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The role of productivity in the ecological and evolutionary dynamics of predator-prey interaction

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ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Biosciences of the University
of Helsinki, for public examination in lecture room YAA303, Ambiotica,
on 14 March 2009, at 12 noon. Jyväskylä 2009

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"Logic will get you from A to B. Imagination will take you everywhere."
- Albert Einstein

Contents

List of original publications

The thesis is based on the following original papers, which will be referred in the text by their Roman numerals I-V.

I   Friman, V-P., Hiltunen, T., Laakso, J. & Kaitala, V. 2008. Availability of prey resources drives evolution of predator-prey interaction. *Proc. R. Soc. Lond. B*, **275**, 1625-1633.

II   Friman, V-P., Laakso, J., Koivu, M. & Hiltunen, T. Temporal variation in productivity can shape the eco-evolutionary dynamics of predator-prey interaction. Manuscript.

III   Friman, V-P. & Laakso J. Temporal variation in productivity can weaken the effect of evolution on ecological dynamics of predator-prey interaction. Manuscript.

IV   Friman, V-P., Laakso, J. & Mikonranta, L. Protozoan predation and prey resource availability affects the adaptive radiation of prey bacterium *S. marcescens*. Manuscript.

V   Friman, V-P., Lindstedt, C., Hiltunen, T., Laakso, J. & Mappes, J. Predation on multiple trophic levels shapes the evolution of pathogen virulence. Manuscript.

Contributions

	I	II	III	IV	V
Original idea	VF, JL & TH	VF & JL	VF	VF & JL	VF, CL, TH, JL & JM
Experimental work	VF, TH & JL	VF & MK	VF	VF & LM	TH, CL, VF
Statistical analysis	VF & JL	VF	VF	VF	VF
Manuscript	VF, JL, TH & VK	VF, JL, TH	VF & JL	VF & JL	VF, CL, TH, JL & JM

VF: Ville-Petri Friman, JL: Jouni Laakso, TH: Teppo Hiltunen, VK: Veijo Kaitala
CL: Carita Lindstedt, JM: Johanna Mappes, LM: Lauri Mikonranta, MK: Maija Koivu

Abstract

Productivity is predicted to drive the ecological and evolutionary dynamics of predator-prey interaction through changes in resource allocation between different traits. However, resources are seldom constantly available and thus temporal variation in productivity could have considerable effect on the species' potential to evolve. To study this, three long-term microbial laboratory experiments were established where *Serratia marcescens* prey bacteria was exposed to predation of protist *Tetrahymena thermophila* in different prey resource environments. The consequences of prey resource availability for the ecological properties of the predator-prey system, such as trophic dynamics, stability, and virulence, were determined. The evolutionary changes in species traits and prey genetic diversity were measured. The prey defence evolved stronger in high productivity environment. Increased allocation to defence incurred cost in terms of reduced prey resource use ability, which probably constrained prey evolution by increasing the effect of resource competition. However, the magnitude of this trade-off diminished when measured in high resource concentrations. Predation selected for white, non-pigmented, highly defensive prey clones that produced predation resistant biofilm. The biofilm defence was also potentially accompanied with cytotoxicity for predators and could have been traded off with high motility. Evidence for the evolution of predators was also found in one experiment suggesting that co-evolutionary dynamics could affect the evolution and ecology of predator-prey interaction. Temporal variation in resource availability increased variation in predator densities leading to temporally fluctuating selection for prey defences and resource use ability. Temporal variation in resource availability was also able to constrain prey evolution when the allocation to defence incurred high cost. However, when the magnitude of prey trade-off was small and the resource turnover was periodically high, temporal variation facilitated the formation of predator resistant biofilm. The evolution of prey defence constrained the transfer of energy from basal to higher trophic levels, decreasing the strength of top-down regulation on prey community. Predation and temporal variation in productivity decreased the stability of populations and prey traits in general. However, predation-induced destabilization was less pronounced in the high productivity environment where the evolution of prey defence was stronger. In addition, evolution of prey defence weakened the environmental variation induced destabilization of predator population dynamics. Moreover, protozoan predation decreased the *S. marcescens* virulence in the insect host moth (*Parasemia plantaginis*) suggesting that species interactions outside the context of host-pathogen relationship could be important indirect drivers for the evolution of pathogenesis. This thesis demonstrates that rapid evolution can affect various ecological properties of predator-prey interaction. The effect of evolution on the ecological dynamics depended on the productivity of the environment, being most evident in the constant environments with high productivity.

1 Introduction

1.1   The ecological play of predator–prey dynamics in an evolutionary theatre

Ecologists have usually treated predator and prey populations consisting of homogenous sets of individuals, rather than genetically diverse populations capable of evolution (Johnson & Agrawal 2003). Recent findings, however, suggest that interactions between predator and prey populations can result from both ecological and rapid evolutionary responses of prey or both prey and predator (Nakajima & Kurihara 1994; Thompson 1998; Yoshida *et al.* 2003; Yoshida *et al.* 2004; Meyer & Kassen 2007). For example, rapid evolution of prey or host defences has been shown to lead to reduction in the strength of top-down regulation on consumers in host-parasite (Bohannan & Lenski 1999) and predator-prey systems (Friman *et al.* 2008), and to changes in the phase of predator-prey cycles (Yoshida *et al.* 2003), or in the course of adaptive radiation in prey (Meyer & Kassen 2007). Rapid evolution of prey traits can also have important correlated consequences on other species interactions. Predation by free-living protozoa has been suggested to increase survival of pathogenic bacteria outside their host (Matz *et al.* 2005) and to maintain genetic diversity required for evading the host immune system (Wildschutte *et al.* 2004). However, at the same time the evolutionary processes are dependent on the prevailing environmental conditions (Bohannan & Lenski 1999; Yoshida *et al.* 2003; Lopez-Pascua & Buckling 2008; Friman *et al.* 2008; Hall *et al.* 2008), which could fundamentally affect species' potential to evolve (Abrams 2000). Thus, not only the evolution can have a profound influence on ecosystem functioning, but also the ecosystem properties can have a profound influence on evolution (Fussmann *et al.* 2007). Yet, despite the growing number of examples (Thompson 1998; Fussmann *et al.* 2007), the ecological and evolutionary processes are still often thought to occur in different timescales and there are rather few experimental studies considering the interplay between ecological and evolutionary processes simultaneously.

1.2   Evolution of prey, predator or both?

From prey's perspective, predation is expected to select for those prey individuals that are capable of defending themselves over those who are not (Abrams 2000). Increase in the prey defensive ability is further thought to select for predators that can evolve to become better at consuming more defensive prey types, giving rise to the co-evolutionary arm's race (Dawkins & Krebs 1979). However, experimental studies and the current theory predict that the rapid evolution of prey is more likely than the evolution of predators (Abrams 2000; Friman *et al.* 2008). This is because predators are

thought to exert stronger selection pressure on preys than preys on predators (Vermeij 1987; Vermeij 1994). This asymmetry is often described as "life versus dinner" dichotomy where unsuccessful predation event means further reproduction opportunity for the prey but only missed lunch for the predator (Dawkins & Krebs 1979). Moreover, preys typically have shorter generation times and larger population sizes compared to predators, which allows their faster evolutionary response compared to predators (de Visser *et al.* 1999; Abrams 2000).

Co-evolutionary dynamics have been found in wide range of interspecific interactions; between vertebrates and their prey (Benkman *et al.* 2001; Brodie *et al.* 2002), insects and plants (Thompson & Cunningham 2002; Zangerl & Berenbaum 2003; Nielsen & Jong 2005), fungi and plants (Thrall *et al.* 2002), parasitoid phage and host bacteria (Bohannan & Lenski 1999; Buckling & Rainey 2002a; Forde *et al.* 2004; Forde *et al.* 2007), and parasite trematodes and host snails (Lively & Dybdahl 2000). Still, in predator-prey systems most often only the prey has been observed to evolve suggesting that the co-evolutionary dynamics could play small role in the predator-prey interaction (Yoshida *et al.* 2003; Meyer and Kassen 2007; Friman *et al.* 2008; Hall *et al.* 2008). Nonetheless, recent studies show that the evolution of predators is also possible. For example, nematode *Caenorhabditis elegans* can evolve to use previously lethal *Pseudomonas aeruginosa* bacteria as its food resource (Navas *et al.* 2007), and the bacterial predator, *Myxococcus xanthus*, can evolve to become more efficient in finding its prey bacteria when the prey density is low (Hillesland *et al.* 2008). Therefore, the evolution of predator, or the co-evolution between prey and predator, could both be possible outcomes of the predator-prey interaction.

The reciprocal selection does not always lead to co-evolution because the strength of selection can vary e.g. geographically (Thompson 2005). For example, some local interactions can show selection on neither or only on one of the species, while some local interactions exhibit strong reciprocal selection on the interacting species (Thompson 2005; Lopez-Pascua & Buckling 2008). Thus, the evolutionary responses of predator-prey interaction could also depend on the abiotic properties of the selective environment.

1.3 The role of productivity in the evolution of predator-prey interaction

Productivity of the environment could have drastic effects on the evolutionary and ecological dynamics of predator-prey interaction (Abrams 2000; Bohannan & Lenski 2000; Yoshida *et al.* 2003; Hall *et al.* 2008). Increase in the productivity is likely to increase the population sizes of prey and predator, which can facilitate the evolution of predator-prey interaction in two ways. First, the increase in population sizes will increase genetic supply for new mutations on which selection for defence and counter-defence can act. Second, encounter rates between preys and predators will be greater, imposing stronger selection for traits of predator and prey (Hochberg & Holt 1995;

Hochberg & van Baalen 1998). However, increasing allocation to defence is often found to incur costs, e.g. the prey defence trades off with its competitive ability. Trade-offs could thus constrain the evolution of prey defence when the productivity is low and the ability to exploit resources efficiently is favoured (Mole 1994; Leibold 1996; Bohannan & Lenski 2000; Yoshida *et al.* 2004; Friman *et al.* 2008; Hall *et al.* 2008). In contrast, when resources are abundant prey should be able to invest in both defensive and competitive traits simultaneously because the excess of resources cancels out the fitness cost of defence (Bohannan *et al.* 2002; Yoshida *et al.* 2004). From these predictions it follows that the evolution of costly defence is more likely in high resource environments where the resource competition has smaller role for prey fitness (Leibold 1996; Hochberg and van Baalen 1998). Recent experiments done with bacterial prey and protozoan predator support these predictions. For example, the evolution of prey defence has been found to be more evident in environments with high productivity (Friman *et al.* 2008; Hall *et al.* 2008). In addition, the studies done with bacterial hosts and their parasitic viruses suggest that increase in productivity can accelerate the rate of host-parasite co-evolution (Lopez-Pascua & Buckling 2008) and the emergence of defending host types (Bohannan & Lenski 1999). Thus, the environmental control of trade-offs offers a potential link by which prey resource availability and predation could affect the ecological properties of predator-prey community through prey evolution (Yamauchi & Yamamura 2005).

