:D) photoperiod. Through a detachable sealed-pore at the lower third of the propagator, plants were watered using a squeezable bottle with a nozzle to squirt water every two days or water and fertiliser [(100 ml of 1/4 strength Hoagland's solution) applied every 4 days throughout the experiment (Hoagland and Arnon 1950; see Rowntree et al. 2011a)]. In order to stimulate induced change in the plant, plants belonging to the treatments 2 and 3 were preconditioned to aphid attack by infesting each plant with 40-50 adult *Myzus persicae* for 10 days. After 10 days (on day 55) all aphids were removed from each plant. Plants were brushed off with a fine paintbrush, washed several times to insure the removal of aphids, and left for two days to rest in the growth cabinets.

Treatments: We used a total of five experimental treatments to test the hypotheses outlined above**.** In all treatments that include aphids, aphids (starting from fixed

number of first and second instars for all replicates) were left on the plants for fourteen days. On day 14, the total numbers of aphids were counted and their locations on the top, middle or bottom thirds of the plants and on stems or leaves were recorded. Plants were then removed from pots, washed, measured for maximum root length and shoot height, cut into shoot and roots, dried and placed in a drying oven at 60°C for one week. After one week plant shoots and roots were weighed. Upon isolation of roots, galls were removed in the same fashion as described above and the number of mature females per gram of root was recorded.

Treatment 1:

These plants were the negative control group and had neither RKN nor aphids added to them.

Treatment 2:

These plants were inoculated with RKN on day zero but had no aphids added to the plants.

Treatment 3:

These plants were inoculated with RKN on day zero and then preconditioned to *Myzus persicae; M.p*, hereafter) attack on day 45 in order to stimulate plant defensive responses. On day 57 each plant was infested with four *M.p* instars and nine instars of *Aphis fabae*; *A.f*, hereafter).

Treatment 4:

These plants were first disturbed by *M.p* attack and then on day 57 infested with four *M.p* instars and nine *A.f* instars. More black aphids were used (9 black instars to four *M.p* ones) to further disturb the focal green peach aphid species.

Treatment 5:

Only green peach aphids (*M.p)* were added to these plants on day 57.

Analyses: Data were analysed as three separate experiments. All statistical analyses were carried out in JMP 9 and illustrated using MS Excel 2003 and Inkscape (Ver. 0.48.01 under GPL).

Experiment 1:

In this experiment we examined the response of the host plants (six genotypes of tomatoes) to herbivory by:

    a- No herbivory (Treatment 1).

    b- Herbivory by RKN (Treatment 2).

    c- Herbivory by RKN, *M.p* and *A.f* (Treatment 3).

    d- Herbivory by *M.p and A.f* (Treatment 4).

The cultivar (genotype) effect (within-tomato genetic variation effect) was composed of 6 cultivars of tomatoes. The analysis was run as a 2-way ANOVA with plant dry weight and root-length:shoot-length ratio fitted using Ordinary Least Squares (OLS).

Experiment 2:

In this experiment we examined the response of RKN to competition with aphids and to community genetic effects. The community genetic effect was composed of six cultivars of tomato. The competition effect comprised 2 levels:

    1) No competition with aphids (Treatment 2).

    2) Competition with both *A.f and M.p* (Treatment 3).

The analysis was run as a 2-way ANOVA using Ordinary Least Squares (OLS) fit. We excluded from our analysis all plants where no RKN were found on Day 71.

Experiment 3:

In this experiment we compared the response of *M.p* (in terms of growth rate and on-plant distribution) to competition and community genetic effects. Six cultivars of tomato comprised the community genetic effect. The competition effect comprised three levels of competition:

1) No competition; *M.p* alone (Treatment 5)

2) Competition with both *A.f* and RKN (Treatment 3).

3) Competition with *A.f* (Treatment 4).

The analysis was run as a 2-way ANOVA with growth rate fit as a normally distributed variable using Ordinary Least Squares (OLS) and behaviours fit as categorical variables in a nominal logistic analysis using Maximum Likelihood (ML). We excluded from our analysis all plants where no *M.p* was found on day 71.
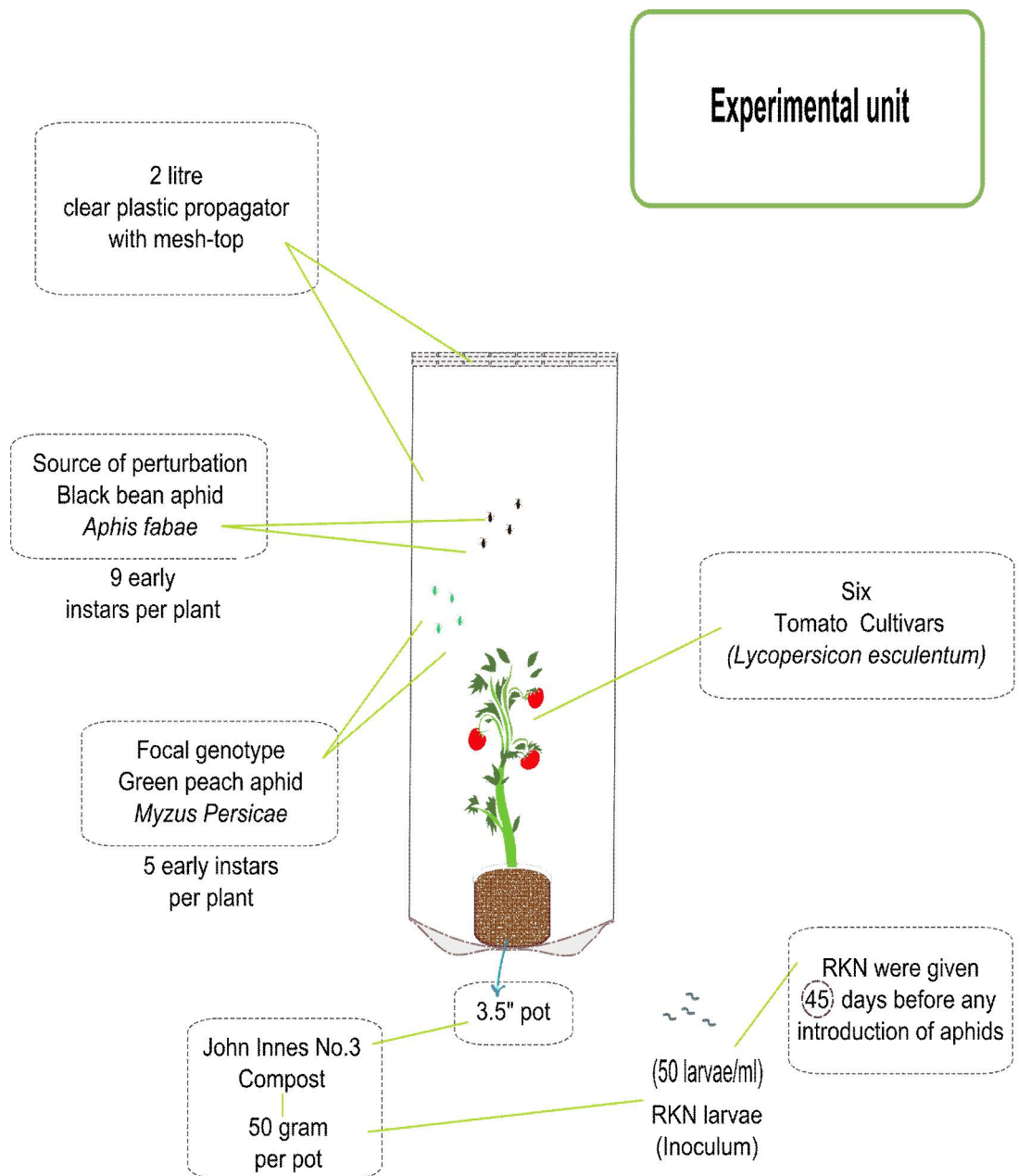
Figure 1: The experimental propagators. (Setup used in all experiments).

Plants were housed individually in propagators. All possible experimental elements are shown and were used as described in the methods. Six tomato genotypes were subjected to three different herbivory treatments; 1) aphids only [black bean (*A.f*) and green peach (*M.p*) aphids], 2) nematodes (RKN) only, 3) nematodes (RKN) and aphids [black bean (*A.f*) and green peach (*A.f*) aphids].

Results

Experiment 1:

Plant dry weight:

We found a significant influence of tomato cultivar ($F_{5,118}$ = 11.942, P < 0.001), and only a marginally significant effect of herbivory ($F_{2,118}$ = 2.918, P = 0.0586). There was a highly significant interaction effect between herbivory and tomato cultivar ($F_{10,118}$ = 6.299, P < 0.001) indicating that plant biomass depended not only on the plant's genotype, but also that cultivars responded differentially to herbivory (**Figure 2,** see also the appendix to this chapter for further illustration of plant biomass differences across treatments).

Treatment (C ▮ , RKN only ▮ , RKN+Aphids ▮ , Aphids only ▮)
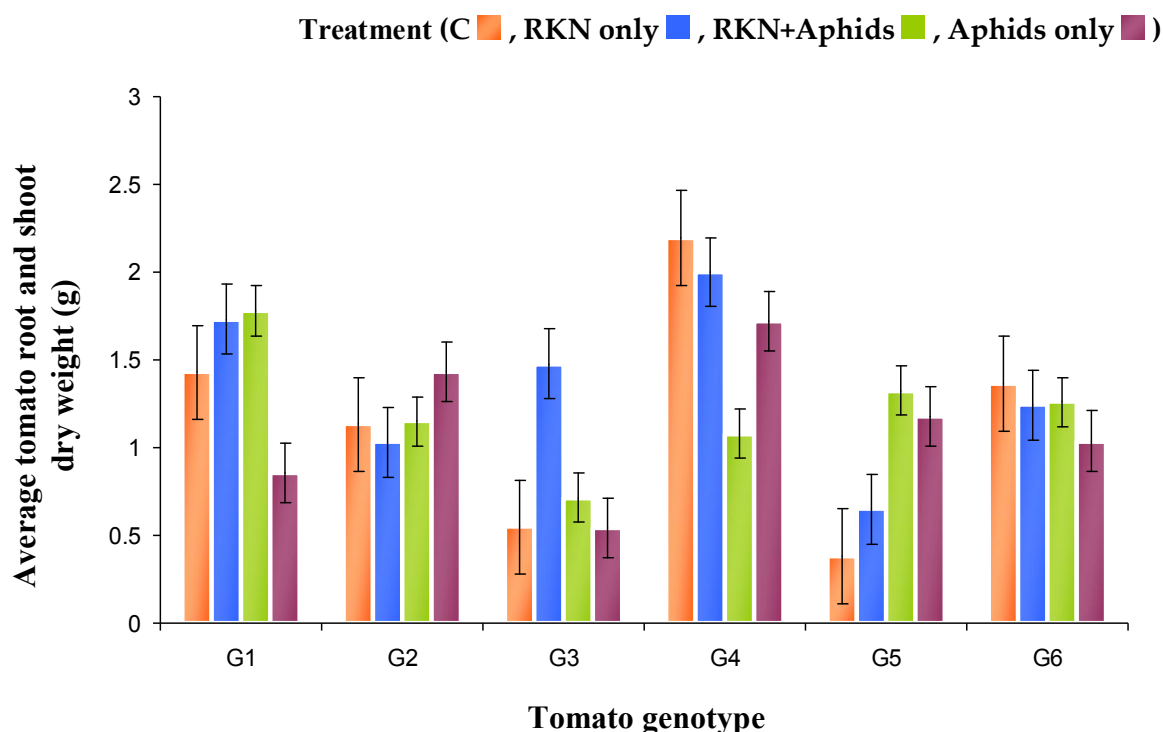
Figure 2: Average plant dry weight (g) of six genotypes of tomato under four levels of herbivory. T1) Controls with neither aphids nor RKN (orange), T2) RKN only, no aphids (blue), T3) RKN and aphids (green), T4) Aphids only (*M.p+A.f*) with no RKN (purple).

Root-length:Shoot-length index:

We found a highly significant effect of tomato cultivar ($F_{5,118}$ = 6.305, P < 0.001) but no significant effect of herbivory ($F_{2,118}$ = 0.889, P = 0.414). However there was a marginally significant interaction between tomato cultivar and herbivory ($F_{10,118}$ = 1.992, P = 0.042) suggesting that root:shoot index of cultivars reflected a differential response to the different types of herbivory we examined (**Figure 3**).
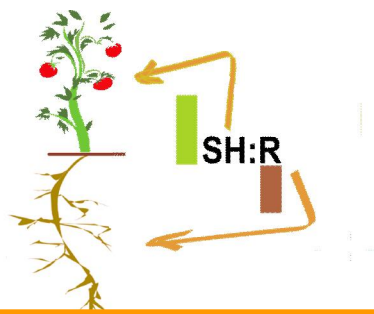
Shoot:Root Index of tomato plant vigour (response to herbivory) across multiple treatments including spatially isolated pests.
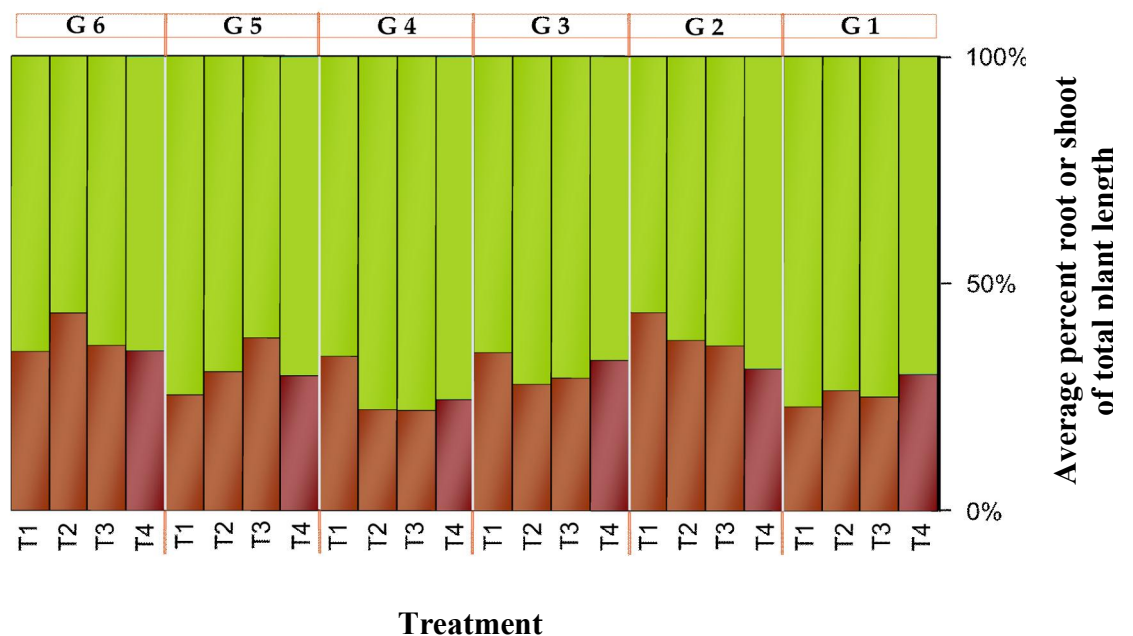
Figure 3: Plant Shoot-length and Root-length percentages. The shoot-root index details the proportional shoot length (green) and root length (brown) for all six genotypes of tomato under four levels of herbivory; T1) Plants with no herbivory, T2) Plants with RKN only, T3) Plants with simultaneous below- and above-ground herbivory, T4) Plants with above-ground herbivory only.

Experiment 2:

We found that the number of RKN females per gram (plant dry weight) was not significantly influenced by tomato cultivar ($F_{5,88}$ = 1.389, P = 0.238) or the presence of above-ground competitors ($F_{1,88}$ = 1.326, P = 0.253) but was strongly influenced by the interaction of cultivar and the presence of competitors ($F_{5,88}$ = 3.356, P = 0.009, **Figure 4**). This indicates that the population growth rate of RKN is influenced by competition but the direction of influence was dependent on the tomato cultivar mediating the interaction.

Treatments

(RKN alone ■, RKN and Aphids ■)

(Across six tomato genotypes)
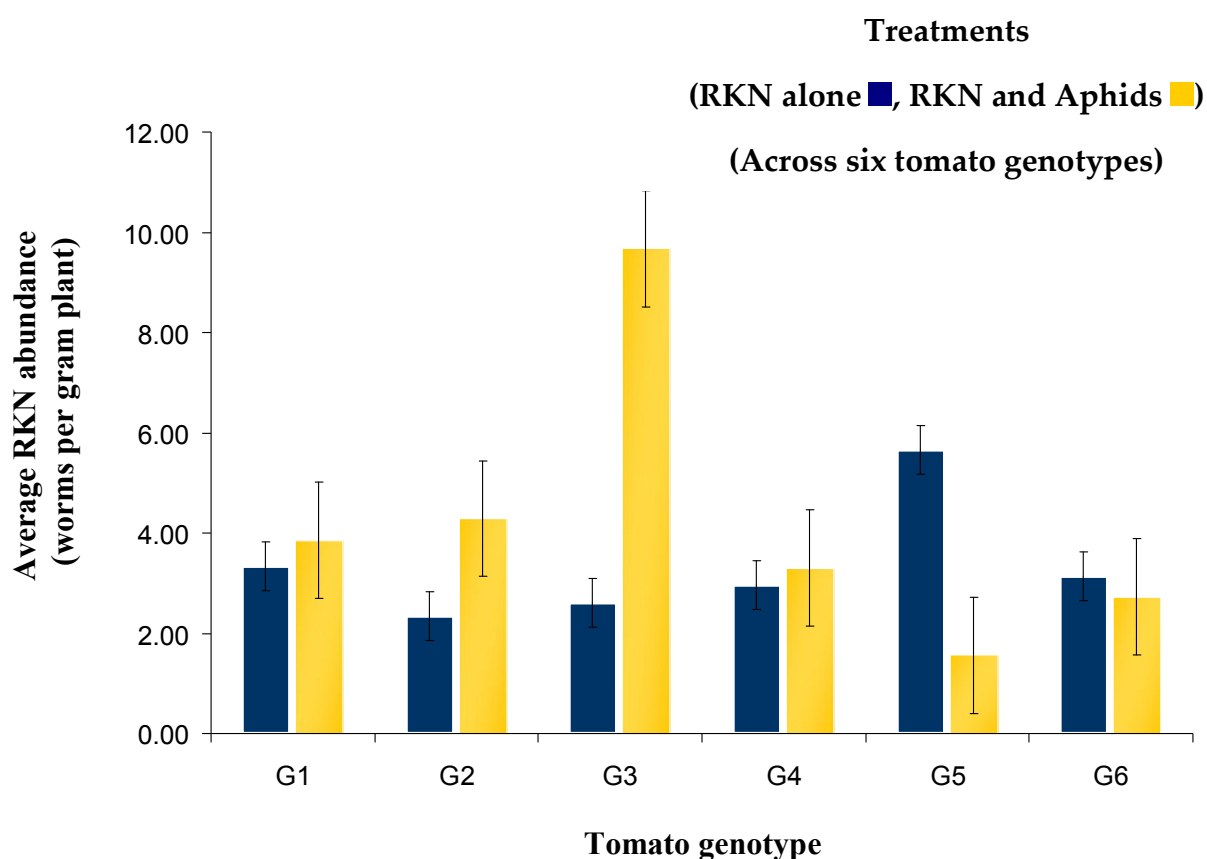
Figure 4: RKN abundance. (A measure of reproductive success).

The RKN fitness index was calculated by relating the estimated density of RKN to the whole plant dry weight. The community genetics effect was composed of six cultivars of tomato. The treatments included either RKN alone (Treatment 2; blue bars) or RKN and aphids i.e. spatially separated interaction (Treatment 3; yellow bars).

Experiment 3:

Aphid abundance: We found no significant main effect of tomato cultivar ($F_{5,81}$ = 1.769, P = 0.132) but a significant effect of competition ($F_{2,81}$ = 10.045, P < 0.001) on aphid growth rate. We also found a significant interaction between cultivar and competition ($F_{10,81}$ = 2.271, P = 0.024) indicating that the response of *M.p* to competition depends on the genotype of the host plant *M.p* resides on (**Figure 5**).
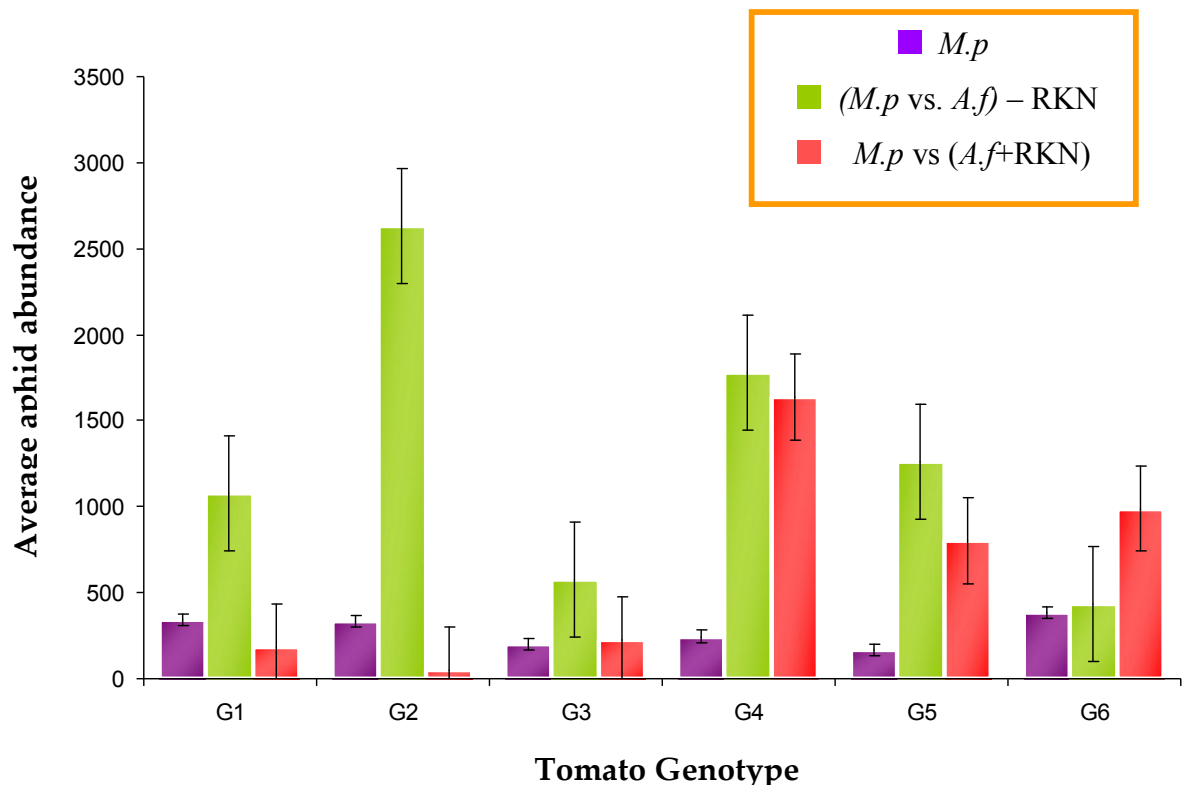
Figure 5: Aphid relative performance (reproductive success). The data illustrated reflect the differential population growth rates of the above-ground focal aphid pest (*M.p*) across six different cultivars of tomato through three scenarios: 1) *M.p* only: Treatment 5 (purple), 2) *M.p* vs. *A.f* in the absence of RKN: Treatment 4(green), 3) *M.p* vs (*A.f* + RKN): Treatment 3 (red).