Many environmental conditions, such as productivity, vary commonly across landscapes and in time (Rosenzweig 1995). For example, the mast fruiting by trees, periodic irruptions of palatable insects, or storm-induced transport of organic nitrogen and phosphorus to aquatic systems are typical properties of terrestrial and aquatic ecosystems characterized by temporal variation in the availability of resources (Ostfeld & Keesing 2000). However, we are currently short of experimental data considering the effects of temporal variability of environmental factors on the ecological and evolutionary dynamics of species interactions, even though it might be common characteristic of natural communities.

Temporal variation in productivity could affect the evolution of predator-prey interaction by increasing the amount of variability in the population densities (Luckinbill & Fenton 1978; Drake & Lodge 2004; Becks *et al.* 2005), and by affecting the species' ability to allocate resources effectively between different traits (Bohannan *et al.* 2002; Yoshida *et al.* 2004). In the absence of predator evolution, variation in predator densities could cause the strength of selection for prey defences fluctuate in time (Levins 1968; Hairston & Dillon 1990; Yoshida *et al.* 2003; Yoshida *et al.* 2007). At the same time, prey ability to allocate resources between defence and other traits can depend on the productivity of the environment because the degree in which allocation to one trait reduces the allocation to another, has been shown to increase when the productivity decreases (Bohannan *et al.* 2002; Yoshida *et al.* 2004; Friman *et al.* 2008). Thus, the trade-offs could constrain the evolution of prey defence when the resources are in short supply and the high cost of defence increases the strength of apparent competition between defending and non-defending prey types (Armstrong 1979; Holt *et al.* 1994; Abrams *et al.* 1998). In contrast, strong prey allocation to defence could be

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selected during the high productivity periods because the resource exploitation is less important for prey fitness and the magnitude of trade-offs are small (Holt *et al.* 1994; Leibold 1996; Bohannan & Lenski 2000; Bohannan *et al.* 2002; Yoshida *et al.* 2004; Friman *et al.* 2008; Hall *et al.* 2008). From these predictions it follows, that the temporal variation in productivity is likely to constrain the evolution of defensive prey types at least in two ways. First, if temporal variability in prey resource availability increases variation in predator densities, also the selection for prey defence could fluctuate in time. Secondly, temporal variation in prey resource availability could lead to alternation of selective environments, some of which allow increased allocation to costly defence (abundance of resources), while other environments favour selection for prey types good at competing for resources (shortage of resources). This could lead the selection for prey defence fluctuate according to the resource availability of the environment. Selection in fluctuating resource environment could lead to cycling of prey genotypes differing in their defensive and competitive ability (Yoshida *et al.* 2003; Yoshida *et al.* 2007), or to evolution of generalist prey genotypes good at defending and competing simultaneously (Fututyama & Moreno 1988; Reboud & Bell 1997; Kassen & Bell 1998; Kassen 2002). Whereas in the constant environment, the selective environment could remain rather similar over time, leading to directional selection for traits conferring the best fitness in the given environment, i.e. evolution of specialists (Fututyama & Moreno 1988; Kassen 2002).

In most of the experimental evolutionary studies to date, temporally varying environment has been imposed on simple one species model systems, e.g. algae to changing light conditions (Kassen & Bell 1998), bacteria (Bennett *et al.* 1992; Bennett *et al.* 1993) or protozoa (Ketola *et al.* 2004) to varying thermal environment and viruses to different toxin combinations (Gao *et al.* 1992). However, there are only few experimental studies considering the effects of temporal environmental variation on the ecological and evolutionary dynamics of the species interactions or more complicated multispecies communities (reviewed in Kassen 2002).

1.4   Rapid prey evolution can shape the ecological dynamics of predator-prey interaction

Predator-prey interaction is fundamental for the transfer of energy from the basal to higher trophic levels across plant and animal kingdoms and is ultimately governed by the productivity of the environment. In food chains, the basal productivity will affect first the growth potential of the consumer (prey) and consequently the densities of predators feeding on prey individuals (Oksanen *et al.* 1981). As a result, increasing productivity should channel to an increase in predator density (Oksanen *et al.* 1981), and thus, increase in the productivity of the environment could increase the food chain length in the absence of evolution (Kaunzinger & Morin 1998). However, this ecological prediction can change qualitatively if prey can evolve defensive strategies in response to predation (Bohannan & Lenski 1999; Bohannan & Lenski 2000; Yoshida *et*

al. 2003; Yoshida *et al.* 2004; Meyer & Kassen 2007; Friman *et al.* 2008; Hall *et al.* 2008). For example, the evolution of defensive prey types can reduce the transfer of the energy from basal to higher trophic levels because resources increase mainly the biomass of defensive prey types less edible for the predators (Bohannan & Lenski 1999; Friman *et al.* 2008). Thus, increasing productivity could lead to decrease in the relative strength of the top-down regulation on prey community, limit the transfer of the energy and possibly the length of the food chains. However, the way rapid prey evolution affects the trophic dynamics of predator-prey system in environments where the productivity varies temporally has not been studied experimentally.

Temporal fluctuations in productivity could affect the stability of predator-prey interaction (Ripa *et al.* 1998; Abrams 2000; Ranta *et al.* 2006). For example, increasing the supply of limiting nutrients is predicted to lead to the "paradox of enrichment", i.e. decreased stability of the prey and predator population dynamics (Rosenzweig 1971). According to this theory the enrichment of ecosystem might not lead to an increase in the yield of the desired predator species, but increase the variability of populations, and at the same time increase the probability of species' extinctions (Rosenzweig 1971). In addition, predation could decrease the stability of interacting populations by causing chaotic (Becks *et al.* 2005) or cyclic dynamics (Yoshida *et al.* 2003). However, recent theoretical studies show that the evolution of prey traits could stabilize species interactions (Abrams & Matsuda 1997; Abrams 2000; Kondoh 2003; Yamauchi & Yamamura 2005; Kondoh 2007; Mougi & Nishimura 2008) and these theoretical predictions are supported by some experimental data (Bohannan & Lenski 1999; Friman *et al.* 2008). However, there is currently no clear consensus about the combined effects of rapid evolution and temporal environmental variation on the stability of predator-prey interaction (Fussmann *et al.* 2007).

The diversification of a lineage into a range of ecologically and phenotypically distinct species, i.e. adaptive radiation, is considered responsible for much of life's diversity (Hedges *et al.* 1996; Benton 1996). Both predation and competition for resources have been suggested as mechanisms driving this process (Van Valen 1974; Schluter 2000). Environmental productivity and intensity of predation are independently expected to have a unimodal relationship with prey diversity (Connell 1978; Tilman 1982; Abramsky & Rosenzweig 1984; Abrams 1995; Flöder *et al.* 1999; Buckling *et al.* 2000; Kassen *et al.* 2000). Productivity driven diversification could be constrained in low-resource environments to phenotypes that can only grow at low resource concentrations (Kassen *et al.* 2000; Hall & Colegrave 2007). Instead, in environments with good resource availability, phenotypes capable to exploit most productive resource or niche are expected to dominate. Predation alone may affect prey adaptive radiation for example by creating additional ecological opportunities in the form of predator-resistant phenotypes (Vamosi, 2005; Nosil & Crespi, 2006). Thus, predation could increase prey diversity because of the combination of direct and apparent competition (indirect competition between prey species through a shared predator, Armstrong 1979; Holt *et al.* 1994), which allows competitive but susceptible prey to coexist with less competitive but more resistant prey. Alternatively, the predator mediated decrease in the prey population density could decrease the intra- or

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interspecific competition between different prey types further limiting the risk of competitive exclusion (Paine 1966; Paine 1969*a*; Paine 1969*b*; Meyer & Kassen 2007). However, if predation is too intense, it may also reduce prey diversity (Sih *et al.* 1985; Cadotte & Fukami 2005). Thus, the effect of predation on prey species diversity can range from positive to negative, depending on various conditions (Chase *et al.* 2002; Chesson & Kuang 2008). Moreover, models combining these two factors predict that the effect of predation on prey diversity depends on the productivity of the environment (e.g. Huston 1994; Kondoh 2001). This is also supported by the recent study by Hall *et al.* (2008) wherein the predation was able to extend the range of resource concentrations where high phenotypic prey diversity was maintained. However, the way predation and productivity interact in adaptive radiation of prey is still somewhat unclear (but see Meyer & Kassen 2007; Hall *et al.* 2008).

In most of the studies to date, the interactive effects of productivity and predation on the evolutionary and ecological dynamics of predator-prey interaction have been tested in constant environments (Meyer & Kassen 2007; Hall *et al.* 2008). However, temporal variation in environmental conditions, such as productivity, could fundamentally change these predictions. Thus, the interplay between evolutionary and ecological dynamics should be also studied in fluctuating environments.

1.5 The effects of predation on evolution of bacterial virulence and host immunity

The pathogen virulence is traditionally thought to co-evolve through reciprocal selection with its host organism. However, in natural communities the pathogens and hosts are typically embedded within a web of interactions with other species, which could affect indirectly the pathogen virulence and host immunity (Schulenberg *et al.* 2008). For example, predation could affect the prevalence of infectious diseases by increasing the frequency of infected individuals in the population (Holt & Roy 2007). Protozoan predation has been suggested to be one of the most important factors affecting the evolution of bacterial virulence (Brüssow 2007). This is because many bacterial defensive traits, such as motility and biofilm formation, are often connected to pathogen virulence factors (Pujol *et al.* 2001; Josenhans & Suerbaum 2002; Matz & Kjelleberg 2005; Malik-Kale *et al.* 2007). For example, protozoan predation can select for more pathogenic bacteria because the increased defence against protozoa confers also resistance for defensive cells of higher organisms (Harb *et al.* 2000). This is due to similarity of digestive enzymes of protozoa and macrophages of multicellular organisms (Harb *et al.* 2000). However, protozoan predation could also lead to evolution of more harmless bacteria if increased allocation to defence is traded off with traits connected to pathogen virulence. Similarly, host's fitness is dependent on its immune system ability to provide protection against parasites and pathogens (Schmid-Hempel 2003; Schulenberg *et al.* 2008). Many selective forces outside the host–pathogen interaction (e.g. predation or sexual selection) could also affect indirectly the strength of the host

immune system through trade-offs because resources are usually limited and the immunity often incurs a cost (Rigby & Jokela 2000). For example, trade-offs between host immune defence and anti-predatory defence has been shown to play important role in determining the structure of natural communities (Edeline *et al.* 2008). However, the evolution of pathogen virulence and host immune defences has been seldom studied experimentally in a wider ecological context where other species interactions typical for natural communities are taken into account.