Aphid Behaviour: When comparing numbers of aphids on the top, middle and bottom thirds of the plant, we found highly significant effects of tomato cultivar (x^2_{10} = 521.26, P < 0.001), competition (x^2_4 = 158.88, P < 0.001) and a significant interaction (x^2_{20} = 541.73, P < 0.001, **Figure 6**) on aphid distribution on the host plant. When comparing numbers of aphids on the stems vs. the leaves of the plants we, found a highly significant effect of cultivar (x^2_5 = 127.12, P < 0.001) and a highly significant interaction (x^2_5 = 62.01, P < 0.001) but no significant effect of competition (x^2_1 = 1.45, P =0.228, **Figure 7**). The results indicate that the behavioural response (preference regarding stem vs. leaf and preference for top, middle or bottom of the plant) of *M.p* is contingent upon the interaction between the community genetic effect (CG) and the effect of competition (**Figure 6** + **7**).

M.p's behaviour explained by on-plant distribution (Top-Mid-Bottom)
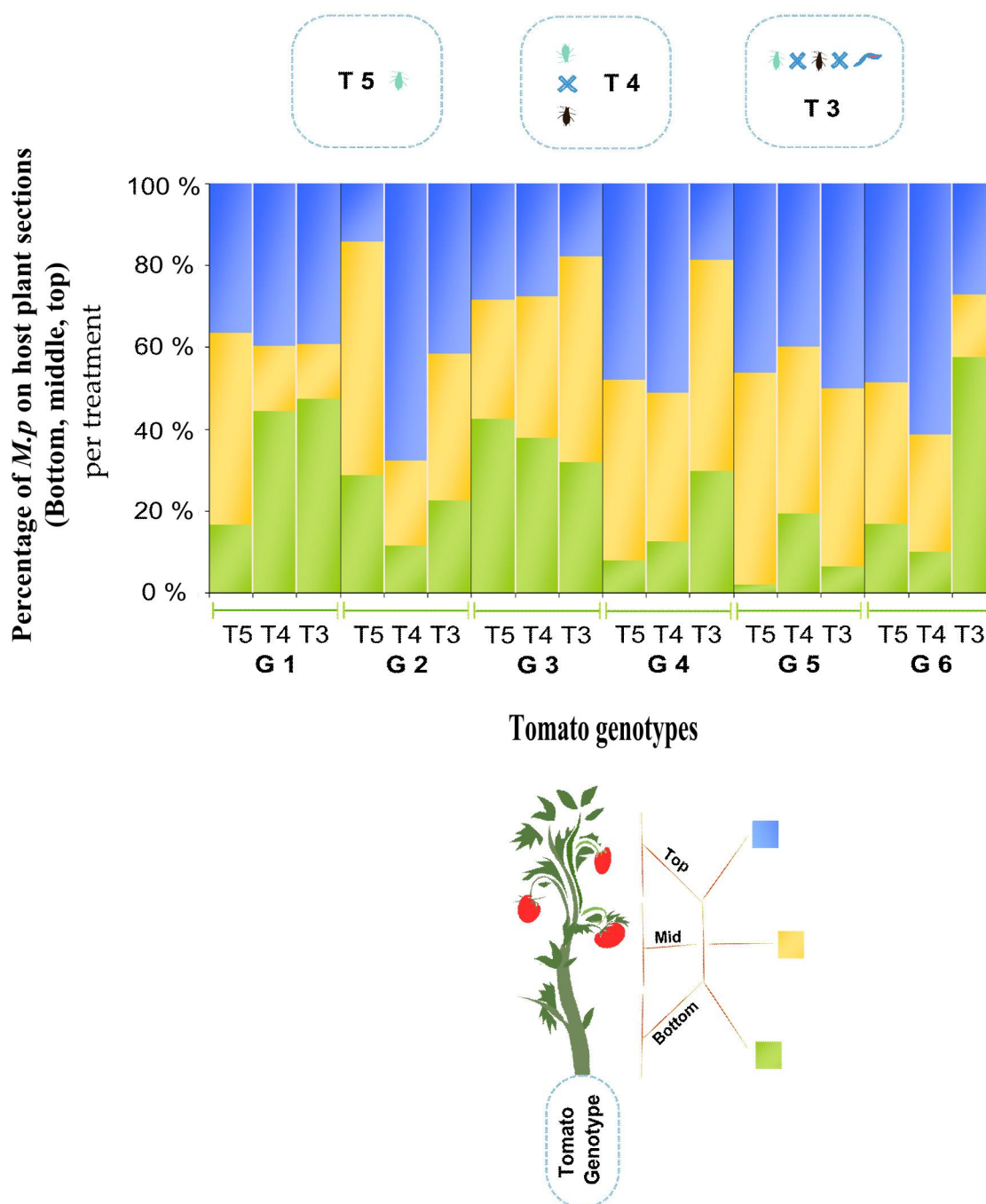
Figure 6: On-plant distribution (Top-middle-bottom) of focal aphid species *M.p.*

Micro-feeding site preference (congregation) of green peach aphid genotype *M.p* across six tomato cultivars under three treatments is illustrated;

T5) *M.p* alone, T4) *M.p* vs *A.f*, T3) *M.p* vs *(A.f* + RKN).

The vector-image of tomato illustrated is adapted from a free public-domain clipart resource; URL: http://www.clker.com/clipart-29009.html

M.p's behaviour explained by on-plant distribution (Stem vs Leaves)

T 5

T 4

T 3

Percentage of *M.p.* on host plant stem *vs.* leaves per treatment

100 %
80 %
60 %
40 %
20 %
0 %

T5 T4 T3 | T5 T4 T3 | T5 T4 T3 | T5 T4 T3 | T5 T4 T3 | T5 T4 T3
G 1 | **G 2** | **G 3** | **G 4** | **G 5** | **G 6**

Tomato genotypes

Leaves

Stem
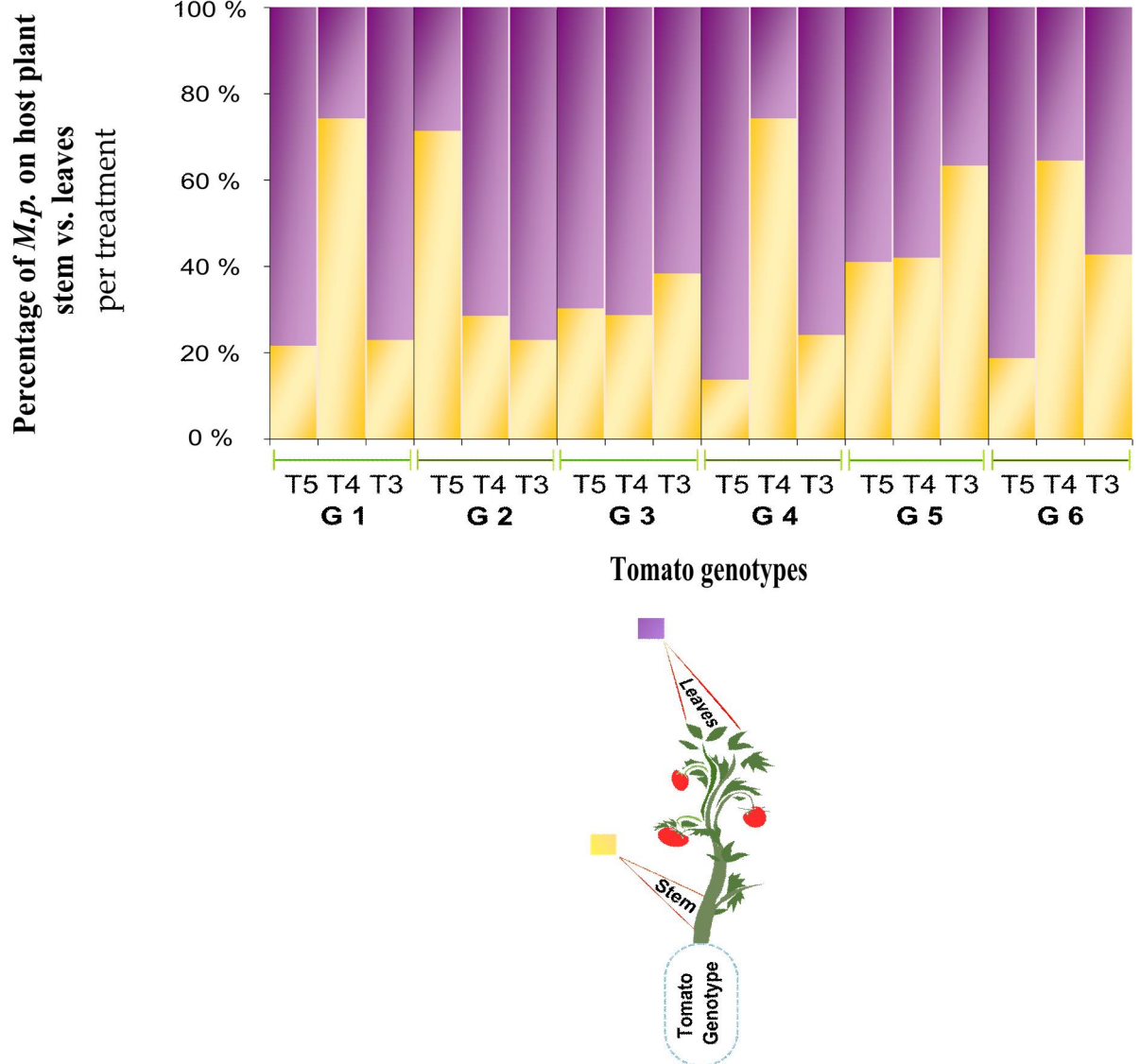
Tomato Genotype

Figure 7: On-plant distribution (stem vs. leaves) of focal aphid species *M.p.* Micro-feeding site preference (congregation) of green peach aphid genotype across six tomato cultivars under three treatments; T5) *M.p* alone, T4) *M.p* vs *A.f*, T3) *M.p* vs *(A.f* + RKN).

The vector-image of tomato illustrated is adapted from a free public-domain clipart resource; URL: http://www.clker.com/clipart-29009.html

Combining perspectives in a multispecies system:

If plant cultivars are ranked by either *M.p* growth rate or RKN abundance the corresponding patterns of competitor abundance are strikingly varied.

This variation in response of the various competitors to the interacting competitors and their common host is shown not simply in changes in the magnitude of response but also in changes in the rank order of responses (**Table 1**).

Source	DF	F	P
Tomato cultivar	5	12.736	<0.001
Competition	1	6.437	0.012
RKN	1	0.599	0.440
Cultivar * Competition	5	11.971	<0.001
Cultivar * RKN	5	3.131	0.010
Competition * RKN	1	0.654	0.420
Cultivar * Competition * RKN	5	1.292	0.270

Table 1: Effects of tomato cultivar (6 genotypes), presence/absence of a competing aphid (*A.f*), and presence/absence of RKN (an IEE), and their interactions on total abundance of the focal aphid (*M.p*).

There are strong effects of tomato cultivar, competition and their interaction. While there is no main effect for RKN, there is a significant interaction between RKN and tomato cultivar. The interplay between the indirect ecological effect and competition is demonstrated to be moderated by tomato variation.

Discussion

This work explored the influence of an IEE (indirect ecological effect) caused by a below-ground pest of tomato (RKN) on plant vigour (plant dry weight) under multiple scenarios of herbivory and across multiple tomato genotypes. The study also investigated the differential reproductive success of RKN on six different genotypes (cultivars) of tomato in the presence and absence of above-ground competitors (clones of two aphid species: *M.p* and *A.f*). In addition, we examined the effect of the RKN on survivorship and behaviour (on-plant feeding site selectivity) of a focal aphid genotype (green peach aphid, *M.p*) when mediated by within-tomato phenotypic variation (community genetic effect). The performance (reproductive success) and on-plant distribution of the focal aphid genotype were also demonstrated in the presence and absence of the other aphid species and RKN competitors. These results may allow a better comprehension of the dynamics of multi-trophic systems within agro-ecosystems.

We found that the fitness of each species in our model system was influenced by interactions between community genetic effects and competition or IEEs. This clearly demonstrates that community genetic effects can significantly alter above-ground and below-ground interactions. As such, our findings have significant implications for the field of community genetics and are directly applicable to agro-ecosystem practices. This also implies that the community genetic effect of host plant cultivar has quite a strong impact on the dynamics of the community of herbivores sharing that host. This possibly occurs through the indirect interactions, between herbivores, that the shared host plant mediates (Wootton 1994, Denno et al. 2000, Wootton 2002, Denno and Kaplan 2007, Anderson et al. 2009, Kaplan et al. 2009, Morriën et al. 2011).

Tomato host plant under pest attack

Tomato dry weight and root-length:shoot-length ratio were significantly influenced by the interaction of the plant's phenotype and the communities of indirectly interacting herbivores. Clearly, our measures of plant fitness here are only correlates of reproductive output, although plant dry weight is a strong predictor of plant fitness (Silim and Saxena 1992). Consequently, the biomass of the host depended not only on the host genotype, but also on its interaction with the presence of competition or RKN. The differential response of tomato cultivar to the influence of single herbivory (above- or below-ground) can be attributed to the different change each type of herbivory induces in the plant host. Metabolically, aphids differ in their influence on their host plant in competition with root-knot nematodes (Williamson and Gleason 2003, Bhattarai et al. 2007, Walling 2008). There is a good body of evidence supporting the ability of species interactions to influence host plant and *vice versa* (Bailey and Whitham 2006b, Bailey et al. 2006, Whitham et al. 2006, Haloin and Strauss 2008, Rowntree et al. 2011a,b). It is possible that the additive effect of spatially separated competition, where aphids and root-knot nematodes cohabit the same host, explains the conditional waxing or waning in plant vigour or stress relative to tomato genotype (White 1978, 1984, Price 1991; see also Lawton 1999, Bailey and Whitham 2006a).

Our findings are concordant with those of Tétard-Jones et al. (2007) who found a (G x G x E) interaction in a system where the aphid-plant interaction was context dependent with regard to the presence or absence of specific rhizosphere bacteria. In such systems, transfer of traits from aphid mothers to their offspring, as well as reproductive success of an aphid genotype, will be influenced by host plant genetic variation (e.g. Cronin and Abrahamson 1999, Underwood and Rausher 2000). Moreover, positive feedback by the soil community will enhance the shoot consumers to flourish while the reverse feedback may be negative (Morriën et al. 2011). Thus,

the influence of community genetic effects (CG) may be extremely important in agro-ecosystems within fields planted as monocultures, and also at the larger scale of a mosaic of agricultural fields that are common across the globe.

RKN (IEE) x CG in the presence and absence of above-ground competition:

The performance of RKN was influenced by the interaction of the presence of competing aphids and host cultivar. Our results also indicate that the mediating community genetic effects of tomatoes influenced above-ground to below-ground IEE. The abundance of RKN increased, decreased or remained relatively constant in response to changes in competition in a manner that was dependent on which tomato cultivar was acting as the host plant. The interaction between RKN and aphids can be usefully visualised and interpreted through negative and positive feedback between the shoot and root consumers (Masters et al. 1993, 2001). However, the model articulated by Masters et al. (1993, 2001) does not account for the plausibility of a community structuring role of competition via the induced changes in the shared host (see Kaplan and Denno 2007, Kaplan et al. 2009). In this regard, it is also important to take into account the additive negative and positive indirect ecological effects (see e.g. Wootton 1994, 2002, Denno and Kaplan 2007, Kaplan et al. 2008 for elucidation on the asymmetric effects among shoot and root consumers mediated by host plant).

Our work encapsulates additional complexity due to the genetic variation of the tomato host plants, IEE (RKN) differential impact and the inter-specific competition between above-ground pests (aphids) as well as competition between the spatially separated herbivores (aphids and RKN).

Species such as root galling nematodes alter gene expression of the plant root system and thus impose a re-differentiation effect on the plant root cells by modifying them into utilisable RKN dietary sinks (Bird 1996, Jammes et al. 2005, Caillaud et al.

2008). These differentially, genomically-altered plants also act as hosts for competing female aphids and, over generations, the genetically identical offspring of these aphids will find themselves involved in three types of competition. The first occurs when the multiplied genotype is competing with itself (mothers, daughters and granddaughters) within the clone on local depleting resources. In this type of competition, the capacity to congregate (i.e. feeding site preference on the plant) may be selected for. The second type is the competition between heterospecifics. The third type of competition is a spatially separated competition between aphids and root-knot nematodes that never meet but share one host (Kaplan et al. 2009). Under such types of interaction, aphid mothers' and daughters' fecundity, plasticity and survival will be influenced by the dilemma of simultaneous intra- and inter-specific types of competition in parallel with kin selection and host exploitation (Hamilton 1964, West-Eberhard 1989, 2003, Pfennig and McGee 2009, Tariq et al. 2010).

In our system (clonal RKN and aphids) herbivores are expected to undergo a process akin to a 'war of attrition' (*sensu* Maynard Smith 1974) whereby each party is engaged in the trade-off explained by the game of 'wait or display' [illuminated by Krebs and Davies (1998)]. Instead of males competing for a female, here we have herbivores competing for survival via their shared host by maintaining an adequate proportion of the resource available (Red Queen Hypothesis; Van Valen 1973). The competitors, highly sensitive and responsive to environmental change, are expected to vary their host manipulation tactics; a process that constantly functions in tandem with the host plant's countermeasures (Dawkins and Krebs 1979, Rawal and Rajagopalan 2010). However, nutritionally, the process of obtaining an adequate proportion of the shared resource will vary dramatically when the competitive relationship takes the form of a co-evolutionary arms-race, occurring between the above- and below-ground plant natural enemies (Dawkins and Krebs 1979, Rawal and Rajagopalan 2010). It is

not clear, however, whether the two enemies will go into interference or exploitative competition. In other words, the 'enemy of my enemy' here might not be a foe. This means that it is possible that plants under attack from one species (pest) may actually be easier to be exploited and manipulated by another species (see Price 1991 for a review on plant vigour and resistance hypotheses).

This will also depend on plant resistance and response to attack (likely to vary among cultivars). Nevertheless, all parties strive to maximise their survival chances. The force behind the variation of scenarios can be the selfish gene effects (Williams 1966, Dawkins 1976, 1982; see also Hull 1980), or more likely a 'hereditary suite' of genetic and non-genetic (epigenetic) routes as envisioned by Jablonka (2004). Note that in both the widespread parthenogenetic aphids and RKN, the replicator (i.e. the genes or hereditary suite) matches the vehicle (i.e. the genetically identical daughters) and hence performs according to the doctrine of the selfish agenda of proliferation (*op. cit.*). The fitter is the replicator that can ensure multiplication and protection of offspring (Van Valen 1973; but see Bergstrom and Lachmann 2003). This also should reflect the ongoing dance between ecology and evolution of the system components in that natural selection is likely modifiable by the activities of the interactors (Odling-Smee et al. 2003, Laland 2004, Marquet 2009, Pelletier et al. 2009, Schoener 2011).

Focal above ground aphid genotype across fluctuating environments:

We found clear evidence that tomato cultivar significantly interacted with competition and altered the population growth rate and behaviour of *M.p*. This indicates that plant genotype is significantly altering the response of the focal aphid genotype to both types of competition (i.e. with heterospecific (black bean aphid) and with RKN). Plant stress and a varying IEE on host cultivar due to the activity of RKN caused all possible responses, that is, aphid numbers increased, decreased or remained

unchanged depending on the tomato cultivar that was acting as a host plant. This suggests that the community genetic effect of tomatoes has a significant mediating effect on other ecological processes in this simplified agricultural model. Therefore, the performance and behaviour of the focal genotype of aphid were influenced by the interaction between competition and host plant cultivar. Consequently, our results indicate that an IEE can modify competition among aphids even when the source of the IEE is spatially separated.

Hong et al. (2010) demonstrated an impact of cyst nematode (in a soybean host system) on aphid behaviour. We argue that the extent and amplitude of such effect should be proportionally influenced by what Smith et al. (2008) proposed as a trade-off occurring between the intra- and inter-specific modes of competition where genetic variation of the host plant affects aphid vitality rates.

We also expect that concentration and availability of nutrients will differ among host genotypes, particularly when subject to loads of pathogens or parasites as seen in the changes to the plant root-system under RKN attack. Thus, host plants in a field will vary in their dietary value to aphids, their attractiveness to aphids and their effects on the reproductive success of aphids. This variation is likely superimposed on the co-variation between susceptibility, resistance and suitability of the host plant to the infesting pest. This mosaic, underlain by the genetics of the plant and in correlation with the IEEs, can cause a facilitative effect for pests to flourish on weakened plant hosts. In contrast, a conflict between plant enemies mediated by CG and IEE would benefit the host plant, possibly via the induction of plant resistance (Cooper and Goggin 2005, Cooper et al. 2005, Bailey and Whitham 2006a). This will enhance a negative competitive effect among aphids, and between aphids and RKN [similar to the findings reported by Denno and Kaplan (2007), Kaplan and Denno (2007) and Kaplan et al. (2009)]. Interestingly, it can be argued that the species

activities leading to such consequences could be themselves mediated by the high level of plasticity exhibited by the interacting species (genotypes), including the plant host (Fordyce 2006, Pfennig and McGee 2009).

We found that the behavioural response (preference for stem vs. leaf and preference for top, middle or bottom thirds of the plant) of *M.p* depended on the interaction between the community genetic effect of tomato and the effect of competition. Aphids examine the suitability of host plants, as well as the suitability of specific parts of the host, before colonisation starts (Karban and Baldwin 1997, Cooper and Goggin 2005, Powell et al. 2006). Root-knot nematodes trigger different metabolic pathways of change than those induced by aphids (Bhattarai et al. 2007). However, it can be argued that the inoculation with RKN in this study induced changes in plant physiology (IEE) i.e. a change in specific plant phenotypic trait/s in terms of palatability and resistance to aphids (Chapman 2003, Cooper and Goggin 2005, Powell et al. 2006; but see Goggin et al. 2006). Concomitantly, aphids will apply a set of logistic and chemical pre- and post ingesting tactics in order to manipulate the host and evade the negative feedback loop of induced resistance in its hosts (Goggin et al. 2006, Powell et al. 2006, Walling 2008). Fine-tuning the phenotype of daughters according to the change of context and suitability of the shared resource, on which the mothers' phenotype experiences the environmental change, may be a mechanism for the response developed by aphids (Dixon 1987, 1998, Agrawal et al. 1999, Podjasek et al. 2005, Zehnder et al. 2007). Such change and subsequent countermeasures are expected to shape the selection of the feeding sites on the plant by aphids so that the uptake of essential nutrients can be optimised (Powell et al. 2006).

The fact that the community genetic effect influenced the performance and propensities of the above-ground interactor (*M.p*) suggests that community genetic

effects may moderate not only simple ecological interactions but also the cyclical interplay among members of the directly plant-associated communities and their consequences on the interactors' activities (Odling-Smee et al. 2003, Laland 2004). This agrees well with the more diverse examples in the literature showing important impacts of variation in host plant quality on the population dynamics of associated insects (e.g. Karban 1992). This is also concordant with the studies reporting an influence of plant genotype on species that are substantially separated from the host plant in time or space (e.g. Whitham et al. 2003, Bailey and Whitham 2006b). Furthermore, similar bottom-up effects of host plant in a multi-trophic system were demonstrated by Johnson (2008) to be independent of the density-dependent factors in their impact on the population growth rates of a focal phytophagous insect. However, very few studies endeavoured to explore the interaction between genetic variation and ecological relationships such as competition (Smith et al. 2008).

We speculate that the activities of the below-ground pests will result in dynamic environments of fluctuating host health and resistance that are analogous to the patterns proposed by Ernebjerg and Kishony (2011) and Khatri et al. (2011). Such fluctuation in host well-being is expected to be ongoing due the constant manipulation of the plant host by RKN (through genome-alteration effect and assimilate-sink effect; Bird 1996, Jammes et al. 2005, Caillaud et al. 2008). The instability in plant induced reactions will vary across host cultivars and is expected to shape the response of on-plant distribution of aphids. In this environment it is possible that the shared phenotype of the two clonal pests will emerge as either a 'conflict avoidance' mechanism or an evolutionary arms-race (Dawkins and Krebs 1979, Dawkins 1982; but see also Araújo et al. 2008, Juarrero 2010, Rawal and Rajagopalan 2010, and Rajagopalan et al. 2010).

Perhaps the most surprising result in our work is that cultivars varied not only in the magnitude of their effect on the fitness of each herbivore, but also that the rank order of effects was different for each of the herbivores we used. Given the varied fluctuation levels in the environmental levels (plant vigour) and likely plant resistance, it is notable that, even though *A.f* initially outnumbered *M.p* in our experimental design, *M.p* performed well. This also occurred in spite of the presence of *A.f* and RKN which suggests that competition amongst plant invertebrate pests can be both direct and plant mediated (Denno et al. 2000, Anderson et al. 2009). However, the relative strength of such changes will vary in response to the induced effects by RKN (the IEE).

Community genetics insights for agro-ecosystems:

In the agricultural arena, the conventional system is the practice of planting areas with a single cultivar or genotype (Revilla-Molina 2009). This practice reduces diversity in the landscape, promotes strong selection on plant enemies and results in specific plant genotypes becoming host traps or host sinks for pests and pathogens (Larson and Whitham 1991, Revilla-Molina 2009). Such man-made homogeneity imposes negative consequences on economic crops that become prone to both pathogen epidemics and pest outbreaks (Karban and Baldwin 1997, Revilla-Molina 2009). However, many elements add to the complexity of relationships in agricultural systems: variation in resistance and usability of hosts, intimate relationships between species, co-evolutionary dynamics between pests and hosts, competition between plant-consumers and finally, interactions with natural enemies (Moran and Whitham 1990, Denno et al. 2000, Strauss et al. 2005, Kaplan et al. 2008; but see also Singer 2000). In such context, the IEEs (*sensu* Wootton 1994 and Bonkowski et al. 2001), underlain and moderated by genetic variability (e.g. Astles et al. 2005, Bailey et al.

2006), will moderate the magnitude of the diffuse co-evolution of hosts and host consumers. Under these conditions changes in the phenotypic traits of the interacting species will be correlated with the fitness and the activities of all the inter-players (Strauss et al. 2005; but see also Dawkins 1976, 1982, Odling-Smee et al. 2003, Laland 2004).

The significant community genetic effects observed in this work highlight the potential importance of inter-cropping of multiple genotypes at a local scale. There is an increasing body of evidence in the literature supporting the value of inter-cropping (see Zhu et al. 2000, Revilla-Molina 2009). While we do not directly address this effect here, our results suggest that there is significant merit in investigating this idea in tomato agricultural systems. Moreover, our results demonstrate that performance (reproductive success) and behaviour (on-plant distributions) of aphid species can be altered by the IEEs (induced by the presence of root-knot nematodes) and competition. The factors considered above can also account for the change in competition strength amongst aphid species. In addition, a disruptive effect resulting from variation in plant resistance and vigour or due to anthropogenic effect (disequilibrium) can be influential in shaping the possible scenarios of interaction within man-made arable plots (Ninkovic et al. 2002, Neuhauser et al. 2003).

Final remark

Our results directly address questions raised in the literature about the relative importance of community genetic effects (Hersch-Green et al. 2011). Our findings show that genetic identity can create important community genetic effects that can be considered on a par with other ecological effects (e.g. competition, IEE). Differential response of cultivars to herbivores is hardly surprising due to relative susceptibility and resistance of tomato genotypes. However, the finding that combinations of

spatially separated herbivores may increase as well as decrease plant biomass (and hence productivity) has potential economic implications and deserves significant further investigation. We have clearly used a simplified model system. Nevertheless, we think this is reasonably representative of a proportion of the community interactions found in agro-ecosystems. More importantly, our results show that community genetic effects interact with other ecological effects and fitness outcomes for all species involved are influenced by such interaction. The findings of this study contribute to the efforts of amalgamating different perspectives to explain the complexity of plant-insect interactions [(concordant with the picture envisaged by Zheng and Dicke (2008)]. Our results highlight the importance of deciphering the relationship between ecology, evolution and genetics of spatially isolated competing plant enemies. This work sheds further light on the dynamics of pest-crop interactions in agro-ecosystems.

References

Agrawal, A. A., & Sherriffs, M. F. (2001). Induced plant resistance and susceptibility to late-season herbivores of wild radish. *Annals of the entomological society of America, 94*(1), 71-75.

Agrawal, A. A., Laforsch, C., & Tollrian, R. (1999). Transgenerational induction of defences in animals and plants. *Nature, 401*, 60-63.

Anderson, K. E., Inouye, B. D., & Underwood, N. (2009). Modeling herbivore competition mediated by inducible changes in plant quality. *Oikos*, *118*, 1633-1646.

Antonovics, J. (1992). Toward community genetics. In R. S. Fritz, & E. L. Simms (Eds.), *Ecology and Evolution of Plant Resistance to Herbivores and Pathogens: Ecology, Evolution, and Genetics* (pp. 426-449). USA: University Of Chicago Press.

Araújo, M. S., Guimarães, P. R., Svanbäck, R., Pinheiro, A., Guimarães, P., Dos Reis, S. F., & Bolnick, D. I. (2008). Network analysis reveals contrasting effects of intraspecific competition on individual vs. population diets. *Ecology, 89*(7), 197-213.

Astles, P. A., Moore, A. J., & Preziosi, R. F. (2005). Genetic variation in response to an indirect ecological effect. *Proceedings of the Royal Society B*, *272*, 2577-2581.

Awmack, C. S., & Leather, S. R. (2002). Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology, 47*, 817-844.

Bailey, J. K. (2011). From genes to ecosystems: a genetic basis to ecosystem services. *Popul Ecol, 53*, 47-52.

Bailey, J. K., & Whitham, T. G. (2006a). Biodiversity is related to indirect interactions among species of large effect. In T. Ohgushi, T. Craig, & P.W. Price (Eds.), *Indirect interaction webs: Nontrophic linkages through induced plant traits* (pp. 306-328). UK: Cambridge University Press. Retrieved from http://web.utk.edu/~jbaile29/publications/Bailey%20and%20Whitham%20indirect%20interaction%20webs.pdf

Bailey, J. K., & Whitham, T. G. (2006b). Indirect trait-mediated interactions between cottonwoods and beavers positively affect sawfly abundance. *Ecological Entomology*, *31*, 294-297.

Bailey, J. K., Schweitzer, J. A., Koricheva, J., Madritch, M. D., LeRoy, C. J., Rehill,B. J., Bangert, R.K., Fisher, D.G., Allen, G., Whitham, T. G. (2009). From genes to ecosystems: An emerging synthesis of eco-evolutionary dynamics. *Philosophical Transactions of the Royal Society B*, *364*, 1607-1616.

Bailey, J. K., Wooley, S. C., & Whitham, T. G. (2006). Importance of species interactions to community heritability: A genetic basis to trophic-level interactions. *Ecology Letters*, *9*, 78-85.

Bengtsson, J. (1993). Interspecific competition and determinants of extinction in experimental populations of three rockpool *Daphnia* species. *Oikos*, *67*(3), 451-464.

Bergstrom, C. T., & Lachmann, M. (2003). The red king effect: When the slowest runner wins the coevolutionary race. *Proceedings of the National Academy of Sciences*, *100*(2), 593-598.

Bhattarai, K. K., Li, Q., Liu, Y., Dinesh-Kumar, S. P., & Kaloshian, I. (2007). The Mi-1-mediated pest resistance requires Hsp90 and Sgt1. *Plant Physiology*, *144*(1), 312-323.

Bird, D. McK. (1996). Manipulation of host gene expression by root-knot nematodes. *Journal of Parasitology*, *82*(6), 881-888.

Bird, D. McK., Williamson, V. M., Abad, P., McCarter, J., Danchin, E. G., Castagnone-Sereno, P. , & Opperman, C. H. (2009). The genomes of root-knot nematodes. *Annu Rev Phytopathol*, *47*(6), 333-351.

Blackman, R. L., & Eastop, V. F. (2000). *Aphids of the world's crops - an identification and information guide,* 2nd ed. New York: John Wiley.

Bonkowski, M., Geoghegann, I., Nicholas, A., Birch, E., & Griffiths, B. (2001). Effects of soil decomposer invertebrates (protozoa and earthworms) on above-ground phytophagous insects (cereal aphid) mediated through changes in the host plant. *Oikos*, *95*, 441-450.

Borges, R. T. (2005). Do plants and animals differ in phenotypic plasticity? *J. Biosci*, *30*(1), 41-50.

Caillaud, M. C., Dubreuil, G., Quentin, M., Perfus-Barbeoch, L., Lecomte, P., Engler, J. A., Abad, P., Rosso, M. N., & Favery, B. (2008). Root-knot nematodes manipulate plant cell functions during a compatible interaction. *J. Plant Physiol*, *165*, 104-113.

Castagnone-Sereno, P. (2006). Genetic variability and adaptive evolution in parthenogenetic root-knot nematodes. *Heredity*, *96*, 282-289.

Chapman, R. F. (2003). Contact chemoreception in feeding by phytophagous insects. *Annual Review of Entomology*, *48*, 455-484.

Connell, J. H. (1983). On the prevalence and relative importance of interspecific competition: Evidence from field experiments. *Am Nat Vol*, *122*(5), 41-50.

Cooper, W. R., & Goggin, F. L. (2005). Effects of jasmonate-induced defenses in tomato on the potato aphid, *Macrosiphum euphorbiae*. *Entomologia Experimentalis et Applicata*, *115*(1), 107-115

Cooper, W. R., Jia, L., & Goggin, F. L. (2005). Effects of jasmonate-induced defenses on root-knot nematode infection of resistant and susceptible tomato cultivars. *J. Chem. Ecol.*, *31*(4), 1953-1967.

Cronin, J. T., & Abrahamson, W. G. (1999). Host-plant genotype and other herbivores influence goldenrod stem galler preference and performance. *Oecologia*, *121*, 392-404.

Dawkins, R. (1976). *The selfish gene*. New York City: Oxford University Press.

Dawkins, R. (1982). *The extended phenotype*. Oxford: W.H. Freeman And Company.

Dawkins, R., and J. R. Krebs. 1979. Arms races between and within species. *Proceedings of the Royal Society London B, 205* (1161), 489-511.

DEFRA: Department for Environment, Food and Rural Affairs. (2011). *Crop areas, yields and production, livestock populations and the size of the agricultural workforce, UK-Final results*. UK. A National Statistics publication http://www.defra.gov.uk/statistics/files/defra-stats-foodfarm-landuselivestock-june-statsrelease-uk-111222.pdf

Denno, R. F., & Kaplan, I. (2007). Plant-mediated interactions in herbivorous insects: Mechanisms, symmetry, and challenging the paradigms of competition past. In Ohgushi T., Craig T. P., Price P. W. (Eds.), *Ecological communities: Plant mediation in indirect interaction webs*. UK: Cambridge University Press. Retrieved from http://www.entm.purdue.edu/ecolab/pdf/07_communities.pdf

Denno, R. F., McClure, M. S., & Ott, J. R. (1995). Interspecific interactions in phytophagous insects: Competition re-examined and resurrected. *Annu. Rev. Entomol, 40*, 297-331.

Denno, R. F., Peterson, M. A., Gratton, C., Cheng, J., Langellotto, G. A., Huberty, A. F., & Finke, D. L. (2000). Feeding-induced changes in plant quality mediate interspecific competition between sap-feeding herbivores. *Ecology, 81*(7), 1814-1827.

Dixon, A. F. G. (1977). Aphid ecology: Life cycles, polymorphism, and population regulation. *Ann. Rev. Ecol. Syst., 8*, 329-353.

Dixon, A. F. G. (1987). Parthenogenetic reproduction and the rate of increase in aphids. In A. K. Minks & P. Harrewijn (Eds.), *Aphids, their biology, natural enemies and control* (pp. 269-287). Amsterdam: Elsevier.

Dixon, A. F. G. (1998). *Aphid ecology*. London: Chapman and Hall.

Dolman, P. M. (1995). The intensity of interference varies with resource density evidence from a field study of snow buntings *Plectrophenax nivalis*. *Oecologia*, *102*, 511-514.

Dombrovsky, A. A., Arthaud, L. L., Ledger, T. N., Tares, S., & Robichon, A. (2009). Profiling the repertoire of phenotypes influenced by environmental cues that occur during asexual reproduction. *Genome Research*, *19*, 2052-2063. (doi: 10.1101/gr.091611.109)

Duc, G. (1997). Faba bean (*Vicia faba* L.). *Field Crops Res*, *53*, 99-109.

Ernebjerg, M., & Kishony, R. (2011). Dynamic phenotypic clustering in noisy ecosystems. *PLoS Computational Biology*, *7*(3). (doi:10.1371/journal.pcbi.1002017)

Fenemore, P. G. (1988). Host-plant location and selection by adult potato moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *J. Insect Physiol*, *34*, 175-177.

Fordyce, J. A. (2006). The evolutionary consequences of ecological interactions mediated through phenotypic plasticity. *The Journal of Experimental Biology*, *209*, 2377-2383.

Goggin, F. L., Jia, L., Shah, G., Hebert, S., Williamson, V. M., & Ullman, D. E. (2006). Heterologous expression of the Mi-1.2 gene from tomato confers resistance against nematodes but not aphids in eggplant. *MPMI*, *19*(4), 383-388.

Goggin, F. L., Shah, G., Williamson, V. M., & Ullman, D. E. (2004). Developmental regulation of mi-mediated aphid resistance is independent of Mi-1.2 transcript levels. *MPMI*, *17*(5), 532-536.

Gonzáles, W. L., Fuentes-Contreras, W. E., & Niemeyer, H. M. (2002). Host plant and natural enemy impact on cereal aphid competition in a seasonal environment. *Oikos*, *96*(3), 481-491.

Haloin, J. R., & Strauss, S. Y. (2008). Interplay between ecological communities and evolution: Review of feedbacks from microevolutionary to macroevolutionary scales. *Ann. N.Y. Acad. Sci*, *1133*, 87-125.

Hamilton, W. (1964). The genetical evolution of social behaviour. I. *Journal of Theoretical Biology*, *7*(1), 1-16.

Hannan, M. M., Ahmed, M. B., Razvy, M. A., Karim, R., Khatun, M., Haydar, A., Hossain, M., & Roy, U. K. (2007). Heterosis and correlation of yield and yield components in tomato (*Lycopersicon esulentum* Mill.). *American-Eurasian Journal of Scientific Research*, *2*(2), 146-150.

Hersch-Green, E. I., Turley, N. E., & Johnson, M. T. J. (2011). Community genetics: What have we accomplished and where should we be going? *Philosophical Transactions of the Royal Society B-Biological Sciences*, *366*, 1453-1460.

Hoagland, D. R., & Arnon, D. I. (1950). The water-culture method for growing plants without soil. *Calif. Agric. Exp. Stn Circ*, *347*, 1-32.

Hong, S. C., Donaldson, J., & Gratton, C. (2010). Soybean cyst nematode effects on soybean aphid preference and performance in the laboratory. *Environ. Entomol*, *39*, 1561-1569.

Hull, D. L. (1980), "Individuality and Selection", *Annual Review of Ecology and Systematics*. *11*, 311–32.

Inbar, M., & Wool, D. (1995). Phloem-feeding specialists sharing a host tree: Resource partitioning minimizes interference competition among galling aphid species. *Oikos*, *73*, 109-119.

Inbar, M., Eshel, A., & Wool, D. (1995). Interspecific competition among phloem - feeding insects mediated by induced host plant sinks. *Ecology*, *76*(5), 1506-1515.

Jablonka, E. (2004). From replicators to heritably varying phenotypic traits. The extended phenotype revisited. *Biology & Philosophy*, *19*, 353-375.,

Jammes, F., Lecomte, P. L., De Almeida-Engler, J., Bitton, F., Martin-Magniette, Marie-Laure, Renou, J. P., Abad, P., & Favery, B. (2005). Genome-wide expression profiling of the host response to root-knot nematode infection in Arabidopsis[a]. *The Plant Journal*, *44*, 447-458.

Janzen, D. H. (1980). When is it coevolution? *Evolution*, *34*(3), 611-612.

Johnson, M. T. J. (2008). Bottom-up effects of plant genotype on aphids, ants and predators. *Ecology*, *89*, 145-154.

Johnson, M. T. J., & Agrawal, A. A. (2005). Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (*Oenothera biennis*). *Ecology*, *86*, 874-885.

Juarrero, A. (2010). Complex dynamical systems theory. "Cognitive Edge Network". Retrieved from http://cognitive-edge.com/uploads/articles/100608%20Complex_Dynamical_Systems_Theory.pdf

Juliano, S. A. (2010). Coexistence, exclusion, or neutrality? A meta-analysis of competition between *Aedes albopictus* and resident mosquitoes. *IJEE*, *56*, 325-351.

Kaplan, I., & Denno, R. F. (2007). Interspecific interactions in phytophagous insects revisited: A quantitative assessment of competition theory. *Ecol Lett*, *10*(1), 977-994.

Kaplan, I., Halitschke, R., Kessler, A., Sardanelli, A., & Denno, R. F. (2008). Constitutive and induced defenses to herbivory in above- and belowground plant tissues. *Ecology*, *89*(2), 392-406.

Kaplan, I., Sardanelli, S., & Denno, R. F. (2009). Field evidence for indirect interactions between foliar-feeding insect and root-feeding nematode communities on *Nicotiana tabacum*. *Ecol. Entomol*, *34*, 262-270.

Karban, R. (1992). Plant variation: Its effects on populations of herbivorous insects. In R. S. Fritz & E. L. Simms (Eds.*), Plant resistance to herbivores and pathogens* (pp. 195-215). Chicago: University Of Chicago Press.

Karban, R., & Baldwin, I. T. (1997). *Induced responses to herbivory.* Chicago and London: University Of Chicago Press.

Karban, R., & Myers, J. H. (1989). Induced plant responses to herbivory. *Annu Rev Ecol Syst*, *20*, 331-348.

Kessler, A., & Halitschke, R. (2007). Specificity and complexity: The impact of herbivore-induced plant responses on arthropod community structure. *Current Opinion in Plant Biology*, *10*(1), 409-414.

Khatri, B. S., Freeb, A., & Allena, R. J. (2011). Noise-driven oscillations in microbial population dynamics. *ARXIV,* Eprint [q-bio.PE]. http://arxiv.org/pdf/1112.4836.pdf

Krebs, J. R., and N. B. Davies. (1998). *An Introduction to Behavioural Ecology.* Oxford, England: Blackwell Science Ltd.

Laland, K. N. (2004). Extending the extended phenotype. *Biology and Philosophy*, *19*, 313-325.

Larson, K. C., & Whitham, T. G. (1991). Manipulation of food resources by a gall-inducing aphid: The physiology of sink–source interactions. *Oecologia*, *88*, 15-21.

Larson, K. C., & Whitham, T. G. (1997). Competition between gall aphids and natural plant sinks: Plant architecture affects resistance to galling. *Oecologia*, *109*, 575-582.

Lawlor, L. R. (1979). Direct and indirect effects of n-species competition. *Oecologia*, *110*, 355-364.

Lawton, J. H. (1999). Are there general laws in ecology? *Oikos*, *84*, 177-192.

Lee, C. T., Miller, T. E. X., & Inouye, B. D. (2011). Consumer effects on the vital rates of their resource can determine the outcome of competition between consumers. *The American Naturalist*, *178*(4), 452-463.

Levin, S. A. (1998). Ecosystems and the biosphere as complex adaptive systems. *Ecosystems*, *1*, 431-436.

Levine, S. F. (1976). Competitive interactions in ecosystems. *Am Nat*, *110*, 903-910.

Luck, G. W., Daily, G. C., & Ehrlich, P. R. (2003). Population diversity and ecosystem services. *Trends in Ecology and Evolution*, *18*(7), 331-336.

Marquet, P. A. (2009). Beyond Darwin: On the role of niche construction and self-organization in evolution. *Revista Chilena De Historia Natural*, *82*, 493-496.

Masters, G. J., Brown, V. K., & Gange, A. C. (1993). Plant mediated interactions between above- and belowground insect herbivores. *Oikos, 66*, 148-151.

Masters, G. J., Jones, T. H., & Rogers, M. (2001). Host-plant mediated effects of root herbivory on insect seed predators and their parasitoids. *Oecologia*, *127*, 246-250.

Maynard Smith, J. (1974). The theory of games and the evolution of animal conflicts. *Journal of Theoretical Biology*, *47*(1), 209-221.

Miller, T. E., & Travis, J. (1996). The evolutionary role of indirect effects in communities. *Ecology*, *77*(5), 1329-1335.

Moens, M., Perry, R., & Starr, J. L. (2009). *Meloidogyne species* – a diverse group of novel and important plant parasites. *Root Knot Nematodes.* Wallingford, UK: CABI Publishing CAB International.

Moore, A. J., Brodie, E. D., & Wolf, J. B. (1997). Interacting phenotypes and the evolutionary process: I. Direct and indirect genetic effects of social interactions. *Evolution*, *51*(5), 1352-1362.

Moran, N. A., & Whitham, T. G. (1990). Interspecific competition between root-feeding and leaf-galling aphids mediated by host-plant resistance. *Ecology*, *71*, 1050-1058.

Morriën, E., Engelkes, T., & Van Der Putten, W. H. (2011). Additive effects of aboveground polyphagous herbivores and soil feedback in native and range-expanding exotic plants. *Ecology*, *92*, 1344-1352.

Neuhauser, C., Andow, D. A., Heimpel, G. E., Ruth, G. M., Shaw, G., & Wagenius, S. (2003). Community genetics: Expanding the synthesis of ecology and genetics. *Ecology*, *84*(3), 545-558.

Ninkovic, V., Olsson, U., & Pettersson, J. (2002). Mixing barley cultivars affects aaphid host plant acceptance in field experiments. *Journal of Parasitology*, *102*(2), 177-182.

Odling-Smee, F. J., Laland, K. N., & Feldman, M. W. (2003). *Niche construction-the neglected process in evolution.* Princeton, NJ: Princeton University Press.

Ohgushi, T. (2005). Indirect interaction webs: Herbivore-induced effects through trait change in plants. *Annual Review of Ecology, Evolution, and Systematics*, *36*, 81-105.

Ohgushi, T. (2008). Herbivore-induced indirect interaction webs on terrestrial plants: The importance of non-trophic, indirect, and facilitative interactions. *Entomologia Experimentalis et Applicata*, *128*(1), 217-229.