1.6 Microbial experimental ecology - studying evolution and ecology in a bottle

Understanding the distribution and abundance of organisms that are embedded in complex, dynamic systems of interactions pose great challenge for general evolutionary ecology. Laboratory experimental systems offer one approach for unravelling this complexity. Microbial experimental systems have played a central, but sometimes underappreciated, role in ecological history (reviewed by Jessup *et al.* 2005). W. D. Dallinger is one of the firs pioneers who described already in 1887 his attempt to discover ''*whether it was possible by change of environment, in minute life-forms, whose life-cycle was relatively soon completed, to superinduce changes of an adaptive character, if the observations extended over a sufficiently long period*'' (Dallinger 1887). Dallinger addressed this question using populations of protists as an experimental system, altering their environment by varying the temperature of the cultures. His experiments -demonstrated that ecological specialization can incur a cost of adaptation (a decline in competitive fitness in environments other than the one to which the organisms have specialized) and that it was possible to study such phenomena with laboratory experimental systems (Jessup *et al.* 2005). Later on G.F. Gause continued this earlier work and asked ''*why has one species been victorious over another in the great battle of life?*'' (Gause 1934). He used experimental systems containing bacteria, yeast, and protists and coupled his laboratory experiments with the mathematical models of competitive and predator-prey interactions first proposed by Alfred Lotka and Vito Volterra (Jessup *et al.* 2005). Gause was able to predict which of two species of *Paramecium* would be competitively dominant by estimating the growth parameters for each of the species grown alone (Gause 1934). Subsequent interpretation of this work led to the development of the principle of competitive exclusion (Hardin 1960).

Since Gause's pioneering experiments, microbial experimental systems have been used to study many central topics in ecology including succession (Gorden *et al.* 1979), the diversity stability relationship (Hairston *et al.* 1968; Van Voris *et al.* 1980), predator-prey interactions (Luckinbill 1973; van den Ende 1973; Luckinbill 1974; Luckinbill 1979), and the co-existence of competitors (Vandermeer 1969; Tilman 1977; Sommer 1984; Tilman & Sterner 1984). Especially the studies done with host bacteria and their parasitic viruses (bacteriophages) have made important contributions

to the study of antagonistic co-evolution (Chao *et al.* 1977; Levin *et al.* 1977, Levin & Lenski 1985; Lenski 1988; Bohannan & Lenski 1997; Bohannan & Lenski 1999; Buckling *et al.* 2000; Buckling & Rainey 2002a; Buckling & Rainey 2002b; Morgan *et al.* 2005).

The popularity of microbial laboratory experiments in ecology has rapidly grown, apart from general interest on microbes, because they offer explicit control and replication of the experiment. In addition, the small size and the short generation time of microorganisms facilitate experiments across a wide range of spatial and temporal scales and enable the study of evolution in action. The abundance of genetic and physiological information available for the most commonly used microorganisms allows detecting the evolutionary changes at the level of genes. Furthermore, microorganisms are amenable to genetic manipulation and to long-term storage in a state of suspended animation, which allows researcher to compare the evolutionary changes of certain selection lines relative to the ancestral populations. With these advantages, the complexity of nature can be dissected into its component parts, which enables ecologists to analyze each part's role in isolation and in combination. Thus, microbial experimental systems provide an important link between theory and the complexity of nature.

These advantages do however confer also some limitations. For example, the small scale of microorganisms can make it difficult to explicitly impose and maintain environmental heterogeneity at small scales. Further, due to their effective use of resources, the studies of small population sizes are challenging. Rapid evolution can be also so fast that the researcher fails to characterize it and the generalization of the results can be at times difficult. However, no experimental study system is perfect. Thus, the major challenge all experimenters face is matching research questions with appropriate experimental systems.

2 Aims of the thesis

The main aim of this thesis is to study experimentally how the productivity of the environment (quality and the temporal variation in resource availability) affects the evolutionary outcomes of predator-prey interaction, and further, how the evolutionary changes in species properties feed back to the ecological properties of the predator-prey community. To study this, three long-term microbial laboratory experiments were established where *Serratia marcescens* prey bacteria was exposed to predation of single celled protist *Tetrahymena thermophila* (Protozoa: Ciliates) in different prey resource environments (Table 1). Low and high productivity environments were used in the experiment I. Constant or temporally varying resource environments were used in the experiments II and III. The species population dynamics and the evolutionary changes in prey and predator traits were measured at the level of populations (I-III). The changes in trophic dynamics, stability and prey diversity were considered as the ecological properties of the predator-prey system. The experiments I-III aimed to answer following questions:

- Which species evolve: prey, predator or is co-evolution observed?
- How the type of prey resource environment affects the evolution of predator-prey interaction?
- How evolution affects the ecosystem properties of predator-prey system depending on the resource environment?

The interactive effect of predation and productivity on the adaptive radiation of prey was studied at the level of bacterial clones (isolated from the experiment I) in the experiment IV (Table 1). In addition, the importance of different prey defence mechanisms was assessed. The experiment IV aimed to answer following questions:

- Does predation-driven adaptive radiation of prey depend on the productivity of the environment?
- What defensive mechanisms prey evolves against protozoan predation?

The role of protozoan predation on the evolution of *S. marcescens* virulence was studied in the experiment V by using insect host (Table 1). Two selection lines of aposematic moth (*Parasemia plantaginis*), differing in the size of orange-black patterned warning signal used to advertise unpalatability to avian predators, were used for pathogen virulence measurements (Lindstedt *et al.* 2008, Fig. 3). The experiment V aimed to answer following questions:

- Does protozoan predation affect the evolution of bacterial virulence?
- Does pathogen success depend on the host allocation to other fitness related traits besides immunity, e.g. effective warning signal?

3 Materials and methods

3.1   The study species

The prey bacterium *Serratia marcescens* is cosmopolite heterotrophic bacterium and a common facultative pathogen with broad host range covering plants, nematodes, insects, fishes and mammals (Tan 2002). It is commonly found from many different habitats ranging from soil to aquatic environments (Grimont & Grimont 1978). Generation times of one hour or less have been reported for *S. marcescens* when cultured in glucose (Tagaki *et al.* 1985). However, bacterial growth is highly dependent on the resource concentration and main source of carbon (Tagaki *et al.* 1985) and could be considerably slower or faster. The *S. marcescens* strain used in all the experiments of this thesis was originally isolated from pond water and was attained from the American Type Culture Collection (ATCC strain #13880). This strain is capable of producing red pigment called prodigiosin, while some other *S. marcescens* strains are completely white, as they have lost the ability of prodigiosin synthesis. The synthesis of prodigiosin in *Serratia* is also unlikely to represent a selectively neutral trait. For example, white colonies of *S. marcescens* have been found to be resistant against phage Kappa (Paruchuri & Harshey 1987) and to be important causal agents of cucurbit yellow vine disease (Bruton *et al.* 2003). In addition, opportunistic *Serratia* strains isolated from human patients are usually white (Grimont & Grimont 1978; Ding & Williams 1983; Tan 2002) while the red pigment has been shown to be important antifungal factor of *S. marcescens* (Someya *et al.* 2001). The prodigiosin-containing supernatant of *S. marcescens* cultures has been also reported to show cytotoxic activity in cancer cell lines (Deorukhkar *et al.* 2007) and to cause apoptosis in haematopoietic cancer cells (Montaner *et al.* 2000).

     The predator *Tetrahymena thermophila* is a well-studied free-swimming particle-feeding protozoon that preys upon numerous bacteria (Hill 1972; Elliot 1974; Fenchel 1987). The *T. thermophila* strain used in all the experiments of this thesis was originally isolated from fresh water and was attained from the American Type Culture Collection (ATCC strain #30008). *T. thermophila* is typically 30 μm long but large changes in size can occur under stress, e.g., food depletion and thermal stress (Laakso *et al.* 2003). *T. thermophila* feeds on bacteria and nonliving particles, and macromolecules (pinocytosis) and it reproduces asexually through binary fission (Elliot 1974). Optimal growth with a generation time close to 2 hours has been observed near 35°C for *T. thermophila* when cultured in proteose-peptone medium (Frankel & Nelsen 2001). However, when grown in 25°C and with bacterial food resource, the *T. thermophila* generation times are longer. *T. thermophila* has been widely used in many microcosm experiments (e.g. Nakajima & Kurihara 1994; Bukharin & Nemtseva 2001; Laakso *et al.* 2003; Ketola *et al.* 2004; Brandl *et al.* 2005; Meyer & Kassen 2007).

     The aposematic wood tiger moth larvae (*Parasemia plantaginis*, Arctiidae) was used as insect model host in the experiment V. The species has a wide

distribution cross the northern Palaearctic region. The larvae of *P. plantaginis* are polyphagous and feed on numerous herbaceous and arborescent plant species (e.g. Chinery 1993, Ojala *et al.* 2005). The larvae are hairy and have moderately conspicuous colouration comprising an orange patch on an otherwise black body (Fig. 3). The size of this orange patch varies continuously (Ojala *et al.* 2007; Lindstedt *et al.* 2008; Lindstedt *et al.* 2009). The larvae have 5-7 instars, the first two of which are cryptically coloured; orange-black colouration develops at the third instar (Ojala *et al.* 2007). In Finland, this species usually has only one generation per year and typically *P. plantaginis* overwinters as 3rd – 4th instar larva.

3.2   The culture media

Three different media were mainly used for cultivating prey bacteria and predatory protozoa in the experiments. When the prey was grown alone or in the presence of predators, prey culture medium made of phosphate-buffered hay extract (Friman *et al.* 2008). The low and high concentration of prey culture media contained 0.54 and 2.15 mg l^{-1} final concentration of plant detritus respectively. As *T. thermophila* is not able to feed directly on prey culture medium, *S. marcescens* and *T. thermophila* occupied separate trophic levels when cultured together. Prey bacteria were also grown on nutrient broth agar plates, e.g. to isolate clones and count the number of individuals in a sample (Friman *et al.* 2008). When predators were grown alone, they were fed with non-living predator culture medium consisting of proteose peptone and yeast extract (Friman *et al.* 2008).

3.3   The microcosms and sampling

Three different kinds of microcosms were used in the experiments. In the experiment I, the microcosms were made of 250 ml polycarbonate Erlenmeyer flasks capped with membrane filters (Corning) and incubated as static batch cultures (Fig. 1). Microcosms were designed to endure contamination risk for long period (6 months) in non-sterile conditions and were thus entirely closed from the outside world. In the experiment II, the microcosms were also made of 250 ml polycarbonate Erlenmeyer flasks but the microcosms were equipped with tubes connected to computer-controlled peristaltic pumps regulating the inflow and outflow of the medium (Fig. 2). Thus, the microcosms in the experiment II resembled chemostats. In order to prevent bacterial contamination, thermostatically controlled heaters were wrapped around the tubes connecting the microcosms to the sterile resource stock bottles in the experiments (I and II). In addition, the outlet tubes were submerged in 80% ethanol. This kind of microcosm setting allowed precise control of the dilution rate of prey culture medium. However, due to length of tubing connecting the microcosms to resource stock bottles and outlet

tube, sampling of microcosms was possible only once a week when it was possible to empty tubing before sampling. In the experiment III, 50 ml loosely capped plastic centrifuge tubes were used as microcosms and incubated as static batch cultures in sterile conditions. This kind of setting allowed sampling of microcosm on daily basis.

In the experiments I and III, microcosms were shaken gently before sampling. In experiment II, the contents of the microcosms were mixed with a magnetic stirrer at 440 rpm for 1 minute prior to sampling. The samples were taken with inbuilt syringes in the experiments I and II while pipette was used in the experiment III. Samples were handled in sterile conditions in all experiments.

3.4 The experimental designs, set up of the experiments and sampling

3.4.1 Experiments I-III

In the experiments I-III, a single clone of prey bacteria *Serratia marcescens* capable of producing red pigment prodigiosin was used to establish the different selection lines. When the experiments were started, bacteria were first grown into carrying capacity in the prey culture medium used in the experiments. After that, bacterized prey culture medium was divided in equal volumes to microcosms. First main factor used in the experiments was predation, i.e. the absence or the presence of predation. Second main factor was the type of the prey resource environment (see below). In the experiments I-III, 1 ml of asexually reproducing strain of the protozoan predator *T. thermophila* was introduced to half of the prey selection lines. The other half was retained as control treatments without predators. Half of the replicates from both treatments were subsequently exposed to different prey resource environments giving four different treatments with equal amount of replicates. In the first experiment, prey was let evolve in the absence or presence of predators in low and high concentration prey culture media (I). In the second experiment, prey was let evolve in the absence or presence of predators in constant and temporally varying prey culture media (II). In the third experiment, prey was also let evolve in the absence or presence of predators in constant and temporally varying prey culture media. In addition, the bacterial biofilm (bacterial communities that are attached to a surface) was removed from the half of the all treatments weekly by transferring the experimental populations to new microcosms after the sampling (III).

In the experiment II, the temporal variation in prey resources was generated by using computer programmed peristaltic pumps: in the constant environment treatment, prey resource flow was kept throughout 1.4 ml h^{-1}, whereas in variable environment it was periodic, alternating between 5 days flow of 2.8 ml h^{-1} to 5 days periods of zero flow. Thus, the average resource flow rate was equal in both environments for the whole duration of the experiment (II). In the experiment III, the

fluctuations in prey resource flow rate were generated manually by transferring the liquid out and in to the microcosms with a pipette. The prey resource turnover was 3 ml day^{-1} (12%) throughout the experiment in the constant environment treatment, whereas in the temporally varying treatment it was periodic, alternating between 6 days flow of 1.5 ml day^{-1} (6%) and 1-day flow of 12 ml day^{-1} (24%)(III). Thus, the average resource flow rate was equal in both environments for the whole duration of the experiment (21 ml week^{-1}) (III). Predators were also grown alone in the experiment I-III on non-living predator culture medium. These control predators were used later as naïve predators when the evolutionary changes in prey defences and predator counter-defences were measured.

Microcosms were incubated at 25°C in all experiments and sampled weekly (I and II) or daily (III) during the experiment. Prey population sizes (biomass) were estimated as turbidity at 460-580 nm with spectrophotometer (Bioscreen C$^{®}$, wideband option) and population sizes of protozoa counted using image analysis (for details see Laakso *et al.* 2003). Temporal stability of population sizes, prey defensive and competitive traits, and predator-to-prey ratio were estimated from the time series data as coefficient of variation (s.d. mean^{-1}) of each microcosm (i.e. the higher the coefficient of variation, the lower the stability).

3.4.2 Experiment IV

In the experiment IV, total of 192 *S. marcescens* bacterial clones were isolated from the frozen samples originating from a long-term evolution experiment (I) and the importance of predation and productivity for the adaptive radiation of prey were analysed at the level of single clones (genotypes). Twelve clones were isolated per replicate selection line and four replicates were used per every treatment, giving 48 isolated clones per treatment (prey evolved alone or in the presence of predators in the low and high concentration of prey culture medium). In addition, 12 ancestral *S. marcescens* clones were isolated for measurements. All the prey clones' traits were measured in low- and high resource concentrations of prey culture medium to determine if the magnitude of the trade-offs between different prey traits was affected by the measurement resource concentration. In order to test the existence of alternative defence strategies and multiple trade-offs in *S. marcescens*, prey resource use ability (maximum growth rate and population size) and potential prey defence mechanisms (prey motility, biofilm formation ability, and toxicity for the predator) were measured from isolated clones.

3.4.3 Experiment V

The experiment V focused on the correlated effects of protozoan predation on prey bacterium's virulence (a pathogen's ability to cause diseases) in insect host (aposematic wood tiger moth, *Parasemia plantaginis*, Arctiidae). Two artificially selected host

larvae lines differing in allocation to defence against avian predation (i.e. having small or large sized warning signal, Lindstedt 2008; Lindstedt *et al. in press*) were infected with three different *S. marcescens* strains (Fig. 3). The bacterial strains included 1) an ancestor *S. marcescens* strain (ATCC strain #13880), 2) a *S. marcescens* strain evolved in the presence of the protozoan predator *T. thermophila* (Friman *et al.* 2008), and 3) a control *S. marcescens* strain, which had been exposed to similar conditions as the evolved *S. marcescens strain* except for the predator (Friman *et al.* 2008). All three strains used for infection consisted of a mixture of 48 randomly selected clones (four microcosm replicates were used per bacterial strain and 12 clones were isolated randomly per replicate). Bacterial infection was done by injection with Hamilton syringes. Larval survival was monitored for 72 h from infection three times per day by scoring the larvae as dead or alive. In addition, sterilized water was injected for groups of larvae from both small and large signal lines to control the physical damage caused by the injection.

3.5 The evolutionary changes in prey and predator traits

The evolutionary changes in prey and predator traits were measured in separate short-term factorial experiments. The subsamples of prey and predator selection lines isolated the main experiments I-III were grown separately for a total of 72 hours before assessing the evolutionary changes. This equals at least ten of prey and predator generations before the evolutionary measurements. During this time, the physiological state of study organisms is likely to reset, and the observed differences can be considered to be caused by genetic factors. The prey clones were separated from predators by plating on agar plates, and antibiotic treatment was used to separate predators from the prey bacteria. In experiments I-III, prey and predator traits were measured at the level of populations (randomly selected sets of clones). In the experiment IV, prey traits were measured at the level of individual prey clones.

3.5.1 Prey resource use ability

Evolutionary changes in prey resource use ability were assessed in the absence of predator as prey maximum growth rate and maximum population size. The maximum population sizes and maximum growth rates of different selection lines were determined from biomass growth data recorded for 96 hours. Prey maximum growth rate at low density and long-term maximum population size indicate how well prey is able to respond to the addition of fresh resource, and how efficiently resources are used to produce biomass in the long term, respectively. In our experimental settings, these traits most likely reflect prey competitive ability at the time when there is surplus of resources ('maximum growth rate' trait), and when the resources becomes well consumed at the end of the resource renewal cycle ('maximum population size').

3.5.2 Prey defence measured as the prey growth in the presence of predators

Evolutionary changes in prey defence were measured as the prey ability to sustain population size in the presence of predators in liquid medium. This measure takes into account the overall defence of prey and does not differentiate between different defence mechanisms. Before the prey defence measurements, prey selection lines were grown to similar high densities in low and high concentration prey culture media after which small inoculums of predators were introduced to prey cultures. The population size prey could sustain in the presence of predators was measured as optical density for 4 days.

3.5.3 Prey defensive mechanisms

Prey biofilm formation ability was measured in the absence, and in the presence of predators. Shortly, prey bacteria were grown alone or in the presence of predators for four days, after which crystal violet solution was used to stain the bacterial cells attached to the walls of microtitre plate wells. The amount of biofilm formed was determined optically as absorbance at 460-580 nm. Prey clones' motility was assessed as the maximum area (mm^2) colonized within 96h on semi-fluid 0.7% agar plates. Prey toxicity for the predator was measured as predator growth on filtered prey culture media that prey clones had been using for growth for 48 hours.

3.5.4 Evolutionary changes in predator traits

To assess if predators evolved during the experiments the maximum population sizes and growth rates of all predator selection lines were measured in separate short-term experiments on 1) non-living predator culture medium, and 2) on prey bacteria that had evolved previously in the absence or presence of predators.

3.6 Statistical analysis

Data with repeated measurements were analysed with repeated measures ANOVA (I-III). If the sphericity assumption of repeated measures ANOVA was violated, Greenhouse-Geisser corrected F-values were used. Two-way ANOVA was used for multiple comparisons and p-values were adjusted with Bonferroni correction when needed. The genetic variation in experiment IV was determined as the standard deviation of prey clone traits between the replicates within a treatment, i.e. high s.d. indicates high genetic variation in a given prey trait. Temporal stability of population sizes, prey diversity and prey traits were estimated from the time series data as

coefficient of variation (s.d. mean^{-1}) of each microcosm (i.e. the higher the coefficient of variation, the lower the stability).