Östman, Ö., (2011). Interspecific competition affects genetic structure but not genetic diversity of daphnia magna. *Ecosphere*, *2*(3). (doi: 10.1890/ES11-00039.1)

Pelletier, R., Garant, D. H., & Hendry, A. P. (2009). Eco-evolutionary dynamics. *Phil. Trans. R. Soc. B*, *364*, 1483-1489.

Pfennig, D. W., & McGee, M. (2009). Resource polyphenism increases species richness: A test of the hypothesis. *Phil. Trans. R. Soc. B*, *365*(1540), 577-591.

Podjasek, J. O., Bosnjak, L. M., Brooker, D. J., & Mondor, E. B. (2005). Alarm pheromone induces a transgenerational wing ployphenism in the pea aphid, *Acyrthosiphon pisum. Can. J. Zool*, *83*, 1138-1141.

Powell, G., Tosh, C. R., & Hardie, J. (2006). Host plant selection by aphids: Behavioral, evolutionary, and applied perspectives. *Annu. Rev. Entomol.*, *51*, 309-330.

Power, M. E. (1992). Top-down and bottom-up forces in food webs: Do plants have primacy? *Ecology*, *73*, 733-746.

Price, P. W. (1991). The plant vigor hypothesis and herbivore attack. *Oikos*, *62*(2), 244-251.

Price, P. W., Westoby, M., Rice, B., Atsatt, P. R., Fritz, R. S., Thompson, J. N., & Mobly, K. (1986). Parasite mediation in ecological interactions. *Annual Review of Ecology and Systematic*, *17*, 487-505.

Rajagopalan, P., Rawal, A., & Miikkulainen, R. (2010). Emergence of competitive and cooperative behavior using coevolution. *Proceedings of the 12th Annual Conference on Genetic and Evolutionary Computation*. Retrieved from http://dl.acm.org/citation.cfm?id=1830681&bnc=1

Rawal, A., & Rajagopalan, P. (2010). Emergence of competitive and cooperative behavior and arms race through coevolution. *Neural Networks Research Group*. Retrieved from http://nn.cs.utexas.edu/?armsrace

Revilla-Molina, I. M. (2009). *Genetic diversity for sustainable rice blast management in china: Adoption and impact*. (Ph.D Dissertation) 144 p. Wageningen,The Netherlands: Wageningen University. Retrieved from http://edepot.wur.nl/122082

Rowntree, J. K., Cameron, D. D., & Preziosi, R. F. (2011a). Genetic variation changes the interactions between the parasitic plant-ecosystem engineer *Rhinanthus* and its hosts. *Phil. Trans. R. Soc. B*, *366*, 1380-1388.

Rowntree, J. K., Shuker, D. M., & Preziosi, R. F. (2011b). Forward from the crossroads of ecology and evolution. *Phil. Trans. R. Soc. B*, *366*(1569), 1322-1328.

Ruhnke, H., Schädler, M., Klotz, S., Matthies, D., & Brandl, R. (2009). Variability in leaf traits, insect herbivory and herbivore performance within and among individuals of four broad-leaved tree species. *Basic and Applied Ecology*, *10*, 726-736.

Ruhnke, H., Schädler, M., Matthies, D., Klotz, S., & Brandl, R. (2006). Are sawflies adapted to individual host trees? A test of the adaptive deme formation hypothesis. *Evolutionary Ecology Research 8*, *8*, 1039-1048.

Schädler, M., Brandl, R., & Kempel, A. (2010). Host plant genotype determines bottom-up effects in an aphid-parasitoid-predator system. *Entomologia Experimentalis et Applicata*, *135*(7), 162-169.

Schoener, T. W. (2011). The newest synthesis: Understanding the interplay of evolutionary and ecological dynamics. *Science*, *331*, 426-429.

Shuster, S. M., Lonsdorf, E. V., Wimp, G. M., Bailey, J. K., & Whitham, T. G. (2006). Community heritability measures the evolutionary consequences of indirect genetic effects on community structure. *Evolution*, *60*, 146-158.

Silim, S. N., & Saxena, A, M. C. (1992). Comparative performance of some faba bean (*Vicia faba*) cultivars of contrasting plant types. 1. Yield, yield components and nitrogen fixation. *Journal of Agricultural Science, Cambridge, 118*, 325-332.

Singer, M. C. (2000). Reducing ambiguity in describing plant-insect interactions: "preference," "acceptability" and "electivity.". *Ecol. Lett, 3*(4), 159-162.

Singh, S. K., & Khurma, R. K. (2007). Susceptibility of six tomato cultivars to the root-knot nematode, *Meloidogyne incognita. The South Pacific Journal of Natural Sci, 13*, 73-77.

Smith, R. A., Mooney, K. A., & Agrawal, A. A. (2008). Coexistence of three specialist aphids on common milkweed, *Asclepias syriaca. Ecology, 89*(8), 2187-2196.

Sorvari, J., & Hakkarainen, H. (2004). Habitat-related aggressive behaviour between neighbouring colonies of the polydomous wood ant *Formica aquilonia. Animal Behaviour, 67*, 151-153.

Strauss, S. Y., Sahl, H., & Conner, J. K. (2005). Toward a more trait-centered approach to diffuse (co)evolution. *New Phytologist, 165*(1), 81-90.

Tagu, D., Klingler, J. P., Moya, A., & Simon, J. C. (2008). Early progress in aphid genomics and consequences of plant-aphid interaction studies. *Mol. Plant Microbe Interact., 21*, 701-708.

Tariq, M., Wright, D. J., & Staley, J. T. (2010). Maternal host plant effects on aphid performance: Contrasts between a generalist and a specialist species on Brussels sprout cultivars. *Agricultural and Forest Entomology, 12*, 107-112.

Tétard-Jones, C., Kertesz, M. A., Gallois, P., & Preziosi, R. F. (2007). Genotype-by-genotype interactions modified by a third species in a plant–insect system. *Am Nat, 170*, 492-499.

Underwood, N., & Rausher, M. D. (2000). The effects of host–plant genotype on herbivore population dynamics. *Ecology*, *81*, 1565-1576.

Utsumi, S., Ando, Y., & Miki, T. (2010). Linkages among trait-mediated indirect effects: A new framework for the indirect interaction web. *Population Ecology*, *52*(4), 485-497.

Van Valen L. (1973). "A New Evolutionary Law", *Evolutionary Theory, 1*, 1-30.

Walling, L. L. (2008). Avoiding effective defenses: Strategies employed by phloem-feeding insects. *Plant Physiology*, *146*(3), 859-866.

West-Eberhard, M. J. (1989). Phenotypic plasticity and the origins of diversity. *Ann. Rev. Ecol. Syst.*, *20*, 249-278.

West-Eberhard, M. J. (2003). *Developmental plasticity and evolution.* Oxford, UK: Oxford University Press.

White, T. C. R. (1984). The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia*, *63*, 90-105.

White, T. R. (1978). The importance of a relative shortage of food in animal ecology. *Oecologia*, *33*, 71-86.

Whitham, T. G., Bailey, J. K., Schweitzer, J. A., Shuster, S. M., Bangert, R. K., LeRoy, C. J., Lonsdorf, E. V., Allan, G. J., DiFazio, S. P., Potts, B. M. Fischer, D. G., Gehring, C. A.Lindroth, R. L., Marks, J. C., Hart, S. C., Wimp, G. M., & Wooley, S. C. (2006). A framework for community and ecosystem genetics: From genes to ecosystems. *Nat Rev Genet*, *7*, 510-523.

Whitham, T. G., DiFazio, S. P., Schweitzer, J. A., Shuster, S. M., Allan, G. J., Bailey, J. K., & Woolbright, S. A. (2008). Extending genomics to natural communities and ecosystems. *Science*, *320*, 492-495.

Whitham, T. G., Young, W. P., Martinsen, G. D., Gehring, C. A., Schweitzer, J. A., Shuster, S. M Wimp, G. M., Fischer, D. G., Bailey, J. K., Lindroth, R. L.,

Woolbright, S. , & Kuske, C. R. (2003). Community and ecosystem genetics: A consequence of the extended phenotype. *Ecology, 84*, 559-573.

Whitman, D. W., & Agrawal, A. A. (2009). What is phenotypic plasticity and why is it important? In D. W. Whitman & T. N. Ananthakrishnan (Eds.), *phenotypic plasticity of insects: Mechanisms and consequences* (pp. 1-54). Moscow, USA: Science.

Williams, G. C. (1966). *Adaptation and natural selection: A critique of some current evolutionary thought.* Princeton, NJ: Princeton University Press.

Williamson, V. M., & Gleason, C. A. (2003). Plant-nematode interactions. *Current Opinion in Plant Biology, 6*, 327-333.

Wolf, B. J. (2000). Indirect genetic effects and gene interactions. In B. J. Wolf, E. D. Brodie, & M. J. Wade (Eds.), *Epistasis and the Evolutionary Process* (pp. 158-176). New York: Oxford University Press.

Wolf, J. B., Brodie III, E. D., Cheverud, A. J., Moore, A. J., & Wade, M. J. (1998). Evolutionary consequences of indirect genetic effects. *Trends Ecol. Evol, 13*, 64-69.

Wootton, J. T. (1994). The nature and consequences of indirect effects in ecological communities. *Annual Review of Ecology and Systematics, 25*, 443-466.

Wootton, J. T. (2002). Indirect effects in complex ecosystems: Recent progress and future challenges, journal of sea research. *Journal of Sea Research, 48*(1), 157-172.

Wurst, S., & Van Der Putten, W. H. (2007). Root herbivore identity matters in plant-mediated interactions between root and shoot herbivores. *Basic Appl. Ecol., 8*, 491-499.

Zehnder, C. B., Parris, M. A., & Hunter, M. D. (2007). Effects of maternal age and environment on offspring vital rates in the oleander aphid (Hemiptera: Aphididae). *Environ Entomol, 36*, 910-917.

Zheng, Si-Jun, & Dicke, M. (2008). Ecological genomics of plant-insect interactions: From gene to community. *Plant Physiol, 146*, 812-817. (doi: http://dx.doi.org/10.1104/pp.107.111542)

Zhu, Y., Chen, H., Fan, J. H., Wang, Y., Li, Y., Chen, J., Fan, J. X., Yang, S., Hu, L., Leung, H., Mew, T. W., Teng, P. S., Wang, Z., & Mundt, C. C. (2000). Genetic diversity and disease control in rice. *Nature, 406*, 718-722

Zytynska, S. E., M. F. Fay, D. Penny, & Preziosi, R. F. (2011). Genetic variation in a tropical tree species influences the associated epiphytic plant and invertebrate communities in a complex forest ecosystem. *Phil Trans R Soc B, 366*, 1329-1336.

Zytynska, S. E., S. Fleming, C. Tétard-Jones, M. A. Kertesz, & Preziosi, R. F. (2010). Community genetic interactions mediate indirect ecological effects between a parasitoid wasp and rhizobacteria. *Ecology, 9*, 1563-1568.

Additional analyses

Plant biomass under multi-herbivory:

Tomato showed the biggest biomass values as follows:

Across cultivars: overall biomass with RKN only (T2) > overall biomass for tomato only (T1) > overall biomass with RKN and aphids (T3) > overall biomass with aphids only (T4). [No data available for (T5) in this regard. Data were for plant dry weight, when cultivars were only infested with *M.p*, were discarded due to out-of-control error].

Across treatments: overall biomass of G4 > overall biomass of G1 > overall biomass of G6 > overall biomass of G2 > overall biomass of G5 > overall biomass of G3

Within cultivar: Intra-cultivar values for maximal and minimal biomass for tomato were quantified and presented in (**Table 1`**) indicating variable response of tomato cultivars to the effect of above- and/or below-ground herbivory.

	C1	**C2**	**C3**	**C4**	**C5**	**G6**
Maximal Biomass	A+RKN	A	RKN	C	A+RKN	C
Minimal Biomass	A	RKN	C	A+RKN	C	A

Table 1`: Within-cultivar differences of biomass across treatments. This table elucidates the highest and lowest values of total tomato biomass per cultivar across the different four treatments. 'C': tomato negative control (T1), 'RKN': tomato inoculated with nematodes only (T2), 'A': tomato infested with aphids (*A.f* and *M.p*) only (T4), while 'A+RKN': tomato infested with both nematodes and aphids (*A.f* and *M.p);* (T3). Shaded cells refer to repeated patterns such as maximal biomass on G4 and G6 where all herbivores were absent. In contrast, minimal values under the same conditions were recorded on G3 and G5.

Chapter 5: Community genetic effects of tank-forming bromeliads on associated invertebrates in a tropical forest ecosystem

*Statement of the nature and extent of my own contribution:

I designed the experiment, collected the bromeliads for the first year work and established the protocols for future sampling. I also sampled and classified the bromeliad-associated faunal communities for the first and second year of the study (with hearty thanks to Dr. David Penney for his help in identifying morphospecies and correcting classification errors). I conducted the first year's molecular work and AFLP analysis and participated in the second year. I completed the initial literature review and was the primary author of the introduction and methods and I wrote the initial outlines of the results and discussion. Dr. Sharon Zytynska conducted the year (2) field and lab work. She re-ran the AFLP analysis for the whole set of samples, performed the analyses for the complete data, provided interpretations, and produced the final complete version of the paper in collaboration with myself, Dr. Richard Preziosi and Dr. Ed Harris. Dr. Ed Harris provided instrumental facilitation for us to run the DNA fingerprinting and sequencing through the molecular Ecology unit he administers at Manchester Metropolitan University.

Community genetic effects of tank-forming bromeliads on associated invertebrates in a tropical forest ecosystem

Sharon E. Zytynska[1*†], Mouhammad Shadi Khudr[1], Edwin Harris[2] and Richard F. Preziosi[1]

[1]Michael Smith Building, Faculty of Life Sciences, University of Manchester, M13 9PT, UK

[2]John Dalton Building, Manchester Metropolitan University, M1 5GD, UK

† Present address: Department of Ecology and Ecosystem Management, Technische Universität München, Freising, Germany

Author email addresses:

Sharon Zytynska: Sharon.zytynska@gmail.com

Mhd.Shadi Khudr: shad1978@gmail.com

Edwin Harris: E.Harris@mmu.ac.uk

Richard Preziosi: preziosi@manchester.ac.uk

Abstract

Within the area of community genetics there is an expanding volume of literature demonstrating how within-species genetic variation in temperate trees can have important effects on structuring animal and plant communities. The influence of community genetic effects in relatively more complex ecosystems is only starting to be appreciated. Within tropical forests, epiphytic bromeliad plants often grow high in the canopy and create unique nutrient-rich microhabitats on which many invertebrate and vertebrate species depend. We investigated the influence of within-species genetic variation in the bromeliad *Aechmea bracteata* on the invertebrate microhabitat community. We found that more genetically similar bromeliad plants were host to more similar communities of juvenile-stage invertebrates, but not adult invertebrates. We discuss possible mechanisms for this, including differential survival and active female oviposition choice. Our work shows that the impact of within-species genetic variation on associated ecological communities may be more general than previously considered. These results agree with recent research suggesting that within-species genetic variation may perform a supporting ecosystem service for maintaining community and ecological processes.

Keywords: genetic interactions, species interactions, ecosystem conservation, amplified fragment length polymorphisms

Introduction

Determining the importance of within-species genetic variation on ecosystem processes and function is a fundamental question in ecology and conservation biology. Biodiversity within an ecosystem consists of multiple levels of diversity including genetic, species, functional group and community levels. Within-species genetic variation provides a basis for adaptation of a population to long-term change in an environment, and can buffer stochastic events reducing the chance of random extinction (Vellend and Geber 2005; Reusch et al. 2005). Recent conservation efforts have placed emphasis on the delivery of ecosystem goods and services and conjecture the importance of supporting ecosystem services, such as genetic variation, as they can be tightly linked with the maintenance of ecosystem function (Luck et al. 2003; Hooper et al. 2005). In addition, interactions between different species are integral to the functioning of an ecosystem, for example predator-prey (Ives et al. 2005) or host-pathogen relationships (Dobson and Crawley 1994). These interactions between species can be mediated by within-species genetic variation, such that the outcome of an interaction depends on the genotypic identity of both the interacting individuals (Service 1984). This has coevolutionary implications for those involved in such interactions (Carius et al. 2001), and can affect organisms involved throughout complex trophic interactions (Zytynska et al. 2010).

There is a growing amount of evidence that within-species genetic variation can have a cascading effect on associated ecological communities (e.g., Whitham et al. 2006; Haloin and Strauss 2008; Zytynska et al. 2010; Rowntree et al. 2011; Hersch-Green et al. 2011; Zytynska et al. 2011). Species interactions mediating community genetic effects are considered intrinsic to the provision of ecosystem services (Bailey 2011),

and thus understanding the ecological and evolutionary impacts such effects have is paramount to understanding ecosystem functioning in order to maximize future benefits. To date, many community genetic studies have focused on temperate systems of limited (species) diversity, using dominant plants as the focal species. It is expected that a dominant plant in a limited diversity ecosystem will have a disproportionate effect on the surrounding ecological communities and, indeed, genetic variation within a number of plant species has been found to influence associated communities of invertebrates (e.g. Fritz and Price 1988; Dungey et al. 2000; Whitham et al. 2003; Johnson et al. 2006), understory plants (Iason et al. 2005), endophytic fungi (Gaylord et al. 1996) and vertebrates (Bailey et al. 2004; Muller et al. 2006). Recently, these community genetic effects have also been detected within a tropical forest ecosystem (Zytynska et al. 2011). Zytynska et al. (2011) suggests that within-species genetic variation of a common (but not dominant) tropical tree species influenced the associated epiphytic plant and invertebrate community structure. This highlights the importance that within-species genetic variation may have even in complex ecosystems. Within a tropical forest, there is high species diversity and typically few dominant species prevail, and as such the effects of genetic variation within one species might be difficult to detect. However, recent thinking suggests that species associations are an important component in maintaining tropical species biodiversity and the genetic component of these interactions may be important but is, as yet, relatively unstudied.

Tropical forests contain a relatively high diversity of habitats, partitioning available resources into many dimensions. For example, epiphytic plants anchor onto trees in order to escape the shady understory and grow higher in the canopy, nearer the sunlight. These plants, themselves, provide unique habitats for other organisms. One

group of epiphytic plants, the bromeliads, have a rosette leaf-morphology such that the radiating leaves overlap one another creating a 'tank' space that gets filled by falling debris and rain water, forming nutrient-rich water bodies called phytotelmata (Frank and Lounibos 2009). Numerous species use these plants for shelter and food, and tank-forming bromeliads have been described as keystone species. Thus, in combination with their associated communities, bromeliads provide an important function in maintaining diversity and resilience in the communities they inhabit (Nadkarni 1994; Stuntz et al. 2002).

To assess the importance of within-species genetic variation in a complex forest ecosystem, the bromeliad-invertebrate system is ideal. Armbruster et al. (2002) determined that 62% of the variation in invertebrate communities between different bromeliads (involving three morphologically similar species) was explained by plant traits, such as the volume of the plant, the number of leaves, the detritus content and the volume of water held in the plant. Further work has shown that bromeliad-invertebrate communities are highly characterized by the forest type the plants reside within (Richardson 1999), thus it is likely that indirect environmental effects are also important for bromeliad-arthropod community structure. Using artificial bromeliad microcosms with one, three or six 'leaves', Srivastara (2006) showed that detrital processing (linked to nutrient cycling) is affected by the structure and habitat complexity of the plant directly, through detritivore action, and indirectly, via the predator community. These studies illustrate that both environmental factors and plant structure strongly influence, and probably interact, to determine bromeliad-arthropod community structure. A component of variance in such communities, including variation in the individual structure plants, factors such as flowering time, leaf chemicals and water retention, is expected to hold some genetic basis. Therefore, it

might be expected that genetic variation within these plants will partly explain variation between the plants of the community of invertebrates, alongside a number of environmental (microclimatic) factors.

In this study, we investigated the influence of bromeliad genetic variation on the associated invertebrate community residing within the plants. By controlling for geographic location and other variables, such as plant size, we directly examined the proportion of variation within the invertebrate community that is due to genetic variation in the plants. We predicted that phenotypic (e.g. plant size), genetic, and environmental (e.g. plant height) variation would, in combination, significantly influence the bromeliad-arthropod community structure.

Materials and Methods

Study site and system

The study site was located in a $4km^2$ area in central Belize near the Las Cuevas Research Station (LCRS) within the Chiquibul forest, a mid-elevation (~550m) broadleaf deciduous forest that is relatively undisturbed. We sampled the total invertebrate community within *Aechmea bracteata* (Swartz) plants, a large epiphytic tank-forming bromeliad. Sample collection occurred over three years (2008-2010), for a period of two weeks per year during the month of July.

Bromeliad collection

Fifty-nine bromeliad plants were collected from trees located along permanent trails situated around LCRS. The collections were therefore not random but sites were chosen so as not to obtain a high degree of clustering across the sampling range. Tree

host species was not identified in this study, and bromeliads were collected from the ground up to a height of 17m. Bromeliad plants that were inaccessible from the ground were collected by rope-climbing. Each plant was detached from the substrate, the leaves were tied together with rope and the whole plant was placed in a robust polyethylene bag, which was secured at the top with rope. If a plant was collected using rope-climbing, the plant was carefully lowered to the ground and then all plants were transferred to the field station in an upright position. The height on the tree at which the bromeliad was growing (to the base of the plant) was measured, as was the length of the longest leaf as a proxy for bromeliad size. Geographic location for each bromeliad plant was recorded using a GPS unit. The GPS coordinates were converted to digital coordinates, then converted to radians before using the spherical law of cosine to calculate the distance between each pair of bromeliads:

$$Distance = a\cos[\sin(lat_1).\sin(lat_2) + \cos(lat_1).\cos(lat_2).\cos(long_2 - long_1)].6371$$

(1)

Invertebrate sampling

Each bromeliad was dismantled completely within a plastic box to collect invertebrates. All invertebrates above 1mm in body size were collected and preserved in 70% isopropanol for identification. Vertebrates inadvertently collected were released at the site of collection. In the UK, the invertebrate samples were identified to order (and family where possible), with additional data collected on the growth stage (adult or juvenile). Distinct morphospecies were identified and these were used in the further analyses of the data. To minimize the variation across the three years of collection, we used only those morphospecies that were found to be present in bromeliads from at least two of the three years. On comparison of results, this did not

qualitatively alter our findings, but reduced the differentiation between the different years of collection.

Genetic analysis

A leaf sample was collected from each bromeliad and preserved in silica gel (Chase and Hills 1991). DNA was extracted using a modified CTAB method (Doyle and Doyle 1987) and eluted DNA was cleaned using spin columns (Sigma-Alrich). Amplified fragment length polymorphisms (AFLP; Vos et al. 1995) were used to calculate Nei's genetic distance using TFPGA (*Tools For Population Genetics Analysis*, M. Miller) between each pair of bromeliads in the study population. For the AFLP genotyping we used *EcoR1* and *Mse1* restriction enzymes with two selective primer combinations (ACA-CAT and AAG-CAG) producing 115 scorable loci. AFLP profiles were visualized using a Beckman-Coulter CEQ™ 8000 Genetic Analysis System. A 5% error rate was used, which removes loci that produce bands in less than 5% of the samples and assumes a locus to be monomorphic if more than 95% of the population produced a band; this ensures the differentiation between plants was not overestimated.

Data analysis

Similarity indices were used to calculate the invertebrate community structure similarity/distance between each pair of bromeliads in CAP4 (*Community Analysis Package 4,* Version 4.1.3, Pisces Conservation Ltd., 2007). Jaccard's (JA) similarity index was used to measure invertebrate community similarity between each pair of bromeliads, using presence/absence data (see Zuur et al. 2007; Zytynska et al. 2011):

$$JA = \frac{a}{a+b+c}$$

(2)

where a is the number of species present in both bromeliads, b and c are the number of species present in only one bromeliad. The JA index gives a range of results from 0 to 1, allowing '1-JA' to be used as a dissimilarity index rather than a similarity index, which is beneficial when comparing the results with plant genetic distance. For abundance data, the Bray-Curtis dissimilarity measure was used (see Bray and Curtis 1957; Bangert et al. 2008):

$$BC = \frac{\sum_{i=1}^{i=n} |s_{i1} - s_{i2}|}{\sum_{i=1}^{i=n} (s_{i1} + s_{i2})}$$

(3)

where, s_{i1} and s_{i2} are the abundances of species i in samples 1 and 2 respectively.

We used Mantel and partial Mantel tests to statistically analyse the data in PASSaGE (*Pattern Analysis, Spatial Statistics and Geographic Exegesis*; Rosenberg and Anderson 2011). Mantel tests use pairwise matrices to test the correlative relationship between two variables, and partial Mantel tests allow for one or more other matrices to be kept constant in the analysis. Here, we analysed the association of genetic distance and invertebrate community difference among the bromeliads whilst controlling for geographic distance, bromeliad size and plant height from the ground. Hereafter, the presence/absence (PA) data will be calculated as '1-JA' to compute community distance (dissimilarity) because JA is a measure of similarity ranging from 0 to 1. Abundance data (AB) directly measures community dissimilarity and thus will be presented as the Bray-Curtis index (BC). In addition, we split the invertebrate community by growth stage and separately analysed the adult and juvenile communities. A significant positive correlation shows there is an association between the matrices compared.

Results

In total, 59 bromeliads were collected containing 1597 individuals from 52 morphospecies.

The majority of morphospecies were classified in the phylum Arthropoda (47 morphospecies), and the remaining in Annelida (2), Mollusca (2) and Platyhelminthes (1) (Table 1). Within Arthropoda, 29 morphospecies were classified in the class Insecta and these accounted for 61.6% of all individuals collected. From these, the most abundant taxa were in the orders Coleoptera and Diptera, which accounted for 30.3% and 14.9% of all the individuals sampled.

Table 1. Summary of the taxa collected, number of morphospecies within each taxa and the developmental stages observed.

Phylum	Class	Order	Morphospecies	Stage
Annelida	Oligochaetes		2	Adult
Arthropoda	Arachnida	Araneae	5	Adult
		Opiliones	2	Adult
		Pseudoscorpiones	1	Adult
		Scorpiones	1	Adult
	Chilopoda	Lithobiomorpha	1	Adult
		Scolopendromorpha	2	Adult
	Crustacea	Isopoda	1	Adult
	Diplopoda	Golida	1	Adult
		Polydesmida	1	Adult
	Insecta	Blattodea	2	Juvenile
		Coleoptera	15	Adult/Juve-nile
		Diptera	6	Juvenile
		Hemiptera	2	Adult
		Hymenoptera (Formicidae)	3	Adult
		Lepidoptera	1	Juvenile
		Unknown	2	Juvenile
Mollusca	Gastropoda		2	Adult
Platyhelminthes			1	Adult

Morphospecies = number of morphospecies within each taxa. Juvenile includes both nymphal and larval stages.

*Effect of geographic location, size and height on tree*

The mean size of bromeliad plant (longest leaf) was 1.83m (range: 0.50-2.84m) and the mean height from the ground was 9.08m (range 0-16.86m). Mean morphospecies richness within bromeliads was 9 (range: 3-21). Larger plants were found to contain a greater number of morphospecies (Regression: $F_{1,56}$=4.15, *P*=0.047); however, bromeliad size did not influence the structure of the invertebrate community for either presence/absence of community members (PA Mantel test: r=-0.004, *P*=0.44) or abundance of community members (AB Mantel test: r=0.029, *P*=0.25). In addition, the height from the ground of bromeliad plants did not influence species richness (Regression: $F_{1,\ 57}$=0.34, *P*=0.56), or the structure of the community (Mantel test: PA r=-0.026, *P*=0.25; AB r=-0.03, *P*=0.19). While invertebrate community structure within bromeliads was significantly associated with geographic location (Mantel test: PA r=0.184, *P*=0.003; AB r=0.162, *P*=0.001, Figure 1), there was also a significant association between genetic distance and geographical distance of bromeliads indicating fine-scale genetic population structure (Mantel test: r=0.166, *P*=0.003). Thus, geographic location was included as a controlled factor in further analyses.
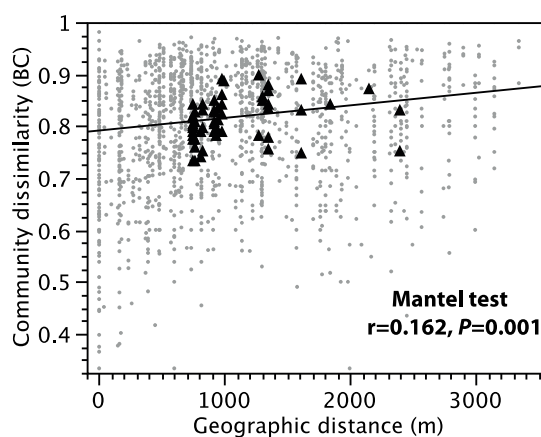
Figure 1. The association between geographic distance and the invertebrate community dissimilarity (abundance) between each pair of bromeliads. BC = Bray Curtis measure. Each grey dot represents one pairwise data point, and the black triangles are the mean values for each bromeliad. Note that a Mantel test is used to statistically analyse the data, to control for non-independence of the pairwise points.

Effect of bromeliad genetics

We found a significant association between bromeliad genetic distance and invertebrate community similarity (Partial Mantel test: PA $r=0.102$, $P=0.025$; AB $r=0.107$, $P=0.01$; Figure 2) and thus bromeliad genotype significantly influences the structure of individual invertebrate community associations.
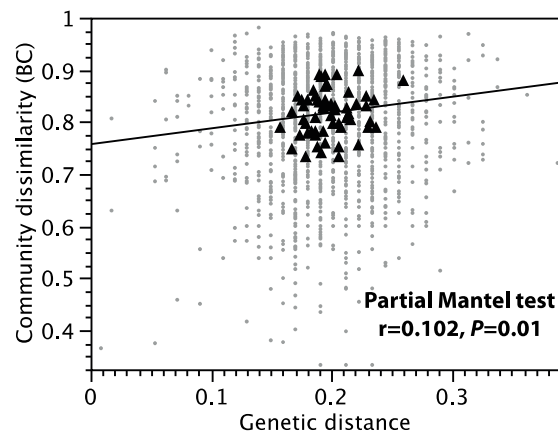
Figure 2. The association between bromeliad genetic distance and community dissimilarity (abundance.) BC = Bray Curtis measure. Each grey dot represents one pairwise data point, and the black triangles are the mean values for each bromeliad. Note that a Partial Mantel test (controlling for geographic distance) was used to statistically analyse the data, to control for non-independence of the pairwise points.

Differences between the adult and the juvenile communities

We identified 20 morphospecies for which the individuals were classified as juveniles (nymphal or larval stages), and 32 morphospecies contained adult individuals. Juvenile morphospecies resided within the orders Blattodea, Coleoptera, Diptera and Lepidoptera within the Insecta (Table 1). We found that bromeliad size influenced the community structure of juveniles (Mantel test: PA r=0.087, P=0.043; AB r=0.105, P=0.021), and therefore include this (as well as geographic location) in further analyses. We found no influence of bromeliad size on the adult community.

We found a significant association between bromeliad genetic distance and the juvenile invertebrate community dissimilarity for the presence/absence data (Partial Mantel test controlling for geographic location and bromeliad size: r=0.108, P=0.009); however, there was a much weaker and only marginally significant association between bromeliad genetic distance and community dissimilarity based on abundance data (Partial Mantel test controlling for geographic location and bromeliad size: r=0.066, P=0.06). We did not find evidence of any association between the bromeliad genetic distance and the adult invertebrate community dissimilarity (Partial Mantel test controlling for geographic location: PA r=0.023, P=0.29; AB r=-0.014, P=0.34). This suggests the influence of bromeliad genetics is largely on the juvenile rather than the adult community (Figure 3).
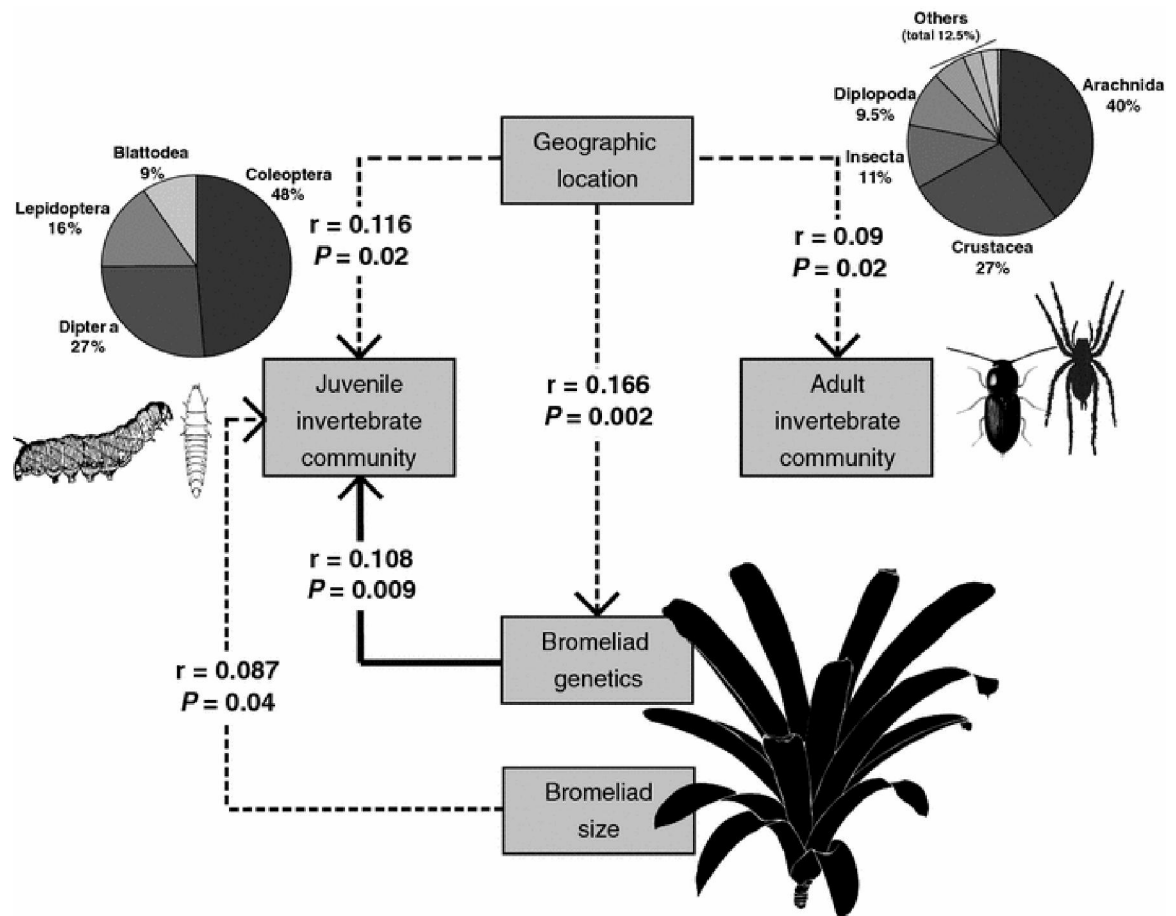
Figure 3. The links and influences of the different factors in the bromeliad-invertebrate system. Bold arrow shows the main effect of interest, that of the bromeliad genetics on the juvenile invertebrate community (presented statistical values for this comparison are controlled for geographic location and bromeliad size). Pie charts show the relative abundances of the different taxa within the invertebrate communities. The adult community gives the different insect classes plus 'Others', which includes Chilopoda, 6%; Oligochaetes, 3%, Gastropoda, 3% and Platyhelminthes 0.5%. The juvenile community shows the different orders within the class Insecta, as all juveniles were classified within Insecta.

Discussion

This paper presents data to show that there is a significant association between bromeliad genetics and the associated invertebrate community structure. We found that genetically similar bromeliads host similar communities of invertebrates. We only found this effect for the juvenile invertebrate community, as we found a significant association between the genetic distance between bromeliads and juvenile community distance but found no association between the bromeliad genetics and the adult community. There was little overlap between the juvenile and adult communities, with the juvenile community all classified in Insecta (Diptera, Coleoptera, Lepidoptera and Blattodea) and the adult community exhibiting higher taxonomic diversity (see Table 1). The only taxonomic grouping to include both adults and juveniles were the Coleoptera, which was also the most abundant group (making up 30% of all individuals sampled).

As predicted, geographic distance between the bromeliad plants influenced invertebrate community structure in our study, with more similar communities found in plants that were located closer together. Bromeliad size has previously been found to influence associated invertebrate communities (Armbruster et al. 2002; Araújo et al. 2007) and we also found an effect of bromeliad size but only on juvenile communities but not the adult communities. Araújo et al. (2007) analysed a faunal community that was dominated by Dipteran larvae and found that richness and abundance was highly correlated with the volume of water held by plants (water held was correlated with plant size). This combined with our findings suggest the bromeliad plants have a stronger influence on the aquatic larvae (juvenile community) than on the leaf-associated, or adult, community. The mechanism for such an effect is unclear, although we have also shown evidence for an association of bromeliad genetic variation on the juvenile invertebrate community structure. There are a number of

possible mechanisms, including active female choice for oviposition sites (bromeliad genotypes) or differential survival of offspring within different bromeliad genotypes. Adult female choice of particular bromeliad genotypes will result in finding only certain species within certain bromeliad genotypes and could lead to the patterns detected within this paper. It is known that many invertebrates will only oviposit on particular groups of host plants, due to the suitability for the offspring, but differentiation between genotypes within a species has also been demonstrated for oviposition preference (Anderson et al. 1999; Wennström et al. 2010; Glinwood et al. 2011; Zytynska and Preziosi 2011). Potential genetically-based mechanisms that could influence active female oviposition choice for bromeliad genotypes could include water quality (Laessle 1961), bromeliad structure (Srivastara 2006), colour perception (Yanoviak 2001) or other chemical cues (Bentley and Day 1989). Additionally, these factors could also influence the survival of the offspring leading to a passive mechanism of host-association. For example, if the different plant genotypes produce variation in water quality (nutrient content, pH) then this could have differential effects on the development and survival of the larval stages of the various species (Diesel and Schuh 1993).

The amount of variation in the juvenile invertebrate community across bromeliads explained by the bromeliad genetic variation is small (about 1%). However, we suggest that this is biologically significant due to the complexity of the tropical ecosystem and the consequently low power to detect the effects of within-species genetic variation. Also, we were unable to consider the identity of the tree host from which the bromeliad was collected, and from previous work (Zytynska and Preziosi 2011) we might predict an association between bromeliad and host tree that could influence the invertebrate community (i.e. indirect ecological effect or indirect genetic

effect). Indeed, we expect that there are also other, non-genetic factors that influence the community structure including geographic location (controlled within this study) and abiotic factors caused by microclimatic differences. Random dispersal of invertebrates due to debris collection resulting from plant structure is also a source of variation (Richardson 1999). By analyzing bromeliad communities over several years, we have tried to minimize these sources of variation.

The molecular markers used (AFLPs) are often considered as neutral markers, such that there is an assumption that they will not be linked to any particular locus that is under selection. This means that there is no expectation to find any link between an AFLP genotype and an environmental trait. We found an association between the bromeliad genetics and the invertebrate community suggesting that one or more of the AFLP loci used in this study are linked to a gene(s) involved in the mechanism that influences the invertebrate community structure of a plant.

Previous work has shown that within-species genetic variation in a tree species can influence the associated epiphytic plant and invertebrate communities in a tropical forest ecosystem (Zytynska et al. 2011). Thus, previous work and our findings here, suggest that community genetic effects may be more general than previously thought. There is now substantial evidence that genetic variation influences community species structure in a number of different ecosystems (see reviews in Haloin and Strauss 2008; Whitham et al. 2006; Rowntree et al. 2011). Exploring the relative influence of within-species genetic variation on the surrounding community and ecosystem as compared to other factors (e.g. intensities of species interactions, disturbance, trophic complexity and the abiotic environment) has been cited as the next step in the area of community genetics (Hersch-Green et al. 2011). It is research in this area that will

determine whether community genetic effects can impact the functioning of the whole-ecosystem or if they are just inherent 'noise' within a system.

A wider implication of community genetics effects on community structure is how ecosystem processes may depend on genetic covariation amongst community members and whether this is important to ecosystem conservation (Schweitzer et al. 2004). Whole-ecosystem conservation efforts can enhance ecosystem services through the long-term maintenance of ecosystem function and stability. Another fundamental question that has not yet been addressed is whether the pattern of biodiversity, especially in spectacularly species rich habitats in the tropics, depends directly or indirectly on community genetic connections.

Here, we show that bromeliad genetic variation influences the invertebrate communities living within individual plants. This suggests that should bromeliad genetic variation be reduced, then the overall species diversity of invertebrates would decline. Loss of species richness in a system can be deterministic, and when this disrupts functional diversity and community structure, ecosystem function can itself be altered (Larson et al. 2005). The loss of genetic variation in a plant population can have cascading effects on the associated communities and lead to reduced species richness and potentially changes at the ecosystem level. The concept of the minimum viable population (MVP), the minimum population census size required to minimize the risk of extinction over some time period, has long been explicitly linked to the quantity of genetic variation contained by a population (Shaffer 1981). More recently this concept been adapted this to include the interacting community; the minimum viable interacting population (MVIP) is the size of a population required to maintain the genetic diversity at a level that is sufficient to maintain the populations of the

interacting species (Whitham et al. 2003). This concept was first applied to dominant species, where the conservation of genetic diversity within a common and non-endangered species would have cascading beneficial effects on the biodiversity of associated communities (Bangert et al. 2005). Here we studied a tropical forest bromeliad-invertebrate system, but it might be expected that bromeliad genetic variation would also influence higher trophic organisms (e.g., salamanders and frogs) that are adapted to living and reproducing in bromeliad plants (Wake 1987; Krügel and Righter 1995). Furthermore, the unique habitat created by a bromeliad can also have an impact on the presence of arthropod-borne disease, the control of which can be of great socio-economic importance, for example malaria vectoring mosquitoes are known to breed within bromeliad plants (Lounibos 2002; Frank and Lounibos 2009).

In conclusion, we found that more genetically similar bromeliad plants are host to more similar communities of juvenile-stage invertebrates in a complex tropical forest ecosystem. This work complements previous work showing that the influence of within-species genetic variation on associated ecological communities could be more important than previously considered (Zytynska et al. 2011). The role of within-species genetic variation as a supporting ecosystem service is beginning to be appreciated to contribute both to the adaptive potential of individual species and for the maintenance of biodiversity within the interacting communities. The relative importance of within-species genetic variation compared to other factors (Hersch-Green et al. 2011), and the link to ecosystem processes are still not fully understood. It is here, we suggest, that future efforts should be focused.

Acknowledgements

References

Anderson S.S., K.D. McCrea, W.G. Abrahamson, and L.M. Hartzel. 1989. Host genotype choice by the ball gallmaker *Eurosta solidaginis* (Diptera: Tephritidae). *Ecology 70*: 1048-1054.

Antonovics J. 1992. Toward community genetics. Pages 426-429 *in* R.S. Fritz and Simms E.L. Plant resistance to herbivores and pathogens: ecology, evolution and genetics. University of Chicago Press, Chicago, Illinois, USA

Araújo V.A., S.K. Melo, A.P. Araújo, M.L. Gomes, and M.A. Carneiro. 2007. Relationship between invertebrate fauna and bromeliad size. *Braz J Biol 67*: 611-617.

Armbruster P., R.A. Hutchinson, and P. Cotgreave. 2002. Factors influencing community structure in a South American tank bromeliad fauna. *Oikos 96*: 225-234.

Bailey J.K. 2011. From genes to ecosystems: a genetic basis to ecosystem services. *Popul Ecol 53*: 47-52.

Bailey J.K., J.A. Schweitzer, B.J. Rehill, R.L. Lindroth, G.D. Martinsen, and T.G. Whitham. 2004. Beavers as molecular geneticists: a genetic basis to the foraging of an ecosystem engineer. *Ecology 85*: 603-608.

Bangert R.K., E.V. Lonsdorf, G.M. Wimp, S.M. Shuster, D. Fischer, J.A. Schweitzer, G.J. Allan, et al. 2008. Genetic structure of a foundation species: scaling community phenotypes from the individual to the region. *Heredity 100:* 121-131.

Bangert R.K., R.J. Turek, G.D. Martinsen, G.M. Wimp, J.K. Bailey, and T.G. Whitham. 2005. Benefits of Conservation of Plant Genetic Diversity to Arthropod Diversity. *Conservation Biology 19*: 379.

Bentley M.D., and J.F. Day. 1989. Chemical ecology and behavioral aspects of mosquito oviposition. *Ann Rev Entomol 34*: 401-421.

Bray J.R., and J.T. Curtis. 1957. An ordination of the upland forest communities of Southern Wisconsin. *Ecological Monographs 27*: 325-349.

Carius H.J., T.J. Little, and D. Ebert. 2001. Genetic variation in a host-parasite association: potential for coevolution and frequency-dependent selection. *Evolution 55*: 1136-1145.

Chase M.W., and H.H. Hills. 1991. Silica gel: an ideal material for field preservation of leaf samples for DNA studies. *Taxonomy 40*: 215-220.

Diesel R., and M. Schuh. 1993. Maternal care in the bromeliad crab *Metopaulias depressus* (Decapoda): maintaining oxygen, pH and calcium levels optimal for the larvae. *Behav Ecol Sociobiol 32*: 11-15.

Dobson A., and M. Crawley. 1994. Pathogens and the structure of plant communities. *TREE 9*: 393-398.

Doyle J.J., and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities

of fresh leaf tissue. *Phytochem Bull 19*: 11-15.

Dungey H.S., B.M. Potts, T.G. Whitham, and H.F. Li. 2000. Plant genetics affects arthropod community richness and composition: evidence from a synthetic eucalypt hybrid population. *Evolution 54*: 1938-1946.

Frank J.H., and L.P. Lounibos. 2009. Insects and allies associated with bromeliads: a review. *Terr Arthropod Rev 1*: 125-153.

Fritz R.S., and P.W. Price. 1988. Genetic variation among plants and insect community structure: willows and sawflies. *Ecology 69*: 845-856.

Gaylord E.S., R.W. Preszler, and W.J. Boecklen. 1996. Interactions between host plants, endophytic fungi, and a phytophagous insect in an oak (*Quercus grisea* x *Q. gambelii*) hybrid zone. *Oecologia 105*: 336-342.

Glinwood R., E. Ahmed, E. Qvarfordt, and V. Ninkovic. 2011. Olfactory learning of plant genotypes by a polyphagous insect predator. *Oecologia 166*: 637-647.

Haloin J.R., and S.Y. Strauss. 2008. Interplay between ecological communities and evolution: review of feedbacks from microevolutionary to macroevolutionary scales. *Ann N Y Acad Sci 1133*: 87-125.