Two-step cluster analysis was used to classify bacterial clones to different functional groups based on prey resource use ability, defensive ability, motility, prodigiosin expression and the ability to form biofilm in the experiment IV. Clustering was done according the clone performance in low and high productivity environments separately (IV). Chi-square tests to investigate if the functionally different clone groups contained more clones with certain evolutionary history compared to what would be expected if the clones were classified to clusters in random.

The changes in bacterial virulence was analysed with survival analysis (V) using Cox-regression Kaplan-Meier log-rank procedures. The Right censoring method was used to include the larvae that did not die within 72 hours in the analysis.
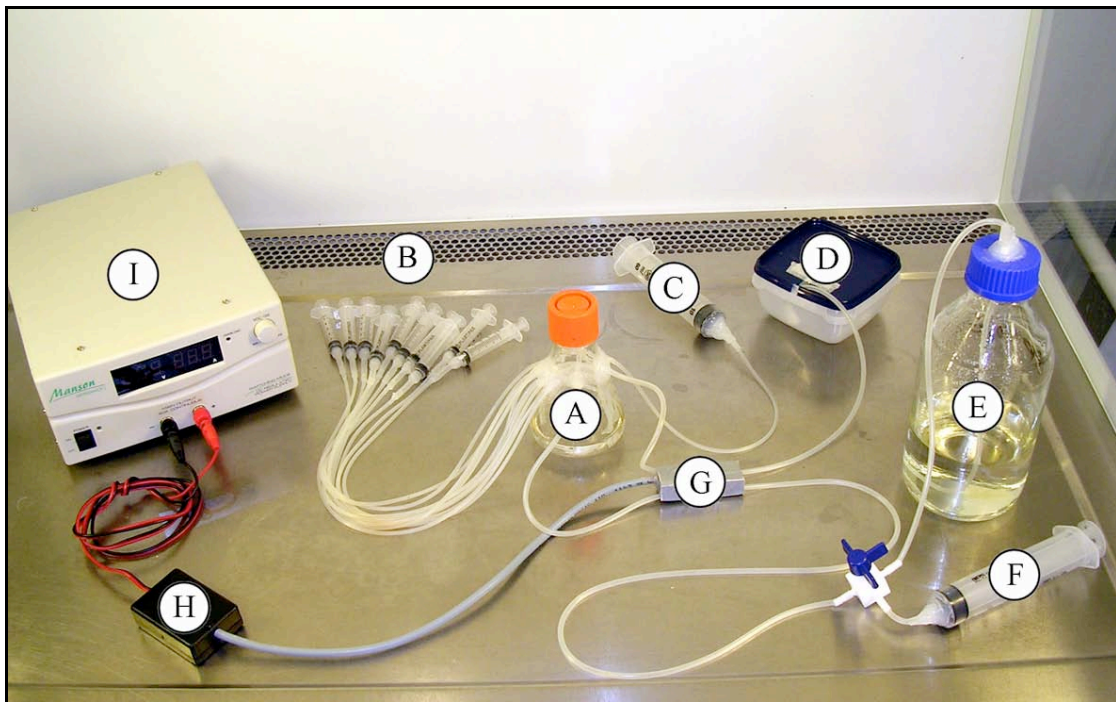
Figure 1. Microcosm design used in the experiment I. A: the microcosm, B: 10 sterile 5 ml syringes for sampling, C: a 50 ml storage syringe, D: Outflow tube submerged in 80 % alcohol, E: the resource stock bottle, F: a 50 ml syringe and 3-way valve for renewal of the resources, G: a heating element, H: a thermostat and I: a power supply for the heating element.
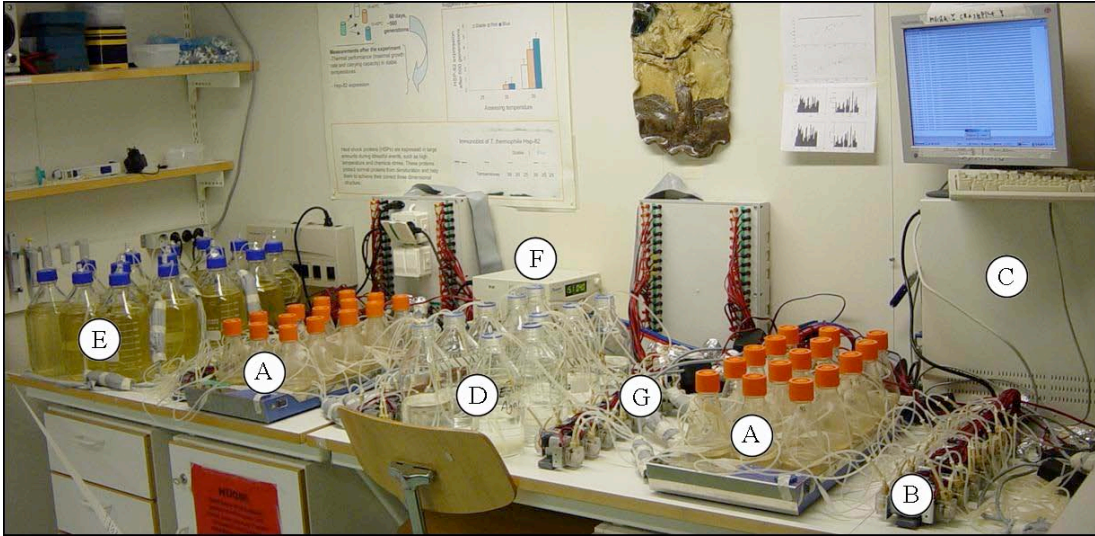
Figure 2. Microcosm design used in the experiment II. A: the microcosms, B: In- and outflow pumps, C: Computer used to control the pumps, D: Outflow tubes submerged in 80 % alcohol, E: the resource stock bottles, G: a heating elements, F: a power supply for the heating elements.
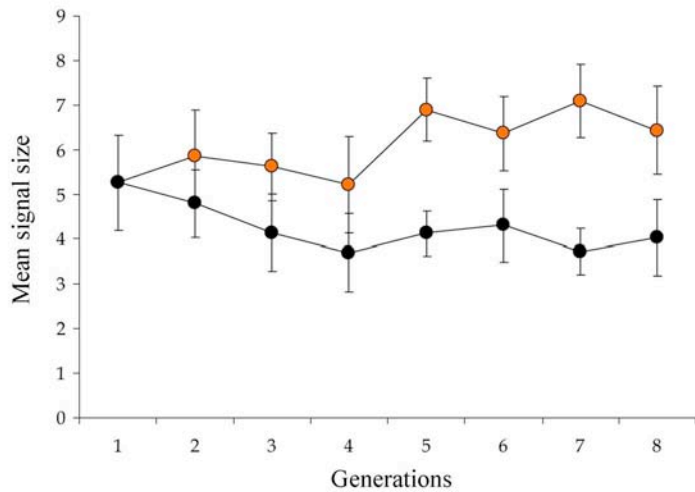
Figure 3. The insect host model used in the experiment V. Left panel photograph (by Eira Ihalainen): host moth *Parasemia plantaginis* larvae with small and large orange warning signal. Right panel: the mean signal sizes (number of orange segments) of the larval selection lines with small (black dots) and large (orange dots) warning signal. Right panel figure originally published in Lindstedt 2008.

TABLE 1   An overview of the experiments included in the thesis

EXPERIMENT	I	II	III	IV	V
Type of study	Microcosm experiment, batch culture	Microcosm experiment, flow-through culture	Microcosm experiment, batch culture	Clone trait measurement from study I	Infection experiment
Outline of the study	Eco-evolutionary dynamics of predator-prey interaction in low and high resource environments, duration: 14 weeks	Eco-evolutionary dynamics of predator-prey interaction in constant and temporally varying resource environments, duration: 8 weeks	Eco-evolutionary dynamics of predator-prey interaction in constant and temporally varying resource environments, duration: 6 weeks	The effect of predation and productivity for the adaptive radiation of prey	The effects of predation on the evolution of pathogen virulence and host immune defence
Experimental manipulations	Predation: yes/no Resources: low/high	Predation: yes/no Resources: constant/temporally varying, Measurement resource concentration: low/high	Predation: yes/no Resources: constant/temporally varying, Biofilm: removal/preservation	Preys' resource history: low/high, Preys' predation history: yes/no, Measurement resource concentration: low/high	Host selection line: small/large warning signal, Preys' predation history: yes/no/ancestor
Main measurements	Population dynamics, prey and predator traits, stability and diversity	Population dynamics, prey and predator traits, stability	Population dynamics, prey and predator traits, stability and diversity	Prey resource use ability, potential defensive traits, genetic variation of prey traits	Bacterial virulence as the host survival, strength of host immune defences between signal lines

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4 Results and discussion

4.1 The evolution of prey defences and predator counter defences

Predation increased prey allocation to defence (measured as the prey ability to sustain population size in the presence of predators in liquid medium) in all the long-term microcosm experiments compared to control treatments where prey evolved alone (I-III). The strength of prey defence depended also on the productivity of the environment and the evolutionary outcomes differed between the experiments (described in more detail below). Predators increased the frequency of white highly defensive *S. marcescens* colony types unable to express red pigment prodigiosin (I, III and IV, Fig. 4). The white colonies of *S. marcescens* have been previously found to be resistant against phage Kappa (Paruchuri & Harshey 1987) and to be important causal agents of cucurbit yellow vine disease (Bruton *et al.* 2003). In addition, opportunistic *Serratia* strains isolated from human patients are usually white (Grimont & Grimont 1978; Ding & Williams 1983; Tan 2002). I suggest that white *S. marcescens* clones could be favoured also because of the resistance against protozoan predation (I, III, IV), and that the colony colour could be related to *S. marcescens* virulence (V).

The bacteria can evolve to avoid protozoan predation by a number of different mechanisms (reviewed in Matz & Kjelleberg 2005). One possible mechanism by which *S. marcescens* could avoid predation is reduced motility, which can increase the prey defence through decrease in predator encounter rate (Monger *et al.* 1992; Gonzáles *et al.* 1993). Interestingly, when the evolutionary changes in prey traits were measured at the level of individual clones the highly defensive white clones were less motile compared to the red clones (IV). This was, however, the case only when the white clones had been previously evolved in the presence of predators (IV). Thus, the *S. marcescens* colony colour can correlate with other fitness traits, but could also be selectively neutral, or phenotypically regulated (Paruchuri & Harshey 1987; Wei & Lai 2006).