Hersch-Green E.I., N.E. Turley, and M.T. Johnson. 2011. Community genetics: what have we accomplished and where should we be going? *Phil Trans R Soc B 366*: 1453-1460.

Hooper D.U., F.S. Chapin III, J.J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J.H. Lawton, et al. 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecological Monographs 75*: 3-35.

Iason G.R., J.J. Lennon, R.J. Pakeman, V. Thoss, J.K. Beaton, D.A. Sim, and D.A. Elston. 2005. Does chemical composition of individual Scots pine trees determine the biodiversity of their associated ground vegetation? *Ecology*

Letters 8: 364-369.

Ives A.R., B.J. Cardinale, and W.E. Snyder. 2005. A synthesis of subdisciplines: predator–prey interactions, and biodiversity and ecosystem functioning. *Ecology Letters 8*: 102-116.

Johnson M.T.J., M.J. Lajeunesse, and A.A. Agrawal. 2006. Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. *Ecology Letters 9*: 24-34.

Krugel P., and S. Richter. 1995. *Syncope antenori* - A Bromeliad breeding frog with free-swimming, nonfeeding tadpoles (Anura, Microhylidae). Copeia 1995: 955-963.

Laessle A.M. 1961. A micro-limnological study of Jamaican bromeliads. *Ecology 42*: 499-517.

Larsen T.H., N.M. Williams, and C. Kremen. 2005. Extinction order and altered community structure rapidly disrupt ecosystem functioning. *Ecology Letters 8*: 538-547.

Lounibos L.P. 2002. Invasions by insect vectors of human disease. *Annu Rev Entomol 47*: 233-266.

Luck G.W., G.C. Daily, and P.R. Ehrlich. 2003. Population diversity and ecosystem services. *TREE 18*: 331-336.

Muller M.S., S.R. McWilliams, D. Podlesak, J.R. Donaldson, H.M. Bothwell, and R.L. Lindroth. 2006. Tri-trophic effects of plant defenses: chickadees consume caterpillars based on host leaf chemistry. *Oikos 114*: 507-517.

Nadkarni N. 1994. Diversity of Species and Interactions in the Upper Tree Canopy of Forest Ecosystems. *Amer Zool 34*: 70-78.

Reusch T.B., A. Ehlers, A. Hämmerli, and B. Worm. 2005. Ecosystem recovery after

climatic extremes enhanced by genotypic diversity. *Proceedings of the National Academy of Sciences of the United States of America 102*: 2826-2831.

Richardson B.A. 1999. The Bromeliad Microcosm and the Assessment of Faunal Diversity in a Neotropical Forest. *Biotropica 31*: 321-336.

Rosenberg M.S., and C.D. Anderson. 2011. PASSaGE: Pattern Analysis, Spatial Statistics and Geographic Exegesis. Version 2. *Methods in Ecology and Evolution 2*: 229-232.

Rowntree J.K., D.M. Shuker, and R.F. Preziosi. 2011. Forward from the crossroads of ecology and evolution. *Phil Trans R Soc B 366*: 1322-1328.

Schweitzer J.A., B.J. Rehill, G.D. Martinsen, R.L. Lindroth, P. Keim, and T.G. Whitham. 2004. Genetically based trait in a dominant tree affects ecosystem processes. *Ecology Letters 7*: 127-134.

Service P. 1984. Genotypic interactions in an aphid-host plant relationship: *Uroleucon rudbeckiae* and *Rudbeckia laciniata*. *Oecologia 61*: 271-276.

Shaffer M.L. 1981. Minimum population sizes for species conservation. *BioScience 31*: 131-134.

Srivastava D.S. 2006. Habitat structure, trophic structure and ecosystem function: interactive effects in a bromeliad-insect community. *Oecologia 149*: 493-504.

Stuntz S., C. Zielger, U. Simon, and G. Zotz. 2002. Diversity and structure of the arthropod fauna within three canopy epiphyte species in central Panama. *Journal of Tropical Ecology 18*: 161-176.

Vellend M., and M.A. Geber. 2005. Connections between species diversity and genetic diversity. *Ecology Letters 8*: 767-781.

Vos P., R. Hogers, M. Bleeker, M. Reijans, T. Van De Lee, M. Hornes, A. Frijters, et al. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res 23*:

4407-4414.

Wake D.B. 1987. Adaptive radiation of salamanders in Middle American cloud forests. *Annals of the Missouri Botanical Garden 74*: 242-264.

Wennström A., L.N. Hjulström, J. Hjältén, and R. Julkunen-Tiitto. 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.

Whitham T.G., J.K. Bailey, J.A. Schweitzer, S.M. Shuster, R.K. Bangert, C.J. LeRoy, E.V. Lonsdorf, et al. 2006. A framework for community and ecosystem genetics: from genes to ecosystems. *Nat Rev Genet 7*: 510-523.

Whitham T.G., W.P. Young, G.D. Martinsen, C.A. Gehring, J.A. Schweitzer, S.M. Shuster, G.M. Wimp, et al. 2003. Community and ecosystem genetics: a consequence of the extended phenotype. *Ecology 84*: 559-573.

Yanoviak S.P. 2001. Container color and location affect macroinvertebrate community structure in artificial treeholes in Panama. *The Florida Entomologist 84*: 265-271.

Zuur A.F., Ieno E.N., and Smith G.M. 2007. Analysing ecological data, Springer New York.

Zytynska S.E., and R.F. Preziosi. 2011. Genetic interactions influence host preference and performance in a plant-insect system. *Evolutionary Ecology* (available online 29/05/2011)

Zytynska S.E., M.F. Fay, D. Penny, and R.F. Preziosi. 2011. Genetic variation in a tropical tree species influences the associated epiphytic plant and invertebrate communities in a complex forest ecosystem. *Phil Trans R Soc B 366*: 1329-1336.

Zytynska S.E., S. Fleming, C. Tetard-Jones, M.A. Kertesz, and R.F. Preziosi. 2010.

Community genetic interactions mediate indirect ecological effects between a parasitoid wasp and rhizobacteria. *Ecology 91*: 1563-1568.

General Discussion

"It takes all the running you can do to keep at the same place" says the red queen [(Lewis Carroll's masterpiece 'Through the looking glass'; adapted by Van Valen (1973)]. Species, while striving to maximise their fitness, get involved in a plethora of interactions on the intra-specific as well as the inter-specific levels. The interaction can be direct or indirect, cooperative or antagonistic, but should be contingent on the variability in the environment, availability of resources, and the presence or absence of perturbation (e.g. Wiens 1977, 1991, Masters et al. 1993, Wootton et al. 1994, Moore et al. 1997, Masters et al. 2001, Wootton et al., 2002, Agrawal 2003, Wade 2003, Kaplan et al. 2009). Furthermore, a significant proportion of the environment of every species can be considered as defined by the expressed genomes of other interactors which are ecologically connected with such species (Rowntree et al. 2011).

First articulated in 1992 by Antonovics, community genetics is a thriving empirical as well as theoretical sphere of biology. Community genetics has provided an innovative interpretation on the ramifications of intra-specific genetic variation of focal species on the reproductive success, distribution, and survivorship of associated communities (Whitham et al. 2003, 2006, 2008). Furthermore, a good body of evidence has been provided through community genetics to support the concept of the 'extended phenotype' where the consequences of 'within-species genetic variation' of a focal species (i.e. keystone species) can extend to affect other interacting species beyond the level of the individual and population of that focal species (reviewed by Agrawal 2003, Johnson and Stinchcombe 2007, Bailey 2011, Rowntree et al. 2011; see also Strauss et al. 2005). It has been well demonstrated that the influence of intra-specific genetic variation of keystone species can even extend across trophic levels to

impact higher levels and hence influence an entire community or ecosystem (Whitham et al. 2003, 2006).

The amalgamation of ecology, genetics, and evolution into the framework of community genetics in both natural ecosystems and man-maintained agro-ecosystems has become an irresistible demand for evolutionary ecologists and agronomists. This is particularly applicable and useful for understanding the community dynamics of ecological networks or communities subject to strong selective pressures (e.g. due to anthropogenic impact; Neuhauser et al. 2003). That amalgamation is useful for deciphering the complexity of species interactions as well as to further our current understanding of the impact of genetic variation of focal taxa on species interactions and *vice versa* (Hersch-Green et al. 2011, Rowntree et al. 2011). This is in addition to exploring the plausible effects of intra-specific genetic variation on ecosystem properties and services (Bailey and Whitham 2006a, Rowntree et al. 2011). However, further research on economic crops and their herbivorous pests is needed. This is also the case for contrasting community genetic effects with other ecological processes such as competition in the presence and absence of environmental variation and perturbation (Hersch-Green et al. 2011, Rowntree et al. 2011). For natural systems, the impact of intra- and inter-specific variation on species associations in highly complex systems such as tropical forests requires a better understanding of the plausible linkage between genetic, species and geographic levels of diversity (e.g. Zytynska et al. 2012).

An opinion on survival and phenotypic plasticity

Biological fitness basically entails the reproductive success and survival of an organism and its offspring. Being fit correlates with being able to achieve adequate reproductive success and successful transition from early developmental stages

through to maturity. However, fitness presents a probabilistic concept because it ripples between viability, fertility and fecundity (Jervis 2005, Rosenberg and Bouchard 2010; see thesis appendix for a 'potpourri' of supporting definitions). As such, plastic phenotypic traits should be favoured in order to encompass individual versus group needs (Hamilton 1964, Dawkins 1982).

Phenotypic plasticity, by definition, describes the capacity of a genotype to express a variety of phenotypes induced by environmental stimuli (Whitman and Agrawal 2009). This capacity can be diverse, including adaptive responses in term of ontogeny, physiology, morphology and behaviour. Furthermore, plasticity represents a spectrum spanning continuous variation (standard plasticity) to discrete phenotypes [polyphenism (i.e. extreme plasticity) such as alate production]; (Whitman and Agrawal 2009). Such plastic response is observable in both the plant and animal kingdoms; making the patterns of relationships between these two major groups exceedingly complex (Sultan 2003, Borges 2005).

Aphids have highly versatile genomes fueling extensive morphological, physiological and behavioural polyphenism (Moran 1991, Zera and Denno 1997, Brisson and Stern 2006, Agrawala 2007). Thus, experience or preconditioning of an adult can be transferred across generations to produce a range of highly plastic, and thus adaptable, individuals (Dixon 1998, Dombrovsky et al. 2009). For example, in response to declining host quality, aphid females are stimulated to produce plastic offspring that can increase their chances of survival e.g. thrifty individuals that are smaller in size and diligent in consumption of the available resource besides dispersal morphs (Dixon 1977, 1987, 1998, Dombrovsky et al. 2009). A surprising observation is that experience (behaviour) in this system can also be inherited as a trait to maintain the ecological functionality of the specialised individual morphs within the clone

(Dixon 1977, 1987, 1998, Dombrovsky et al. 2009; see also Odling-Smee 2007, Odling-Smee et al. 2003).

To ensure longevity and continuity, an organism governed by trade-offs, is expected to manage between evading enemies, maintaining reproduction and sustaining the survivorship of juveniles (e.g. Dixon 1977, 1987, 1998; see also Eshel et al. 1998). It is expected for genetically identical individuals of a specific lineage of aphids to face a dilemma between intra- and inter-specific types of competition, where changes in the negative interactions within the clone can become advantageous for the population of a rival one (Smith et al. 2008). In this regard, the mathematical work developed by Bürger (2002) may illuminate the premise that a putative interplay can occur between additive genetic variation, competition and selection. Bürger (2002) concluded that: "For equal effects, the equilibrium fitnesses of the genotypic values exhibit disruptive selection if and only if competition is strong enough to maintain a two-locus polymorphism. Interestingly, with unequal effects, disruptive selection may be observed even if competition is not strong enough to change the equilibrium maintained by stabilising selection alone".

More on ecological interactions and ongoing change

In their efforts to replicate their genetic constitution species will develop a changeable set of tactics and strategies while interacting with the environment they populate. Consequently, species interactions, throughout the ecological networks they comprise in both wild and arable systems, continuously induce changes in their environment (biotic and abiotic) and, in turn, are changed by their environment. Such actions will be responded to by the species sharing that selective environment and so forth (Odling-Smee et al. 2003, Laland 2004, Juarrero 2010; see also Levin 1998). The environment-modifying species are embedded within the environment they strive

to modify (e.g. Juarrero 2010). Furthermore, the above mentioned cyclical relationship encompasses positive and negative feedback loops between the selective environment and the environment-modifying species a.k.a. 'agents of change' (Laland 2004, Juarrero 2010). This can result in an endless co-evolution (e.g. Dawkins and Krebs 1979; but see Maynard Smith 1974).

The constant process of change and response to change correlates with the entropy of the system (Mejer and Jørgensen 1979, King 2003, Bonchev et al. 2005). This process is also affected by the plasticity of the interactors and certainly the genetic basis for their interactions (Antonovics 1992, Whitham et al. 2003, 2006, Rowntree et al. 2011). This is also the case for systems where inter-players experience strong or frequent interactions and escalating dependence on each other's, or on the genetics of a keystone species (e.g. host-parasite, plant-pest, epiphytes and their associated invertebrate communities in the canopy; Moore 2002, Neuhauser et al. 2003, Whitham et al. 2003, Poulin 2006, Powell et al. 2006, Whitham et al. 2006). Thus, a complex system could develop through which genetic variation within ecologically important species will have a great influence on the productivity and stability of the entire ecosystem (Whitham et al. 2003, 2006, Bailey 2011, Zytynska et al 2012). In such systems simple relationships occur between species but may develop in complexity, dependent on how adaptive or evolvable the interactions between the species may be (Watts and Strogatz 1998, Yong and Miikkulainen 2001, King 2003, Montoya et al. 2006, Yong and Miikkulainen 2007, Araújo et al. 2008, Juarrero 2010, Rawal and Rajagopalan 2010). In such a context, each party develops a response to one or more other members of the community or ecological network. This should depend on how complex and reticulate the ecological network is among the interacting species (Montoya et al. 2006). Co-evolutionary interactions can occur between community members, for example, between competitors or between hosts and

parasites (Dawkins and Krebs 1979). However, one of the most pertinent requirements for co-evolution to occur is genetic variation for phenotypic traits (e.g. Henter 1995, Henter and Via 1995, Ferrari et al. 2006). This entails that a change in a trait will be contingent on the presence or absence of, or degree in influence from, other interacting species (Mauricio and Rausher 1997, Russo et al. 2005, Strauss et al. 2005; but see Weiher and Keddy 1999, Ackerly 2003). It can also be argued that a portion of the notable adaptive responses shown by aphids to the varying conditions they experience can be attributed to the mutualistic symbionts they carry (e.g. Chen et al. 2000; see also McLean et al. 2011). However, it is not clear how the feedback from symbionts can alter the evolution of the aphid clone (Alex et al. 2003).

Under these context-dependent conditions, community genetics is progressively of heuristic importance for ecologists because this new frontier in evolutionary biology provides an innovative toolkit of approaches in order to decipher the complexity of species interactions under the influence of intra-specific genetic variation (Agrawal 2003, Neuhauser et al 2003, Wade 2003, Whitham et al. 2003, 2006, Johnson and Stinchcombe 2007, Rowntree et al. 2011).
(See also the general thesis introduction for discussion of the prime perspectives of community genetics).

The foregoing arguments demonstrate that fitness can vary depending on the disparity of opportunities encountered by an organism or population (Watts and Strogatz 1998, Araújo et al. 2008). It can be argued that parthenogenetic aphids operate on the 'selfish-gene's agenda' in that they display all the creative combinations possible to ensure proliferation in order to remain fit and plastic (Dawkins 1976). Genes have their own agendas when extending phenotypic expressions and chances of proliferation of an organism, qua survival, beyond its individual and population levels (Dawkins 1976, 1982). The effects may extend to

influence entire communities and perhaps ecosystems (Whitham et al. 2003, 2006). The gene's goal is replication (i.e. continuity and survival). Whether the extended phenotype of a gene increases the fitness (or not) of the individuals which carry them in the short run is irrelevant. What does matter is how to extend the gene's existence. To do so, 'replicators' (*sensu* Dawkins 1982, Hull 1980, Jablonka 2004; see also Laland 2004) can develop plasticity in morphology, physiology and behaviour to fulfil their target of maximising their own fitness. This plasticity can be heritable, especially in genomically versatile species such as aphids (Tagu et al. 2008). However, Jablonka (2004) devised an extension to the gene's eye concept of Dawkins by advocating a 'hereditary suite' of genetic, as well as alternative, routes [see also (Danchin et al. 2011)]. The ability of aphid clones to produce several environmentally stimulated phenotypes that have the capacity to fine-tune their 'repertoire' (*sensu* Dombrovsky et al. 2009) suggests that a set of shared (extended) phenotypes will emerge (Dawkins 1982).

Therefore, the genotypes associated with higher fitness are expected to become more common since they can be associated with advantageous traits in the organism itself or extended phenotypes shared with other interacting species (Dawkins 1982, Whitham et al. 2003, Brodie 2005, Whitham et al. 2006). It may take a substantial amount of investment to maintain a position or fitness status (Red Queen hypothesis; Van Valen 1973), although a slower runner could win at some feedback crossroads (Red King hypothesis; Bergstrom and Lachmann 2003). A continuum between cooperation i.e. conflict avoidance (e.g. Rajagopalan et al. 2010, 2011) and antagonism (e.g. an evolutionary arms-race; Dawkins and Krebs 1979; see also Rawal and Rajagopalan 2010) amongst the interacting species, when considered in conjunction with community genetic effects, can perhaps help explain the complexity

of the relationship between ecology and evolution (but see Pelletier et al. 2009, Schoener 2011).

However, co-evolution is not only 'pairwise', but also can be 'diffuse' among assemblages of species, and thus expected to be active within communities (Fox 1988; see also Strauss et al. 2005). I advocate Stewart Brand's comprehension of co-evolution stating that: "Evolution is adapting to meet one's need. Coevolution, the larger view, is adapting to meet each other's need." [Quotation conveyed by Kelly (1994)]. Such comprehension allows us to construe the causation for the emergence of antagonistic as well as cooperative behaviours (Dawkins and Krebs 1979, Wade 2003, Rajagopalan et al. 2010, 2011) and probable adaptations (Futuyma and Slatkin 1983, Karban 1989, Kelly 1994; but see Nesse 2005).

As such, selection will favour the pay-off of the behavioural pattern opted for (Dawkins and Krebs 1979, Abrams 1995, 2000, Weiblen 2003). On average, I tend to adopt the opinion formulated on selection by Lewontin (1978): "...natural selection over the long run does not seem to improve a species' chance of survival but simply enables it to 'track', or keep up with, the constantly changing environment."

Community genetics vs. niche construction theory and eco-evolutionary dynamics; integrity or contrast?

Niche construction (NC) represents a process where species go beyond typological levels of interaction by modifying resources and conditions shared by other species (i.e. environment), and hence engineering (altering) the selection pressures (selection paradigms) imposed on themselves (Lewontin 1983, Odling-Smee et al. 2003, Laland and Boogert 2008, Marquet 2009). While natural selection operates through 'Darwinian algorithms' (Brodie 2005), niche construction activities, both 'inceptive' and 'counteractive', promote changes in states which in turn comprise the

feedback loops which recalibrate the selective influence (Odling-Smee et al. 2003; see also Brodie 2005). In parallel, these activities will fuse into a cyclical interplay with selection (Laland 2004), fuelling and maintaining adaptation or maladaptation (Lewontin 1978, Karban 1989, Ackerly 2003, Nesse 2005).

However, there is another school of thought close to that of NC; that is 'eco-evolutionary dynamics' (Pelletier et al. 2009, Schoener 2011). The former and the latter have coined their own terminologies, but they share several common factors in theory and application, blurring the borders between these two sets of frameworks.

Niche construction can be considered synonymous with the term 'ecosystem engineers' (Jones et al. 1994, 1997) and to some extent with the concept of natural 'molecular geneticists' (Bailey et al. 2004), where CG meets NC. Consider the impact of a beaver on a lake ecosystem, the indirect impacts of root-knot nematodes on plant health/productivity and hence on plant associated communities (Odling-Smee et al. 2003, Whitham et al. 2003, Brodie 2005, Bailey and Whitham 2006b, Whitham et al. 2006, Kaplan et al. 2009). Moreover, interestingly, the articulation of EMGAs (Environmentally Mediated Genotypic Associations; Odling-Smee et al. 2003) in NC bears notable similarity with the 'genetic basis of species interactions' postulated by CG researchers. The framework of NC also entails the concept of ecological inheritance that implicates the maintenance of trans-generational functional, ecological roles in response to specific environmental stimuli (Odling-Smee 2007, Odling-Smee et al. 2003). This occurs through the transition of induced traits from parents to offspring (in aphids usually from mothers to daughters) in order to increase survivorship within communities (Odling-Smee 2007, Odling-Smee et al. 2003). Ecological inheritance of NC in conjunction with genetic inheritance, form the most complete picture (another basis where CG meets NC).

The perspectives proposed in NC and eco-evolutionary dynamics integrate smoothly with those developed via community genetics regarding the functionality of intra-specific genetic variation beyond the levels of species and population. However, further research is needed to profoundly explore how species interactions affect genetic variation and *vice versa*.

Furthermore, the general concept of the extended phenotype devised by Dawkins (1982) outlines that the influence of a genotype (replicator) is not strictly confined to the organism it represents [(see also the perspective devised by Hull (1980)]. The 'Cottonwoods research group' formulated this concept into the platform of community genetics by Whitham (2003, 2006, 2008) in order to describe how genetic variation in keystone species can influence associated communities and ecological processes and thus affect the entire ecosystem. Interestingly, this approach led to the suggestion of community heritability (Dickson and Whitham 1996, Bailey et al. 2006, Whitham et al. 2006), where functional associated ecosystems may be 'inherited' across generations (see the thesis introduction for further insights).

Final Remarks

My research in community genetics takes into consideration that part of each organism's biotic environment is the outcome of the interplay of the expressed genomes of other organisms (Rowntree et al. 2011).

I ran experiments in simplified agricultural model systems and complex tropical forest systems. The studies included the use of several genotypes (cultivars) of two crops of global economic importance (the broad bean *Vicia faba* and the tomato *Lycopersicon esculentum*). These species are important sources of food, fodder, green manure, and also have industrial and medicinal applications. They are grown in temperate regions in general and in Syria in particular. I also used important

economic pests including generalist aphids, such as the pea aphid *Acyrthosiphon pisum* Harris, the black bean aphid *Aphis faba* Scopoli, the vetch aphid *Megoura viciae* Buckton, and the green peach aphid *Myzus persicae* Sulzer. In addition, the root-knot nematode *Meloidogyne hapla* was used in a study on spatially separated competition against community genetic effects and indifferent ecological effects (IEEs). Root-knot nematodes (RKN) are a group of serious plant pests across the globe (Bird 1996). These root-feeders are parthenogenetic and show a high level of plasticity (Jammes et al. 2005). They manipulate their hosts through modifying the genetics of the plant roots (Jammes et al. 2005). The infestation symptoms include the formation of assimilate-sinks or knots (galls) in the infected roots, which serve to maintain a food supply. These artificial sinks violate the normal plant metabolic pathways and are considered to have tremendous effects on plant vigour and plant-mediated effects (Kaplan et al. 2009).