The bacteria generally exhibit two distinct modes of behaviour. The first is planktonic form in which single cells swim independently in liquid medium. The second is an attached state in which cells excrete exopolymers, pack closely together, and attach firmly to each other and on surfaces, i.e. form biofilm (O´Toole & Kolter 1998). The shared evolutionary history with predators increased prey ability to biofilm in the presence of predators when measured at the level of populations (II and III) or single prey clones (IV). Especially the white defensive *S. marcescens* clones were efficient in forming the biofilm in the presence of predators (IV). Growing as a biofilm has been shown to give resistance against grazing of *T. thermophila* with *Vibrio cholerae* (Matz *et al.* 2005) and *Pseudomonas fluorescens* (Meyer & Kassen 2007), while predation by flagellate *Rhynchomonas nasuta* can select for grazing-resistant microcolonies in *Pseudomonas aeruginosa* biofilms (Matz *et al.* 2004). Thus, the evolution of predation

resistant biofilm forming prey genotypes could have also conferred direct defence against protozoan grazing for *S. marcescens* (II-IV). Interestingly, the bacterial biofilm defence has been shown to often accompany with cytotoxic chemicals that have anti-protozoal activity (Matz *et al.* 2004; Matz *et al.* 2005; Matz *et al.* 2008). According to my results, the filtrated supernatant of prey bacterial cultures reduced the cell numbers of *T. thermophila* when the prey clones shared evolutionary history with predators (IV). This suggests that the predation resistant biofilm of *S. marcescens* could have also had cytotoxic activity against protozoa.

The white clones that were most efficient in formation of biofilm in the presence of predators, were also the least motile (IV). Recent findings suggest that the bacterial motility and biofilm formation could be antagonistic properties (Àlvarez *et al.* 2006). Thus, it is possible that increasing allocation to predation resistant biofilm was traded off to maintain the energy demanding motility (Josenhans & Suerbaum 2002) and prodigiosin expression. Alternatively, different defence mechanisms could have been used independently of each other, or in different combinations, yielding similar prey fitness.

Even though co-evolutionary dynamics have been shown to play important role in host-parasite interactions between phage and bacteria (Bohannan & Lenski 1999; Buckling & Rainey 2002a; Forde *et al.* 2004), and trematode parasite and snail host (Lively & Dybdahl 2000), it has been seldom observed in the predator-prey interaction. The predator *T. thermophila* was able to evolve better at consuming bacterial resource in one of the three microcosm experiments (II). Becoming more efficient in consumption of bacteria was costly for predator in terms of reduced maximum population size on non-living predator culture medium (II). This result is consistent with recent studies, where *Caenorhabditis elegans* (Navas *et al.* 2007) and bacterial predator *Myxococcus xanthus* (Hillesland *et al.* 2008) has been shown to evolve more efficient in using their prey bacteria as resource. Also in these studies, predator adaptation decreased other important fitness traits such as metabolism (Navas *et al.* 2007), and ability to survive for prolonged starvation (Hillesland *et al.* 2008). Thus, antagonistic fitness costs could constrain the evolution of predator counter-adaptations.

The main difference between the microcosm experiments (I-III) was that the resource turnover was highest in the experiment where the predator evolution was observed (II). Lopes-Pascua & Buckling (2008) have showed recently that increasing productivity can increase the rate of co-evolution between bacterial resistance and phage infectivity. Thus, relatively high productivity probably increased the prey and predator densities, which could have increased the strength of selection for prey defences and predator counter defences by increasing the prey and predator encounter rates. My results suggest that the predator ability to evolve in response to its prey should not be ignored even in relatively short-term microcosm experiments.

4.2   Evolution of prey defence in low and high productivity environments

The prey defence evolved stronger in the high resource environment (I). However, increasing allocation to defence was costly in terms of decreased prey growth rate in the absence of predators in both resource environments (I). Interestingly, the cost of defence was especially clear in the low resource environment (I). These results support the theoretical prediction that when anti-predatory adaptation is costly, evolution of predator-prey interaction can be constrained by prey resource availability (Hochberg & van Baalen 1998; Abrams 2000; Yamauchi & Yamamura 2005). Thus, prey evolution could influence predator prey-interaction less in low resource environments where the allocation to prey defensive traits is constrained more strongly by resource competition.

4.3   Evolution of prey defence in constant vs. temporally varying resource environments

4.3.1   Experiment II

Temporal variability in productivity could weaken the selection for prey defensive traits, because of the productivity-induced fluctuations in predator densities and costly trade-offs associated with defence against predation (Yoshida *et al.* 2004). We found evidence that temporal variation in prey resources at 10-day wavelength increased the variability of prey and predator densities (II). This probably caused the selection for prey defence vary according the predation pressure, which could have led to increase in the variability of prey defensive ability (II). Further, allocation to defence was costly in terms of reduced prey resource use ability (II), suggesting that resource competition could constrain the evolution of prey defence in resource-limited conditions. However, predation increased the prey defence equally in the constant and variable resource environment (II).

I suggest that this could be explained in several ways. It is possible that the fluctuating selection was not strong enough to cause divergence in the strength of prey defence because the magnitude of prey trade-off between defence and resource use ability was small, i.e. increasing allocation to defence decreased the prey resource ability only in minor degree. Alternatively, the realised resource availability of the environment did not differ enough between the environments to create resource-limited conditions in the temporally varying environment. I found evidence for both of these explanations. The magnitude of the prey trade-off between defence and competitive ability was considerably small and only evident when measured in fourfold diluted resource concentration (II). In addition, the magnitude of this trade-off diminished during the experiment (II). The temporal variation in prey resources increased also the prey population densities in the absence of predators, despite the fact that the average

resource flow between the environments was the same (II). Therefore, prey resource pulses increased prey productivity, which is consistent with recent study of Lennon & Cottingham (2008) where this was explained with physiologically based theory on bacterial metabolism. Thus, even though evidence for the fluctuating selection was found, the effect of resource competition may not have been strong enough to constrain the evolution of defensive prey types even in the temporally varying resource environment (II).

Prey ability to form predation-resistant biofilm increased clearly during the experiment especially when the prey was exposed to predation in temporally varying resource environment (II). The variation in resource flow rate could have had larger negative effect on the bacteria living in the free water in the temporally varying resource environment because of the periodically higher mortality caused by the outflow rate (II). Thus, the reduction in the resource competition between bacteria occupying the free-water habitat could have favoured the biofilm forming bacteria more in the temporally varying environment. Formation of biofilm has been also shown to be costly (Spiers *et al.* 2003; MacLean *et al.* 2004). Thus, it could have been also more easily maintained in the temporally varying environment where the bacterial productivity was higher (II).

4.3.2   Experiment III

Clear weeklong cycles in predator densities were observed in the temporally varying resource environment in the experiment III, whereas the predator densities remained more stable in the constant environment (III). This suggests that also the selection for prey defence fluctuated more in temporally varying prey resource environment. Also, increasing allocation to defence was generally costly in terms of reduced prey maximum growth rate. I found that the prey evolved more defensive in the constant resource environment wherein also the increase in the frequency of small and highly defensive white clones (incapable of synthesizing red pigment prodigiosin) was the most evident (III). The high peaks in predator densities observed in temporally varying resource environment suggest that weekly resource pulses turned effectively into biomass of edible prey (III). The resulting high predation pressure was not however able to increase the frequency of defending prey types but only in a small amount in the temporally varying productivity environment (III). Thus, the cost of defence and competition for resources probably constrained the emergence of defensive prey genotypes more in the temporally varying productivity environment (III). The defending prey genotypes emerged in low frequency eventually also in the temporally varying productivity environment (III). This suggests that the temporal variation in prey resources was not able to fully prevent, but rather considerably weakened, the evolution of defending prey genotypes (III).

4.3.3 Summary for experiments II and III

My results suggest that temporal variation in prey resource availability can affect the variability of prey and predator population dynamics. Variation in predation pressure could potentially lead the selection for prey traits fluctuate in time, and consequently, predation could have smaller role for the adaptive radiation of prey in environments where prey resource availability varies in time (III). However, temporal variation in prey resource availability constrained the evolution of defensive prey types only in the experiment III but not in experiment II. This was probably because the magnitude of prey trade-off between defence and resource use ability was smaller in the experiment II. In addition, the temporal variation in resource availability did not probably create strongly resource-limited conditions in the experiment II, suggesting that resource competition was not of big importance in this experiment. In contrast, the magnitude of prey trade-off was clearer in the experiment II. Thus, temporal variation in prey resources could constrain the evolution of defensive prey types only when it creates resource-limited conditions where the magnitude of prey trade-off between defence and resource use ability is large enough to reduce prey ability to compete for resources.

4.4 The effect of measurement resource concentration on prey trade-offs

The magnitude of prey trade-off between defence and resource use ability, i.e. the degree in which allocation to one trait reduces allocation to another, can depend on the resource concentration where the prey traits are measured, i.e. current environmental conditions (Yoshida *et al.* 2004). Therefore, the trade-offs should be more easily observed in resource-limited conditions where the common pool of resources is more easily depleted (Bohannan *et al.* 2002). This pattern was observed in the experiment II, where the more defensive prey types suffered from the decreased resource use ability only when measured in resource-limited conditions. Moreover, the magnitude of this trade-off diminished during the experiment, which suggests that some compensatory mutations could lessen the role of trade-offs for the prey evolution (Björkman *et al.* 2000; Andersson 2003; MacLean *et al.* 2004). In addition, according to the clone level measurements in the experiment IV, increasing the measurement resource concentration diminished the magnitude of the prey trade-off between defence and resource use ability (IV). More clones in general were also able to defend against protozoan predation when measured in the high measurement resource concentration (IV). However, some prey clones were effective at defending regardless of the measurement resource concentration in the experiment IV. Interestingly, these clones were able to produce predator-resistant biofilm in the air-liquid interface of the culture vessels. This suggests that the energetic cost of biofilm formation could be possibly outweighed by the benefits of growing at the oxygen rich air-liquid interface (Hall *et al.* 2008). I suggest that resource-controlled trade-offs can constrain the evolution of prey defence, but also

that the resource-independent prey defensive strategy is possible outcome of predator-prey interaction (IV).