Both the aphids and RKN used in the studies are considered amongst the most noxious agricultural pests on the planet, which result in serious annual negative impacts on crop yields, including in Syria (Oerke et al. 1994, Bird 1996, Blackman and Eastop 2000).

The parasitoid wasp *Aphidius ervi* Haliday was also investigated as an aphid natural enemy. Parasitoids show complex foraging and host manipulation patterns and are proposed to show host preference (Godfray 1994, Shaw 1994, Quicke 1997, Rehman and Powell 2010, Cheng et al. 2011). The relationship between parasitoids and their aphid hosts is envisaged to follow a co-evolutionary arms-race trajectory where each interactor strives to maintain the upper hand (Dawkins and Krebs 1979). Recent studies advocated a genetic variation basis for the interaction (Henter and Via 1995, Ferrari et al. 2001). However, little is known about the effects of intra-specific genetic variation on host manipulation by parasitoids and countermeasures displayed

by hosts under attack. The elements of these systems, qualified in detail within the chapters of the thesis, offer unique models for eco-evolutionary studies on the genetic basis of species interactions at the crossroads between agronomy and community genetics.

In tandem, composite field and lab work focused on the epiphytic tank-forming bromeliad species *Aechmea bracteata* in the tropics (Belize). The aim was to investigate the relationship between the genetics of bromeliads and the age-structured species diversity of their associated invertebrate communities.

As such, my doctoral research provides clear support for the influence of 'within-species' genetic variation on species interactions. I also presented evidence for a genetic variation basis for host manipulation by parasitoid wasps and demonstrated the influence of community genetic effects on the above- and below-ground interactions between sap feeding aphids and root-knot nematodes. My findings support the emergence of shared (extended) phenotypes between the interactors affecting potency of competition, behaviour and reproductive success of aphid species and/or genotypes. Moreover, the indirect genetic effects displayed in my studies across several cultivars of host plant have been rarely shown, particularly for the interaction between aphids and root-knot nematodes that never meet but cohabit the same plant. The findings also exemplify the genetic basis of the co-evolutionary-arms race between the interacting species. In addition, they imply a probability for intra-specific genetic variation to underpin the formation of guild (cooperative behaviour) as an extended (shared) phenotype when competition effects and community genetic effects modulate each other. The interpretations provided in each chapter-paper highlight and provide mechanisms to understand the plausible shifts in the antagonistic or synergistic nature of the relationships between species.

In conclusion, this doctoral research will increase our understanding of:

a) The dynamics of Genotype × Genotype (G × G) and Genotype × Genotype × Environment (G × G × E) interactions.

b) The fundamental basis of [Community Genetic effects (CG) × Competition].

b) The ecological genetics of the shared and extended phenotypes.

c) The genetic complexity of ecological interactions.

d) How pests and biological control agents (natural enemies) interact and co-evolve.

e) The relative impact of species assemblages on ecosystem properties and services.

f) The genetics of competition and Indirect Ecological Effects (IEEs).

Another facet of my research is to delve into the causation and teleology of the paradigms observed. There is more than one explanation for a biological phenomenon. A simple yet elucidating example in this regard is "why a bird migrates when winter approaches", well articulated by Mayr (1961); but see (Ariew 2003, Laland et al. 2011). Mayr (1961) dissected the causation of this phenomenon into 'ultimate' causes (qua genetic and ecological causes) and 'proximate' causes (qua intrinsic and extrinsic causes). As such, Mayr adopted Scriven's definition of 'cause' to be "…a member of a set of jointly sufficient reasons without which the event would not happen" (Mayr 1961, references therein). Surprisingly, such integration of elements for examining and interpreting observations is unfortunately underused in evolutionary biology. Nevertheless, the community genetics synthesis freely facilitates the use and restructure of Mayr's seminal work towards a much more inter-disciplinary development of community genetics tools that can provide more maneuverability of the investigation and interpretation of how intra-specific genetic variation affects species interactions and *vice versa*.

Gradually, the research within the community genetics arena will allow us to understand the genetic basis of the relationships among host plants, pests and natural enemies. The community genetics toolbox of theory, empiricism and methodologies

will also help in decoding the unknown facets of above- and below-ground plant-mediated interactions. Thus, community genetics research helps evolutionary ecologists and agronomists to establish collaborative modern laboratories that offer un-paralleled possibilities for unravelling more threads of the interwoven interplay between ecology and evolution (Rowntree et al. 2011).

I conducted further research on the influence of biotic heterogeneity on the magnitude of community genetic effects besides a study on intra-guild competition at the crossroads between community genetics and niche construction. In addition, I accomplished an extensive study on aphid phenotypic plasticity and polyphenism (induced plasticity by a wide spectrum of biotic and abiotic environmental stimuli). The manuscripts for these experiments are still in preparation as time was insufficient for their completion prior to submission of this thesis.

My foreseen future directions are to complete and publish several additional articles produced throughout the research of this doctoral thesis. I am also in preparation to go back home to Syria, once I obtain my degree, in order to work on the foundation of my own community genetics research laboratory at the University of Damascus.

Last, but certainly not least, I believe that the theoretical, ecological and applied aspects of this work have numerous applications and will influence the development of our current comprehension of how nature functions and how the genetic variation in one organism influences the reproductive success and behaviour of other organisms through reciprocal mediation.

A journey of a thousand miles commences with a smile and a single step.

References

Abrams, P. A. (1995). Implications of dynamically variable traits for identifying, classifying, and measuring direct and indirect effects in ecological communities. *American Naturalist, 146*, 112-134.

Abrams, P. A. (2000). The impact of habitat selection on the heterogeneity of resources in varying environments. *Ecology, 81*, 2902-2913.

Ackerly, D. D. (2003). Community assembly, niche conservatism and adaptive evolution in changing environments. *International Journal of Plant Sciences, 164*, S165-S184.

Agrawal, A. A. (2003). Community genetics: New insights into community ecology by integrating population genetics. *Ecology, 84*, 543-544.

Agrawala, B. K. (2007). Phenotypic plasticity in aphids (Homoptera: Insecta): components of variation and causative factors. *Current Science, 93*, 308-313.

Alex, C., Wilson, C., Sunnucks, P., & Hales, D. F. (2003). Heritable genetic variation and potential for adaptive evolution in asexual aphids (Aphidoidea). *Biological Journal of the Linnean Society, 79*(1), 115–135.

Antonovics, J. (1992). Toward community genetics. In R. S. Fritz, & E. L. Simms (Eds.), *Ecology and Evolution of Plant Resistance to Herbivores and Pathogens: Ecology, Evolution, and Genetics* (pp. 426-449). USA: University Of Chicago Press.

Araújo, M. S., Guimarães, P. R., Svanbäck, R., Pinheiro, A., Guimarães, P., Dos Reis, S. F., & Bolnick, D. I. (2008). Network analysis reveals contrasting effects of intraspecific competition on individual vs. population diets. *Ecology, 89*(7), 197-213.

Ariew, A. (2003). Ernst Mayr's 'ultimate /proximate' distinction reconsidered and reconstructed. *Biology and Philosophy, 18*, 553-565.

Bailey, J. K. (2011). From genes to ecosystems: a genetic basis to ecosystem services. *Popul Ecol, 53*, 47-52.

Bailey, J. K., & Whitham, T. G. (2006a). Biodiversity is related to indirect interactions among species of large effect. In T. Ohgushi, T. Craig, & P.W. Price (Eds.), *Indirect interaction webs: Nontrophic linkages through induced plant traits* (pp. 306-328). UK: Cambridge University Press. Retrieved from http://web.utk.edu/~jbaile29/publications/Bailey%20and%20Whitham%20indirect%20interaction%20webs.pdf

Bailey, J. K., & Whitham, T. G. (2006b). Indirect trait-mediated interactions between cottonwoods and beavers positively affect sawfly abundance. *Ecological Entomology, 31*, 294-297.

Bailey, J. K., Schweitzer, J. A., Rehill, B. J., Lindroth, R. L., Martinsen, G. D., & Whitham, T. G. (2004). Beavers as molecular geneticists: A genetic basis to the foraging of an ecosystem engineer. *Ecology, 85*, 603-608.

Bailey, J. K., Wooley, S. C., Lindroth, R. L., & Whitham, T. G. (2006). Importance of species interactions to community heritability: A genetic basis to trophic-level interactions. *Ecology Letters, 9*, 78-85.

Bergstrom, C. T., & Lachmann, M. (2003). The red king effect: When the slowest runner wins the coevolutionary race. *Proceedings of the National Academy of Sciences, 100*(2), 593-598.

Bird, D. McK. (1996). Manipulation of host gene expression by root-knot nematodes. *Journal of Parasitology, 82*(6), 881-888.

Blackman, R. L., & Eastop, V. F. (2000). *Aphids of the world's crops - an identification and information guide,* 2nd ed. New York: John Wiley.

Bonchev, D., Rouvray, D. H., & Mezey, P. G. (2005). *Complexity in chemistry, biology, and ecology.* London: Springer.

Borges, R. T. (2005). Do plants and animals differ in phenotypic plasticity? *J.Biosci*, *30*(1), 41-50.

Brisson, J. A., & Stern, D. L. (2006). The pea aphid, *Acyrthosiphon pisum*: An emerging model system for the molecular investigation of ecologically-relevant trait variation. *BioEssays*, *28*, 747-755.

Brodie, E. D. (2005). Caution: Niche construction ahead. *Evolution*, *59*(1), 249-251.

Bürger, R. (2002). Additive genetic variation under intraspecific competition and stabilizing selection: A two-locus study. *Theoretical Population Biology*, *61*, 197-213.

Chen, D. Q., Montllor, C. B., & Purcell, A. H. (2000). Fitness effects of two facultative endosymbiotic bacteria on the pea aphid, *Acyrthosiphon pisum*, and the blue alfalfa aphid *A. kondoi*. *Entomol. Exp. Appl*, *95*, 315–323.

Cheng, R., Meng, L., Mills, N. J., & Li, B. (2011). Host preference between symbiotic and aposymbiotic *Aphis fabae*, by the aphid parasitoid*, Lysiphlebus ambiguus*. *J Insect Sci*, *11*(81), 1-13. (doi: 10.1673/031.011.8101)

Danchin, E., Charmantier, A., Champagne, F. A., Mesoudi, A, Pujol, B., & Blanchet, S. (2011). Beyond DNA: Integrating inclusive inheritance into an extended theory of evolution. *Nature Reviews Genetics*, *12,* 475-486.

Dawkins, R. (1976). *The selfish gene.* New York City: Oxford University Press.

Dawkins, R. (1982). *The extended phenotype.* Oxford: W.H. Freeman And Company.

Dawkins, R., and J. R. Krebs. 1979. Arms races between and within species. *Proceedings of the Royal Society London. B, 205*(1161), 489-511.

Dickson, L. L., & Whitham, T. G. (1996). Genetically-based plant resistance traits affect arthropods, fungi, and birds. *Oecologia*, *106*, 400-406.

Dixon, A. F. G. (1977). Aphid ecology: Life cycles, polymorphism, and population regulation. *Ann. Rev. Ecol. Syst., 8*, 329-353.

Dixon, A. F. G. (1987). Parthenogenetic reproduction and the rate of increase in aphids. In A. K. Minks & P. Harrewijn (Eds.), *Aphids, their biology, natural enemies and control* (pp. 269-287). Amsterdam: Elsevier.

Dixon, A. F. G. (1998). *Aphid ecology.* London: Chapman and Hall.

Dombrovsky, A. A., Arthaud, L. L., Ledger, T. N., Tares, S., & Robichon, A. (2009). Profiling the repertoire of phenotypes influenced by environmental cues that occur during asexual reproduction. *Genome Research*, *19*, 2052-2063. (doi: 10.1101/gr.091611.109)

Eshel, L., Feldmanb, M. W., & Bergmanc, A. (1998). Long-term evolution, short-term evolution, and population genetic theory. *Journal of Theoretical Biology*, *191*(4), 391-396.

Ferrari, J., Godfray, H. J., Faulconbridge, A., Prior, K., & Via, S. (2006). Population differentiation and genetic variation in host choice among pea aphids from eight host plant genera. *Evolution*, *60*, 1574-1584.

Ferrari, J., Müller, C. B., Kraaijeveld, A. R., & Godfray H.C. J. (2001). Clonal variation and covariation in aphid resistance to parasitoids and a pathogen. *Evolution*, *55* (9), 1805-1814.

Fox, L. R. (1988). Diffuse coevolution within complex communities. *Ecology*, *69*, 906-907.

Futuyma, D. J., & Slatkin, M. (1983). *Coevolution.* Sunderland, MA.: Sinauer Associates, Inc.

Godfray, H. C. J. (1994). *Parasitoids: Behavioral and Evolutionary Ecology.* Princeton, New Jersey, USA: Princeton University Press.

Hamilton, W. (1964). The genetical evolution of social behaviour. I. *Journal of Theoretical Biology*, *7*(1), 1-16.

Henter, H. J. (1995). The potential for coevolution in a host-parasitoid system: II. Genetic variation within a population of wasps in the ability to parasitize an aphid host. *Evolution*, *49* (3), 439-445.

Henter, H. J., & Via, S. (1995). The potential for coevolution in a host-parasitoid system: I. Genetic variation within an aphid population in susceptibility to a parasitic wasp. *Evolution, 49*(3), 427-438.

Hersch-Green, E. I., Turley, N. E., & Johnson, M. T. J. (2011). Community genetics: What have we accomplished and where should we be going? *Phil. Trans. R. Soc. B*, *366*(1569), 1453-1460.

Hull, D. L. (1980), "Individuality and Selection", *Annual Review of Ecology and Systematics*, *11*, 311–32.

Jablonka, E. (2004). From replicators to heritably varying phenotypic traits. the extended phenotype revisited. *Biology & Philosophy*, *19*, 353-375.

Jammes, F., Lecomte, P. L., De Almeida-Engler, J., Bitton, F., Martin-Magniette, Marie-Laure, Renou, J. P., Abad, P., & Favery, B. (2005). Genome-wide expression profiling of the host response to root-knot nematode infection in Arabidopsis[a]. *The Plant Journal*, *44*, 447-458.

Jervis, M. A. (2005). *Insects as Natural Enemies: A Practical Perspective*. Kluwer Academic Publishers.

Johnson, M. T. J., & Stinchcombe, J. R. (2007). An emerging synthesis between community ecology and evolutionary biology. *Trends in Ecology and Evolution*, *22*, 250-257.

Jones, C. G., Lawton, J. H., & Shachak, M. (1994). Organisms as ecosystem engineers. *Oikos*, *69*, 373-386.

Jones, C. G., Lawton, J. H., & Shachak, M. (1997). Positive and negative effects of organisms as physical ecosystem engineers. *Ecology*, *78*, 1946-1957.

Juarrero, A. (2010). Complex dynamical systems theory. "Cognitive Edge Network".
Retrieved from http://cognitive-
edge.com/uploads/articles/100608%20Complex_Dynamical_Systems_Theory.
pdf

Kaplan, I., Sardanelli, S., & Denno, R. F. (2009). Field evidence for indirect
interactions between foliar-feeding insect and root-feeding nematode
communities on *Nicotiana tabacum*. *Ecol. Entomol*, *34*, 262-270.

Karban, A. (1989). Fine-scale adaptation of herbivorous thrips to individual host
plants. *Nature*, *340*, 60-61.

Kelly, K. (1994). Coevolution, the unreasonable point of life. In *Out of control the
new biology of machines.* Perseus Books. Retrieved from
http://www.kk.org/outofcontrol/ch5-b.html

King, C. C. (2003). Biocosmology 2: Evolutionary epoch: Complexity, chaos and
complementarity. *340*. Retrieved from
http://www.dhushara.com/pdf/biocos2.pdf

Laland, K. N. (2004). Extending the extended phenotype. *Biology and Philosophy*, *19*,
313-325.

Laland, K. N., & Boogert, N. J. (2008). Niche construction, co-evolution and
biodiversity. *Ecol Econ*, *69*, 731-736. (doi:10.1016/j.ecolecon.2008.11.014)

Laland, K., Sterelny, K., Odling-Smee, J., Hoppitt, W., & Uller, T. (2011). Cause and
effect in biology revisited: Is Mayr's proximate-ultimate dichotomy still
useful? *Science*, *334*(6062), 1512-1516.

Levin, S. A. (1998). Ecosystems and the biosphere as complex adaptive systems.
Ecosystems, *1*, 431-436.

Lewontin, R. C. (1978). "Adaptation". *Scientific American*, *293*(3), 212-230.

Lewontin, R. C. (1983). Gene, organism, and environment. In D. S. Bendall (Ed.), *Evolution from molecules to men* (pp. 273-285). Cambridge: Cambridge University Press.

Marquet, P. A. (2009). Beyond Darwin: On the role of niche construction and self-organization in evolution. *Revista Chilena De Historia Natural, 82*, 493-496.

Masters, G. J., Brown, V. K., & Gange, A. C. (1993). Plant mediated interactions between above- and belowground insect herbivores. *Oikos, 66*, 148-151.

Masters, G. J., Jones, T. H., & Rogers, M. (2001). Host-plant mediated effects of root herbivory on insect seed predators and their parasitoids. *Oecologia, 127*, 246-250.

Mauricio, R., & Rausher, M. D. (1997). Experimental manipulation of putative selection agents provides evidence for the role of natural enemies in the evolution of plant defense. *Evolution, 51*, 1435-1444.

Maynard Smith, J. (1974). The theory of games and the evolution of animal conflicts. *Journal of Theoretical Biology, 47*(1), 209-221.

Mayr, E. (1961). Cause and effect in biology. *Science, 134*(3489), 1501-1506.

McLean, A. H. C., van Asch, M., Ferrari, J., & Godfray, H. C. J. (2011). Effects of bacterial secondary symbionts on host plant use in pea aphids. *Proc. R. Soc. B, 278*(1706), 760-766.

Mejer, H. F., & Jørgensen, S. E. (1979). Energy and ecological buffer capacity, State-of-the-art of ecological modelling. In: S.E. Jørgensen, editor, Environmental sciences and applications, Proc. 7th Conf. Ecological modelling, 28 august–2 September 1978, Copenhagen international society for ecological modelling, Copenhagen.

Montoya, J. M., Solé, R. V., & Pimm, S. L. (2006). Ecological networks and their fragility. *Nature, 442*, 259-264.

Moore, A. J., Brodie, E. D., & Wolf, J. B. (1997). Interacting phenotypes and the evolutionary process: I. Direct and indirect genetic effects of social interactions. *Evolution*, *51*(5), 1352-1362.

Moore, J. (2002). *Parasites and the Behavior of Animals*. New York, USA: Oxford University Press.

Moran, N. A. (1991). Phenotype fixation and genotypic diversity in the complex life cycle of the aphid *Pemphigus betae*. *Evolution*, *45*, 957-970.

Nesse, R. M. (2005). Maladaptation and natural selection. *The Quarterly Review of Biology*, *80*(1), 1435-1444.

Neuhauser, C., Andow, D. A., Heimpel, G. E., Ruth, G. M., Shaw, G., & Wagenius, S. (2003). Community genetics: Expanding the synthesis of ecology and genetics. *Ecology*, *84*, 554-558.

Odling-Smee, F. J., Laland, K. N., & Feldman, M. W. (2003). *Niche construction: The neglected process in evolution*. Princeton, NJ: Princeton University Press.

Odling-Smee, J. (2007). Niche inheritance: A possible basis for classifying multiple inheritance systems in evolution. *Biological Theory*, *2*(3), 276-289.

Oerke, E. C., Dehne, H. W., Schonbeck, F., & Weber, A. (1994). *Crop production and crop protection – estimated losses in major food and cash crops*. Amsterdam: Elsevier Science.

Pelletier, F., Garant, D., & Hendry, A. P. (2009). Eco-evolutionary dynamics. *Philos Trans R Soc Lond B Biol Sci*, *364*(1523), 1483-1489.

Poulin, R. (2006). *Evolutionary Ecology of Parasites*. New Jersey, USA: Princeton University Press.

Powell, G., Tosh, C. R., & Hardie, J. (2006). Host plant selection by aphids: Behavioral, evolutionary, and applied perspectives. *Annu. Rev. Entomol.*, *51*, 309-330.

Quicke, D. L. J. (1997). *Parasitic wasps*. London: Chapman and Hall.

Rajagopalan, P., Rawal, A., & Miikkulainen, R. (2010). Emergence of competitive and cooperative behavior using coevolution. *Proceedings of the 12th Annual Conference on Genetic and Evolutionary Computation*. Retrieved from http://dl.acm.org/citation.cfm?id=1830681&bnc=1

Rajagopalan, P., Rawal, A., Miikkulainen, R., Wiseman, M. A., & Holekamp, K. E. (2011). The role of reward structure, coordination mechanism and net return in the evolution of cooperation. *Proceedings of the IEEE Conference on Computational Intelligence and Games (CIG 2011), Seoul, South Korea*. Retrieved from http://bit.ly/rPXszL

Rawal, A., & Rajagopalan, P. (2010). Emergence of competitive and cooperative behavior and arms race through coevolution. *Neural Networks Research Group*. Retrieved from http://nn.cs.utexas.edu/?armsrace

Rehman, A., & Powell, W. (2010). Host selection behaviour of aphid parasitoids (Aphidiidae: Hymenoptera). *Journal of Plant Breeding and Crop Science*, *2*(10), 299-311.

Rosenberg, A., & Bouchard, F. (2010). Fitness. In *The Stanford Encyclopedia of Philosophy*. Zalta, E. N. (Ed.). Retrieved from http://plato.stanford.edu/entries/fitness/

Rowntree, J. K., Shuker, D. M., & Preziosi, R. F. (2011). Forward from the crossroads of ecology and evolution. *Phil. Trans. R. Soc. B*, *366*(1569), 1322-1328.

Russo, S., C. Campbell, J. Dew, P. Stevenson, and S. Suarez. (2005). A multi-forest comparison of dietary preferences and seed dispersal by *Ateles* spp. *International Journal of Primatology*, *26*, 1017-1037.

Schoener, T. W. (2011). The newest synthesis: Understanding the interplay of evolutionary and ecological dynamics. *Science*, *331*, 426-429.

Shaw, M. R. (1994). Parasitoid host ranges. In B. A. Hawkins & W. Sheehan (Eds.), *Parasitoid community ecology* (pp. 111-144). Oxford: Oxford University Press.

Smith, R. A., Mooney, K. A., & Agrawal, A. A. (2008). Coexistence of three specialist aphids on common milkweed, *Asclepias syriaca*. *Ecology*, *89*(8), 2187-2196.

Strauss, S. Y., Sahl, H., & Conner, J. K. (2005). Toward a more trait-centered approach to diffuse (co)evolution. *New Phytologist*, *165*(1), 81-90.

Sultan, S. E. (2003). Phenotypic plasticity in plants: A case study in ecological development. *Evol. & Dev.*, *5*(1), 25-33.

Tagu, D., Klingler, J. P., Moya, A., & Simon, J. C. (2008). Early progress in aphid genomics and consequences of plant-aphid interaction studies. *Mol. Plant Microbe Interact.*, *21*, 701-708.

Van Valen, L. (1973). "A New Evolutionary Law", *Evolutionary Theory, 1*, 1-30.

Wade, M. J. (2003). Community genetics and species interactions. *Ecology*, *84*, 583-585.

Watts, D., & Strogatz, S. H. (1998). Collective dynamics of 'small-world' networks. *Nature*, *393*, 440-442. (doi:10.1038/30918)

Weiblen, G. D. (2003). Interspecific coevolution. In *Nature Encyclopedia of Life Sciences* (pp. 1-12). London: Nature Publishing Group. Retrieved from http://geo.cbs.umn.edu/Weiblen2003.pdf

Weiher, E., & Keddy, P. (1999). *Ecological assembly rules: Perspectives, advances, retreats*. UK: Cambridge University Press.

Whitham, T. G., Bailey, J. K., Schweitzer, J. A., Shuster, S. M., Bangert, R. K., LeRoy, C. J., Lonsdorf, E. V., Allan, G. J., DiFazio, S. P., Potts, B. M. Fischer, D. G., Gehring, C. A., Lindroth, R. L., Marks, J. C., Hart, S. C., Wimp, G. M.,

& Wooley, S. C. (2006). A framework for community and ecosystem genetics: From genes to ecosystems. *Nat Rev Genet*, *7*, 510-523.

Whitham, T. G., DiFazio, S. P., Schweitzer, J. A., Shuster, S. M., Allan, G. J., Bailey, J. K., & Woolbright, S. A. (2008). Extending genomics to natural communities and ecosystems. *Science*, *320*, 492-495.

Whitham, T. G., Young, W. P., Martinsen, G. D., Gehring, C. A., Schweitzer, J. A., Shuster, S. M Wimp, G. M., Fischer, D. G., Bailey, J. K., Lindroth, R. L., Woolbright, S. , & Kuske, C. R. (2003). Community and ecosystem genetics: A consequence of the extended phenotype. *Ecology*, *84*, 559-573.

Whitman, D. W., & Agrawal, A. A. (2009). What is phenotypic plasticity and why is it important? In D. W. Whitman & T. N. Ananthakrishnan (Eds.), *phenotypic plasticity of insects: Mechanisms and consequences* (pp. 1-54). Moscow, USA: Science.

Wiens, J. A. (1977). On competition and variable environments. *Am Nat*, *65*, 590-597.

Wiens, J. A. (1991). Ecological crunches and competition. *Am Nat*, *CC/N14*. Retrieved from http://garfield.library.upenn.edu/classics1991/A1991FC51200001.pdf

Wootton, J. T. (1994). The nature and consequences of indirect effects in ecological communities. *Annual Review of Ecology and Systematics*, *25*, 443-466.

Wootton, J. T. (2002). Indirect effects in complex ecosystems: Recent progress and future challenges, journal of sea research. *Journal of Sea Research*, *48*(1), 157-172.

Yong, C. H., & Miikkulainen, R. (2001). Cooperative coevolution of multi-agent systems. *Technical Report "AI07-338"*, Department of Computer Sciences, the University of Texas at Austin.

Yong, C. H., & Miikkulainen, R. (2007). Coevolution of role-based cooperation in multi-agent systems. *Technical Report "AI07-338"*, Department of Computer Sciences, the University of Texas at Austin.

Zera, A. J., & Denno, R. F. (1997). Physiology and ecology of dispersal polymorphism in insects. *Annu Rev Entomol, 42*, 207-230.

Zytynska, S. E., Khudr, M. S., Harris, E., & Preziosi, R. F. (2012). Genetic effects of tank-forming bromeliads on the associated invertebrate community in a tropical forest ecosystem. *Oecologia*. (In press). Retrieved from http://www.springerlink.com/content/p700k6v52tt82xr7/fulltext.pdf

Appendix

Additional supporting notes and definitions on 'Fitness'

"survival of the fittest; Spencer (1864) and "the fittest survive"; Mills and Beatty (2006).

- **Fitness:**

"Fitness is a measure of the survival and reproductive success of an entity. This entity may be a gene, individual, group or population."; Day and Otto (2001).

- **Absolute fitness and Relative fitness:**

The term absolute fitness refers to the absolute number of copies (offspring/descendants) of an entity. Absolute fitness represents the differential reproductive success within generations and between generations. Furthermore, the fecundity and survival of the replicated/propagated copies vary according to the environment, spatiotemporal effects, and age-structured differences within populations (Orr 2009); see also (Engen et al. 2009). Such differential fecundity and survival will also be relative to genotype i.e. genotypes differ in their reproductive values (Fisher 1930, Carroll and Corneli 1999, Day and Otto 2001, Orr 2009, Engen et al. 2009).

- **Reaction norms:**

Norms of reaction are 'functions' that explain the differential expression of a focal genotype across environments through relating environmental variation to phenotypic variation (Carroll and Corneli 1999, Carr 2004; see also Stearns 1989, Whitman and

Agrawal 2009). Reaction norms vary according to genotype within and between generations/populations (Whitman and Agrawal 2009). The reaction norms may differ relative to environment [e.g. spatial effects (Orr 2009), stochasticity (Engen et al. 2009)], and can be plastic between inter-players with greater capacity, possibly leading to a state of 'plasticity co-evolution' between the interactors (Whitman and Agrawal 2009).

- **Phenotypic plasticity:**

Phenotypic plasticity is the ability of a specific genotype to variably alter its phenotypic expression in terms of physiology, morphology and behaviour in response to environmental change (Whitman and Agrawal 2009, Fusco and Minelli 2010).

- **Polyphenism:**

Polyphenism is an extreme type of phenotypic plasticity explained by the production/expression of phenotypes that are discretely plastic, e.g. the production of winged morphs in aphids and the phase-dependent extreme plasticity of 'solitarius' and 'gregarious' locusts (Whitman and Agrawal 2009, Simpson et al. 2011).

- **Canalisation:**

It can be argued that 'buffering' the genetic replication (a.k.a. homeostasis) against both environmental change and the selective forces requires balancing robustness in one trait (trait-stabilising) while displaying plasticity in another (Sultan 1987, Carroll and Corneli 1999, Whitman and Agrawal 2009). Such canalisation should be underpinned with plasticity (maintenance of the 'buffer' effect); (Whitman and Agrawal 2009, Bateson and Gluckman 2011). Therefore, canalisation and phenotypic

plasticity (morphological, physiological and behavioural) should act in tandem and/or reciprocate (Whitman and Agrawal 2009, Bateson and Gluckman 2011).

- **Behavioural traits:**

Behavioural traits are complex, context-dependent and cost-sensitive i.e. adjustable in response to environmental cues, and thus varyingly plastic and strategic in order to cope with the ongoing changes in the environment (Slobodkin and Rapoport 1974, Carroll and Corneli 1999, Odling-Smee et al. 2003, Odling-Smee 2007, Bergmüller and Taborsky 2010, Gardner et al. 2011).

- **The phenotypic gambit:**

"The phenotypic gambit is a simplifying assumption that phenotypes of biological interest are controlled by a simple genetic architecture, such that evolution proceeds on an essentially adaptationist basis without being upset by recombination between epistatically-interacting loci and other such factors"; Gardner et al. (2011).

- **Multi-agent ESP (Multi-agent Enforced Sub-Populations), Emergence of Cooperation and Guild-Fitness Reward*:**

The fitness reward of functioning as a guild with reduction of conflict can be, under specific conditions, high if contrasted with the state of being selfish or an antagonistic inter-player (agent). Arguably, the fitness reward, 'frivolous', for cooperation entails that conspecifics and probably heterospecifics can develop a set of different strategies to maximise fitness through indirectly perceiving changes in the shared environment brought about by other interactors, and hence act accordingly. Thus, a network of interactions can develop comprising a complex system that is conceived to be adaptive or evolving (Iseda 1996, Rees 2004, Yong and Miikkulainen 2009, Lieberman et al.

2009, Juarrero 2010, Levin 1998). Surprisingly, cooperation may not follow Hamilton's rule of thumb on kin selection i.e. cooperation can emerge amongst unrelated individuals under specific conditions (Fletcher and Doebeli 2009, Gardner et al. 2011).

---

* "These roles are assigned through simultaneous adaptive 'niching': as one agent begins to converge to a particular behaviour, the other agents that behave complementarily are rewarded, and themselves begin to niche into such roles. This adaptation in turn yields a higher fitness, and all predators begin to converge into cooperative roles. In this way, a stigmergic form of coordination between team members quickly emerges."; Yong and Miikkulainen (2009).

Box1: Fitness; a set of definitions.

The definitions provided in **Box 1** are adopted from the significant works of West et al. (2007, 2011); see also (Oli 2003, Griffin and West 2002). The 'greenbeard' effect entails relatedness through the function of 'role' rather than the effects exerted by a neighbour (kin); (Queller 1985, Odling-Smee et al. 2003, Odling-Smee 2007, Bergmüller and Taborsky 2010, Gardner et al. 2011; but see below the 'Neighbour-modulated fitness'.

 The concepts defined above shed light on further facets of the intricacy of fitness as a concept. This set of definitions is pertinent to the supportive parts on fitness within this appendix and in general discussion besides chapters one and two. Together, they illuminate the term (fitness) as well as heighten the need to devise an amalgamated approach in order to help further our understanding of the interaction between ecology

and evolution. In this regard, I advocate community genetics a promising synthesis that helps bridge the gap between theory and application in evolutionary biology.

- **Neighbour-modulated fitness:**

This term refers to the reproductive success due to 'self', and reproductive success due to help from other interactors i.e. neighbours (Hamilton 1964a, Iseda 1996, West et al. 2007, Rosas 2010, West et al. 2011).

- **Kin selection:**

"Kin selection is the name of a process, parallel to Darwinian selection or natural selection, that causes individuals to behave differently (and generally more favourably) towards more closely than to less closely related conspecifics"; Graften (2009); see also (Maynard Smith 1964) and **Box 1** above.

- **Inclusive fitness:**

Hamilton (1964a,b, 1970) formulated a novel concept that is considered to be an expansion for the current understanding of individual fitness. In his theory of kin selection and the lesser known theory of "Neighbour-modulated fitness" (Iseda 1996), Hamilton proposed that genes duplicate/replicate themselves through generations in two ways:

1) Replication of the holder (self).

2) Increasing the chances of kin survival (replication of the relatives, i.e. individuals that share varying portions of their genome).

As such, "inclusive fitness is the sum of direct and indirect fitness"; (Hamilton 1964a,b, Griffin and West 2002, Oli 2003, West et al. 2011). Hamilton's theory of kin selection should not be in conflict with the Darwinian perspective of natural selection. This is because what appears to be suboptimal or fitness-minimised in the short term

for a specific 'kin' (due to context-dependent conditions), can end up to be fitness-optimising in the long term (see Dawkins 1979, Iseda 1996 and Graften 2009). This is especially conceivable, as well as observable, in parthenogenetic aphids where genetically identical copies, which occur solo or mingled with other congeners, comprise populations of copies of 'oneself' (for each specific clone). In this regard, it is no surprise that the gene's eye view of evolution known as the 'selfish gene agenda' (*sensu* Dawkins 1976) including the expansion of the concept by Jablonka (2004), can be considered with kin selection theory, to represent two opposing faces of a coin (Iseda 1996, Lieberman et al. 2009; see also Dawkins 1979, 1981, 1982). Genes or 'hereditary suites' (*sensu* Jablonka 2004) strive to replicate and pass through generations via different genetic and epigenetic routes.

- **Epigenetics:**

"Epigenetics is the study of emergent properties in the origin of the phenotype in development and in modification of phenotypes in evolution" as defined by Hallgrímsson and Hall (2011).

- **Note:**

Genetically identical individuals comprise the clone in the case of parthenogenetic aphids under preferable conditions, and obligatory parthenogenetic aphids (i.e. aphids that display anholocyclic or incomplete life cycle); (Van Emden and Harrington 2007). The more copies a genotype can produce, and the greater the degree of plasticity in morphology, ontogeny, physiology and behaviour are maintained, the fitter the genotype is, in such populations with high levels of relatedness (e.g. Mondor and Messing 2007).

Concluding remarks

The fitness of a focal genotype (aphid clone) can be explained by the number of copies propagated (reproduction) and the survival of these copies (Crow and Kimura 1970, Grant 1977, Sober 2006). Aphids increase the chances of their reproductive success through:

a) Higher exponential rates of reproduction [viviparity and telescoping of generations; (Dixon 1985, 1987, 1998)].

b) Intensive plasticity (morphological, ontogenetic, physiological and behavioural); (Agrawala 2007, Whitman and Agrawal 2009).

Although certain expressed phenotypes such as alates are less fecund when compared with apterae, alates are dispersive and therefore increase the chances of survival of the clone when it is threatened by the presence of natural enemies or resource depletion. What appears to be suboptimal from one perspective, may actually be optimal from another, e.g. where alates, through dispersal, increase the fitness of the clone and their wingless sisters concurrently. The same principle applies for altruistic aphids, which commit suicide under parasitoid attack in order to increase the probability of kin survival (McAllister et al. 1990; see also Hamilton 1964b). Another key example in aphids is the development of abdominal cornicles, proposed by Mondor and Messing (2007) to be determined by both direct and inclusive fitness, and to be more pronounced under parthenogenesis. Similarly, based on the observations made by Inbar and Wool (1995), it is probable that the length of stylets in gall aphids, under competition, is shaped by both direct and inclusive fitness in order to maximise resource utilisation, and hence reproductive success and survival.

In this sense, fitness can be interpreted as a "propensity" as proposed by Mills and Beatty (2006). These authors elaborated on the 'propensity' qualification of fitness by suggesting: "The fitness of an organism explains its success at survival and

reproduction in a particular environment in the same way that the solubility of a substance explains the fact that it has dissolved in a particular liquid. When we say that an entity has a propensity (disposition, tendency, capability) to behave in a particular way, we mean that certain physical properties of the entity determine, or are causally relevant to, the particular behavior whenever the entity is subjected to appropriate ''triggering conditions.''

References

Agrawala, B. K. (2007). Phenotypic plasticity in aphids (Homoptera: Insecta): components of variation and causative factors. *Current Science, 93*, 308-313.

Bateson, P., & Gluckman, P. (2011). *Plasticity, robustness, development and evolution.* UK: Cambridge University Press.

Bergmüller, R., & Taborsky, M. (2010). Animal personality due to social niche specialisation. *Trends in Ecology & Evolution, Trends in Ecology and Evolution-1267*, 1-8. (doi:10.1016/j.tree.2010.06.012)

Carr, S. M. (2004). Norm of reaction. Retrieved from Memorial University, Biology, Scarr, Directory website:

http://www.mun.ca/biology/scarr/6390_Norm_of_Reaction.html

Carroll, S. P., Corneli, P. S., (1999). The evolution of behavioral norms of reaction as a problem in ecological genetics. In S. F. Foster & J. A. Endler (Eds.), *Geographic variation in behaviour* (pp. 51-68). New York: Oxford University Press. Retrieved from http://bit.ly/ShUWCi

Crow, J. F., and Kimura, M. (1970). *An Introduction to Population Genetics Theory,* New York: Harper.

Dawkins, R. (1976). *The selfish gene.* New York City: Oxford University Press.

Dawkins, R. (1979). Twelve Misunderstandings of Kin Selection. *Zeitschrift für Tierpsychologie*, *51*(2), 184–200. (doi: 10.1111/j.1439-0310.1979.tb00682.x)

Dawkins, R. (1981). In defence of selfish genes. *Philosophy*, *56*(218), 556-573.

Dawkins, R. (1982). *The extended phenotype.* Oxford: W.H. Freeman And Company.

Day, T. & Otto, S. P. (2001) Fitness. *Encyclopaedia of Life Science*. Nature Publishing Group, http://www.els.net. Retrieved from: http://onlinelibrary.wiley.com/doi/10.1038/npg.els.0001745/pdf

Dixon, A. F. G. (1985). Structure of aphid populations. *Annual Review of Entomology*, *30*, 155-174.

Dixon, A. F. G. (1987). Parthenogenetic reproduction and the rate of increase in aphids. In A. K. Minks & P. Harrewijn (Eds.), *Aphids, their biology, natural enemies and control* (pp. 269-287). Amsterdam: Elsevier.

Dixon, A. F. G. (1998). *Aphid ecology.* London: Chapman and Hall.

Engen, S., Lande, R., & Sæther, B. E. (2009). Reproductive value and fluctuating selection in an age-structured population. *Genetics*, *183*(2), 629-637. (doi: 10.1534/genetics.109.105841)

Fisher, R. A., (1930). *The Genetical Theory of Natural Selection*. Oxford: Clarendon Press.

Fletcher, J. A. & Doebeli, M. (2009). A simple and general explanation for the evolution of altruism. *Proc. R. Soc. Lond. B Biol. Sci., 276*: 13–19.

Fusco, G., & Minelli, A. (2010). Phenotypic plasticity in development and evolution: Facts and concepts. *Phil. Trans. R. Soc. B, 365*(1540), 547-556. (doi:10.1098/rstb.2009.0267)

Gardner, A., West, S. A., & Wild, G. (2011). The genetical theory of kin selection. *J. Evol. Biol.*, *24*(1533), 1020-1043. (doi: 10.1111/j.1420-9101.2011.02236.x)

Graften, A. (2009). Formalizing Darwinism and inclusive fitness theory. *Philos Trans R Soc Lond B Biol Sci.*, *364*(1533), 3135-3141. (doi: 10.1098/rstb.2009.0056)

Grant, V. (1977). Organismic Evolution. San Francisco: W. H. Freeman.

Griffin, A. S. & West, S. A. (2002). Kin selection: Fact and fiction. *Trends Ecol. Evol., 17*, 15–21.

Hallgrímsson, B., & Hall, B. K. (2011). *Epigenetics: Linking genotype and phenotype in development and evolution.* Retrieved from http://bit.ly/NXSUVg

Hamilton, W. D. (1964a). The genetical evolution of social behaviour I. *Journal of Theoretical Biology*, *7*, 1-16.

Hamilton, W. D. (1964b). The genetical evolution of social behaviour II. *Journal of Theoretical Biology*, *7*, 17-32.

Hamilton, W. D. (1970). Selfish and spiteful behaviour in an evolutionary model. *Nature*, *228*, 1218–1220.

Inbar, M., & Wool, D. (1995). Phloem-feeding specialists sharing a host tree: Resource partitioning minimizes interference competition among galling aphid species. *Oikos*, *73*, 109-119.

Iseda, T., (1996). Changes in the concept of 'fitness' in evolutionary biology. *Jissentetsugaku-Kenkyu* (Studien zur Praktischen Philosophie), *19*, 1-31. Retrieved from http://bit.ly/OljwgI

Jablonka, E. (2004). From replicators to heritably varying phenotypic traits. the extended phenotype revisited. *Biology & Philosophy*, *19*, 353-375.

Juarrero, A. (2010). Complex dynamical systems theory. "Cognitive Edge Network". Retrieved from http://cognitive-edge.com/uploads/articles/100608%20Complex_Dynamical_Systems_Theory.pdf

Levin, S. A. (1998). Ecosystems and the biosphere as complex adaptive systems. *Ecosystems*, *1*, 431-436.

Lieberman, D. L., Haselton, M. G., & von Hippel, W. (2009). Kin selection. In H. Reis & S. Sprecher (Eds.), *Encyclopedia of Human Relationships*: Vol. 2 (pp. 954-957). Thousand Oaks, CA: Sage. Retrieved from: http://www.sscnet.ucla.edu/comm/haselton/papers/downloads/lieberman_et_al_reis_and_sprecher.pdf

Maynard Smith, J. (1964). Group selection and kin selection. *Nature*, *201*, 1145–1147.

McAllister, M. K., B. D. Roitberg, & K. L. Weldon. (1990). Adaptive suicide in pea aphids: Decisions are cost sensitive. *Animal Behaviour*, *40*(1), 167-175.

Mills, S. K., Beatty, J. H. (2006). The propensity interpretation of fitness. In E. Sober (Ed.), *Conceptual issues in evolutionary biology* (pp. 3-24). Cambridge, Massachusetts London, England: Bradford Books The MIT Press.

Mondor, E. B., & Messing, R. H. (2007). Direct vs. inclusive fitness in the evolution of aphid cornicle length. *Journal of Evolutionary Biology*, *20*(2), 807-812. (doi: 10.1111/j.1420-9101.2006.01271.x)

Odling-Smee, F. J., Laland, K. N., & Feldman, M. W. (2003). *Niche construction-the neglected process in evolution.* Princeton, NJ: Princeton University Press.

Odling-Smee, J. (2007). Niche inheritance: A possible basis for classifying multiple inheritance systems in evolution. *Biological Theory*, *2*(3), 276-289.

Oli, M. K. (2003). Hamilton goes empirical: Estimation of inclusive fitness from life-history data. *Proc. R. Soc. Lond. B*, *270*(1512), 307-311. (doi: 10.1098/rspb.2002.2227)

Orr, H. A. (2009). Fitness and its role in evolutionary genetics. *Nat Rev Genet*, *10*(8), 531-539. (doi: 10.1038/nrg2603)

Queller, D. C. (1985). Kinship, reciprocity, and synergism in the evolution of social behaviour. *Nature*, *318*, 366–367.

Rees, T. (2004). An Introduction to Evolutionary Game Theory. UBC Department of Computer Science. Retrieved from http://bit.ly/MsauEM

Rosas, A. (2010). Beyond inclusive fitness? On a simple and general explanation for the evolution of altruism. *Philos Theor Biol 2:e104*, *2*(e104), 1-9. Retrieved from http://hdl.handle.net/2027/spo.6959004.0002.004

Simpson, S. J., Sword, G. A. & Lo, N. (2011). Polyphenism in insects. *Current Biology*, *21*(18), R738-749. (doi: 0.1016/j.cub.2011.06.006)

Slobodkin, L. B., & Rapoport, A. (1974). An optimal strategy of evolution. *Quurt. Rev. Biol*, *49*, 181-200.

Sober, E. (2006). *Conceptual issues in evolutionary biology*. Cambridge, Massachusetts London, England: Bradford Books The MIT Press.

Spencer, H. (1864). The principles of biology, vol. 1. London: Williams and Norgate.

Stearns, S. C. (1989). The evolutionary significance of phenotypic plasticity. *BioScience*, *39*, 436-445.

Sultan, S. E., (1987). Evolutionary implications of phenotypic plasticity in plants. *Evolutionary Biology*, *2*, 127-178.

Van Emden, H. F., & Harrington, R. (2007). *Aphids as crop pests.* London: CABI Publishing.

West, S. A., Griffin, A. S., & Gardner, A. (2007). Evolutionary explanations for cooperation. *Current Biology*, *17*(16), R661-R672. (doi:10.1016/j.cub.2007.06.004)

West, S. A., Mouden, C. E., & Gardner, A. (2011). Sixteen common misconceptions about the evolution of cooperation in humans. *Evolution and Human Behavior*, *32*, 231-262.

Whitman, D. W., & Agrawal, A. A. (2009). What is phenotypic plasticity and why is it important? In D. W. Whitman & T. N. Ananthakrishnan (Eds.), *phenotypic plasticity of insects: Mechanisms and consequences* (pp. 1-54). Moscow, USA: Science.

Yong, C. H., & Miikkulainen, R. (2009). Coevolution of role-based cooperation in multiagent systems. *IEEE Transactions on Autonomous Mental Development*, *1*(30), 170-186.

'Facts do not "speak for themselves"; they are read in the light of theory. Creative thought, in science as much as in the arts, is the motor of changing opinion. Science is a quintessentially human activity, not a mechanized, robotlike accumulation of objective information, leading by laws of logic to inescapable interpretation'

Stephen Jay Gould (1979); Ever Since Darwin

Gould, S. J. (1979). *Ever since Darwin: Reflections in natural history.* New York: W.W. Norton.