4.5 The effects of prey evolution on the ecological dynamics of predator-prey interaction

4.5.1 Trophic dynamics

Ecologists have long debated if the prey populations are controlled more strongly by their resources or by their predators. According to my result, the relative strength of top-down and bottom-up forces can change because of the rapid evolution of prey defences. However, the effect of prey evolution is affected by the availability (I) and the temporal variability (II-III) of resources in the given environment.

In the experiment I, the fourfold increase in prey resource availability increased mainly the biomass of prey, having no long-term effects on predator numbers (I). This was due to the emergence of less edible, white prey clones that did not suffer markedly from reduced resource use ability under high resource environment (I and IV).

Similar results were obtained also in the experiment II and III. In the experiment III, prey biomass was converted least efficiently to predator biomass in constant prey resource environment where the evolution of prey defence was strongest (III). In the experiment II, predators reached higher biomasses on prey in the temporally varying prey resource environment resulting in higher predator-to-prey ratio (II). However, the predator-to-prey ratios converged between the constant and temporally varying environments towards the end of the main experiment, even though no difference in the overall strength of prey defence between environments was found (II). This was probably due to increased allocation to predator-resistant biofilm observed especially clearly in the temporally varying prey resource environment.

These results suggest that the rapid evolution of defensive traits of prey can be important factor contributing to the weak propagation of enrichment effects to higher trophic levels in aquatic systems. However, low productivity conditions and temporal variation in prey resource availability can weaken the effect of prey evolution on the trophic dynamics of predator prey interaction.

4.5.2 Stability

Resource enrichment and predation are both expected to destabilise food chains in non-evolving predator-prey systems (Rosenzweig 1971; Johnson & Agrawal 2003). However, recent theoretical studies show that the evolution of traits can stabilize species interactions (Abrams & Matsuda 1997; Abrams 2000; Kondoh 2003; Yamauchi & Yamamura 2005; Kondoh 2007; Mougi & Nishimura 2008). Predation destabilized the

prey population dynamics in general in the experiment I. However, predation destabilised the dynamics of prey populations, prey genetic diversity and resource use ability less in the high productivity environment (I). This supports the idea that the resource enrichment can stabilize the predator-prey interaction when the prey is able to evolve and the fitness costs of anti-predatory traits diminish with resource enrichment.

Temporal variation in prey resources increased the variability of predator populations in experiment II and caused clear weeklong predator cycles in the experiment III. This suggests that the temporal variation in productivity can transfer to the population dynamics of predators, which can further affect the strength and direction of selection for prey defensive traits. As a result, prey defensive ability varied more with prey selection lines that had been exposed previously to predation in temporally varying compared to constant resource environment (II). However, the peaks of predator densities decreased towards the end of the experiment in temporally varying resource environment in both experiments (II and III). This was probably due to emergence of white small defensive prey clones (III) and increased allocation to predator resistant biofilms (II). Thus, the evolution of defensive prey types could weaken the destabilization of predator population dynamics forced by the temporally varying resource environment.

4.5.3 Prey diversification

Both predation and competition for resources have been suggested as mechanisms driving rapid prey diversification into a range of ecologically and phenotypically distinct species or prey types (Van Valen 1974; Schluter 2000). However, the way predation and productivity interact in adaptive radiation of prey is still somewhat unclear (but see Meyer & Kassen 2007; Friman *et al.* 2008; Hall *et al.* 2008).

In the experiments I and III, predation caused rapid prey diversification measured as the frequency of different prey colony colour types (I, IV). Predation increased especially the frequency of white non-pigmented defensive *S. marcescens* clones (I, III and IV) (Fig. 4). Predation increased prey diversity in general in the experiment I (Shannon diversity calculated from the frequencies of *S. marcescens* colour variants differing in their ability to synthesize prodigiosin), and the productivity of the environment affected only the initial dynamics of prey diversification (I). However, the increase in the frequency of white defensive prey clones was more evident in the constant compared to temporally varying resource environment in the experiment III. This suggests that temporal variation in prey resources can weaken the predator-induced adaptive radiation of prey. The white prey clones were not able to exclude the less defensive but more competitive prey types in neither of the experiments (I and III). This suggests that predation facilitated the coexistence of different prey clones probably through apparent competition between defending and non-defending prey types (Armstrong 1979; Holt *et al.* 1994; Abrams *et al.* 1998). However, also the existence of prey clones poor in defensive and resource use ability was observed in the experiment IV. Surprisingly, these clones were not driven to extinction by predation or resource

competition. One reason for this could be that prey bacteria use also direct competition in addition to resource competition. In addition, some other fitness traits that were not measured in this study could alternatively explain this result. Alternatively, these poor genotypes could have adopted some kind cheater strategy, where they gained resistance against predators e.g. as a part of exopolymer biofilm matrix. Follow-up experiment is however needed to test these hypotheses.
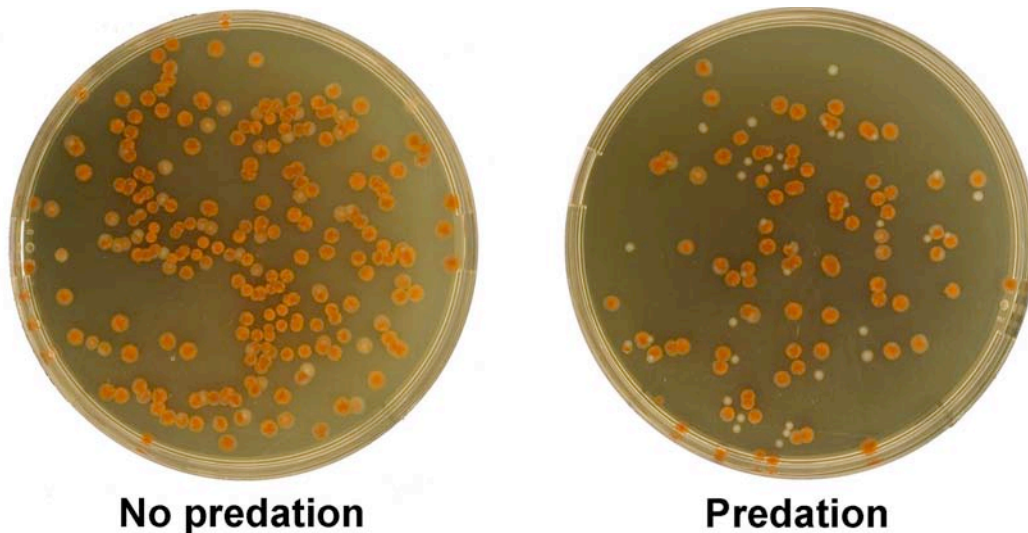
Figure 4. The predation-driven adaptive radiation of prey bacterium *S. marcescens* into white non-pigmented defensive prey types in the constant prey resource environment (III).

4.6   Predation and the evolution of pathogen virulence and host immunity

The pathogens and hosts are typically embedded within a web of interactions with other species in natural communities, which could affect indirectly the pathogen virulence and host immunity (Holt & Roy 2007). For example, protozoan predation can select for more pathogenic bacteria because the increased defence against protozoa confers also resistance for defensive cells of higher organisms (Harb *et al.* 2000). However, protozoan predation could also lead to a decrease in bacterial virulence if increased allocation to anti-predatory traits is traded off with pathogen virulence factors. According to my results, the *S. marcescens* virulence decreased because of protozoan predation (V). This was probably because increasing allocation to defence traded off with traits affecting bacterial ability to cause infection in insect host (IV-V). First, protozoan predation decreased bacterial motility (IV), which has been shown to be connected to the decreased virulence of *Campylobacter jejunum* in piglets (Malik-Kale *et al.* 2007) and that of Db1140 *S. marcescens* strain in C. elegans (Pujol *et al.* 2001).

Second explanation for the decreased virulence could lie in the predator-induced decrease in prey resource-use ability (I, IV); the less virulent *S. marcescens* could simply be inefficient in obtaining resources within the host, leading to poorer reproduction, and thus a less harmful infection. In addition, the laboratory conditions seemed to decrease the pathogen virulence in some degree (V).

The small warning signal was positively linked with defence against pathogenic bacteria (V). However, when the less virulent bacterial strains were used for infection, the signal line had no effect on larval survival (V). Based on predators' learning efficiency, selection is assumed to lead to uniformity and conspicuousness in warning signal expression thereby decreasing the variation in signal size (Ruxton *et al.* 2004; Lindstedt *et al.* 2008). Thus, the observed variation in warning signal pattern of *P. plantaginis* larvae (Ojala *et al.* 2007) could be explained with the selection by avian predators and bacterial pathogens from different trophic levels. The large warning signal size could be favoured when birds are the main cause of larval mortality (Lindstedt *et al.* 2008). Conversely, when the risk of bacterial infection is high (e.g. during the winter hibernation period), larvae with small warning signals, and better immune defence should be favoured. However, the bacterial strains had different effects on larval survival when analyzed within the large and small signal lines separately (V). This suggests that a pathogen's ability to cause infections does not only depend on its own past evolutionary history, but is also affected by the genetic background of its host.

These results demonstrate that virulence is a function of both past evolutionary histories and present ecological interactions of hosts and pathogens. Thus, in order to understand the emergence and dynamics of diseases it could be necessary to understand how evolution affects the pathogen's ability to cause diseases and the host's ability to resist infections in communities with multiple species interactions.

5 Conclusions

The productivity of the environment can have drastic effects on the evolution of predator and prey (Abrams 2000; Thompson 2005). However, there is little experimental evidence considering the effects of temporal variation in productivity on the ecological and evolutionary dynamics of predator-prey interaction. This thesis adds more support to the current view according which the evolutionary dynamics can have considerable effect on the ecological properties of ecosystems (Thompson 1998). The prey resource availability was an important driver of the evolutionary and ecological outcome of the predator-prey interaction. In general, the prey allocation to defence was stronger in high productivity environments, while the costs of defence were realized more clearly in the resource-limited conditions (I). Increasing the measurement resource concentration diminished the magnitude of the prey trade-off between defence and resource use ability (II and IV) suggesting that the resource-controlled trade-offs could limit the evolution of prey defence. In addition, the magnitude of prey trade-off between defence and resource use ability diminished in experiment II probably because of beneficial compensatory mutations. Interestingly, some prey clones evolved effective at defending regardless of the measurement resource concentration (IV). This suggests further that resource-independent prey defensive strategy is also a possible outcome of predator-prey interaction (IV).

Bacteria have been shown to use various defence mechanisms against protozoan predation (Matz & Kjelleberg 2005). I found that the predators selected for white, non-pigmented and highly defensive prey clones (I and III). These clones were able to form predation resistant biofilm, which was the most prominent mechanism behind the prey defence. The biofilm defence was also potentially accompanied with cytotoxicity for predators, which is commonly observed in various bacteria (Matz *et al.* 2004; Matz *et al.* 2005; Matz *et al.* 2008). The increased prey ability to form predation resistant biofilm could also have been traded off with high motility, which has been shown to be energetically costly (Josenhans & Suerbaum 2002). Evidence for the evolution of predator ability to use bacterial resource more efficiently was also found in the experiment II. Predator adaptation was also costly in terms of reduced growth ability in its previous resource environment. It has been shown recently that the co-evolutionary rate between bacterial host and its viral parasite increases according the productivity of the environment (Lopes-Pascua & Buckling 2008). The resource turnover was highest in the experiment II, suggesting that co-evolutionary dynamics could affect the evolution of predator-prey interaction more in high productivity environments.

Temporal variation in productivity is predicted to constrain the evolution of prey defence at least in two ways. First, by increasing the variation in predator densities (Luckinbill & Fenton 1974; Drake & Lodge 2004; Becks *et al.* 2005) leading the selection for prey defences to fluctuate in time (Levins 1968; Hairston & Dillon 1990; Yoshida *et al.* 2003). Second, by affecting the prey potential to evolve, the abundance of resources favouring and the shortage of resources disfavouring the prey

allocation to defence. Temporal variation in prey resources increased the variability of the predator population dynamics in the experiment II and caused clear weeklong predator cycles in the experiment III. However, temporal variation was able to constrain evolution of prey defence only in the experiment III. This was probably because the temporal variation in productivity increased also the bacterial productivity in the experiment II. This could have decreased the effects of costly trade-offs and facilitated the evolution of predation-resistant biofilm forming prey (II). In the experiment III, the magnitude of prey trade-off between defence and competitive ability was larger and the resource turnover considerably lower, which probably constrained the emergence of defending prey genotypes more strongly. Thus, temporal variation in productivity could constrain the evolution of prey defence only when the allocation to defence incurs high cost, and the resource availability of the environment is low.

The evolution of prey defences affected also many ecological properties of predator prey system. The rapid evolution of prey defence reduced the transfer of the energy from basal to higher trophic levels in experiments I-III. In the experiment I, the predation induced destabilization of population dynamics, diversity and prey traits was less pronounced in the high productivity environment, where the evolution of prey defence was strongest. In addition, evolution of prey defence weakened the environmental variation induced destabilization of predator population dynamics in experiments II and III. Temporal variation in prey resource availability was also able to weaken the predator-induced adaptive radiation of prey (III).

The impact of protozoan predation was not only limited to the predator-prey interaction. Predation decreased the *S. marcescens* virulence in the insect host suggesting that species interactions outside the context of host-pathogen relationship could be important indirect drivers for the evolution of pathogenesis (V). This was probably because increased allocation to defence against protozoan predation was traded-off with traits connected to bacterial virulence (e.g. motility and resource use ability) (V). The pathogen success depended also from the host allocation to effective warning signal against avian predation (V). Thus, the pathogen virulence is a function of both past evolutionary histories and present ecological interactions of hosts and pathogens.

To conclude, this thesis demonstrates that evolution can affect multiple ecological properties of predator-prey interaction. The effect of evolution on ecosystem properties can depend on the productivity of the environment, being most evident in the constant environments with high productivity. Thus, not only the evolution can have a profound influence on ecosystem functioning, but also the ecosystem properties can have a profound influence on evolution.

6 Acknowledgements

I owe big gratitude to my supervisor Jouni Laakso and fellow co-workers Teppo Hiltunen and Tarmo Ketola for introducing me to the world of microbes. I especially like to thank Jouni Laakso for being there when ever needed and for the understanding towards my band activities and touring schedule. It has been long road, probably for both of us, but I feel that we did really accomplish something scientifically important with this thesis. I would also like to thank my fellow co-workers Tarmo Ketola for stimulating conversations and Teppo "suokkari" Hiltunen for the assistance in all the practical matters related to work with microcosms. Also, big thanks goes to my fellow PhD student of this group, Minna Pekkonen, not only from fruitful co-operation but also from valuable peer support during this process. I would also like to thank Veijo Kaitala for comments with presentations and manuscripts and for funding during my master thesis. Jouni and Teppo were the ones who gave me the first spark but you provided the fuel. What I am most thankful is that my relationship with this group goes beyond the work matters and I am glad to have such good friends as you.

I have been also privileged to work with Predator-prey interaction group in Jyväskylä and big thanks goes especially to Johanna Mappes and Carita Lindstedt and all the people belonging to Jyväskylä Centre of Excellence in Evolutionary Research. I honestly believe that we have done good science together. Johanna has especially supported me since my master thesis in numerous ways. For me the most important thing has been the value and belief she has had in our work and experimental approach. From Carita I cannot say but good things; she is great personality, hard-working, top-notch scientist. It has been great to work and be friends with you.

Moreover, I would not have been able to complete all the work without help from my graduate students Maija Koivu and Lauri Mikonranta. It was pleasure to work with both of you and I hope you feel the same. In addition, I like to thank all our trainees who did valuable job in keeping the lab in order and things running. Further, I am anxiously waiting for the forthcoming collaboration with our newest group member, Ji Zhang, on the evolution of bacterial virulence. Big thanks go also to Kari Nissinen and Salme Kärkkäinen from the department of mathematics and statistics from prolific discussion and help with the data analyses. I think that also all other PhD students here in Jyväskylä deserve big thanks for the peer support and meetings in the sauna and support-group. Sometimes the little things make all the difference. In addition, I want to thank the Academy of Finland, Vanamo and Societas Pro Fauna et Flora Fennica foundations dearly for the funding which made his thesis possible.

Besides the science, I am who I am because of my family, friends, band mates and last but definitely not least, my dear fiancé Leena. I would probably never become that interested in biology if I have not been dragged to my roots in the Lapland every summer and winter during my childhood. It is good that your parents make you sometimes walk that extra mile even though all that you want to do is watch cartoons and play with He-man toys. Another job and vent hole during this process has been my band INSOMNIUM. It has been truly an adventure to make music and tour with you

guys, even though combining the lifestyle of an amateur musician to a lifestyle of a scientist has been sometimes bit troublesome to say at least. Still, from all the things Leena have had the greatest influence on me becoming the person who I am today. Life might not be always easy but spending it with person, you truly love and who truly loves you back, makes it something special. Thank you for your endless support. Without you I would not be writing these words.

References

Abrams, P.A. 1995. Monotonic or unimodal diversity productivity gradients: what does competition theory predict? *Ecology*, **76**, 2019–2027.

Abrams, P.A. & Matsuda H. 1997. Prey adaptation as a cause of predator-prey cycles. *Evolution*, **51**, 1742-1750.

Abrams, P.A., Holt, R.D. & Roth, J.D. 1998. Apparent competition or apparent mutualism? Shared predation when populations cycle. *Ecology*, **79**, 201-212.

Abrams, P.A. 2000. The evolution of predator-prey interactions. Theory and evidence. *Annu. Rev. Ecol. Syst.*, **31**, 79–105.

Abramsky, Z. & Rosenzweig, M.L. 1984. Tilman's predicted productivity–diversity relationship shown by desert rodents. *Nature*, **309**, 150–151.

Àlvarez, B., Secades, P., Prieto, M., McBride, M. J. & Guijarro, J. A. 2006. A mutation in *Flavobacterium psychrophilum tlpB* inhibits gliding motility and induces biofilm formation. *Appl. Environ. Microbiol.*, **72**, 4044–4053.

Andersson D.I. 2003. Persistence of antibiotic resistant bacteria. *Current Opinion in Microbiology*, **6**, 452-456.

Armstrong, R. A. 1979. Prey species replacement along a gradient of nutrient enrichment: graphical approach. *Ecology*, **60**, 76–84.

Becks, L., Hilker, F. M., Malchow, H., Jürgens, K. & Arndt, H. 2005. Experimental demonstration of chaos in a microbial food web. *Nature*, **435**, 1226–1229.

Benkman, C.W., Holimon, W.C., & Smith, J.W. 2001. The influence of a competitor on the geographic mosaic of coevolution between crossbills and lodgepole pine. *Evolution Int. J. Org. Evolution*, **55**, 282–294.

Bennett, A.F. & Lenski, R.E. 1993. Evolutionary adaptation to temperature. II. Thermal niches of experimental lines of *Escherichia coli*. *Evolution*, **47**, 1-12.

Bennett, A.F., Lenski, R.E. & Mittler, J.E. 1992. Evolutionary adaptation to temperature. I. Fitness responses of *Escherichia coli* to changes in its thermal environment. *Evolution*, **46**, 16-30.

Benton, M. J. 1996. Diversification and extinction in the history of life. *Science*, **268,** 52-58.

Björkman, J., Nagaev, I., Berg, O.G., Hughes, D. & Andersson, D.I. 2000. Effects of environment on compensatory mutations to ameliorate costs of antibiotic resistance. *Science*, **287**, 1479-1482.

Bohannan, B.J.M. & Lenski, R.E. 1997. Effect of resource enrichment on a chemostat community of bacteria and bacteriophage. *Ecology*, **78**, 2303-2315.

Bohannan, B.J.M. & Lenski, R.E. 1999. Effect of prey heterogeneity on the response of a model food chain to resource enrichment. *Am. Nat.*, **153**, 73-82.

Bohannan, B.J.M. & Lenski, R.E. 2000. The relative importance of competition and predation varies with productivity in a model community. *Am. Nat.*, **156**, 329–340.

Bohannan, B.J.M., Kerr, B., Jessup, C.M., Hughes, J.B. & Sandvik, G. 2002. Trade-offs and coexistence in microbial microcosms. *Anton. Leeuw.*, **81**, 107–115.

Brandl, M.T., Rosenthal, B.M., Haxo, A.F. & Berk, S.G. 2005, Enhanced survival of *Salmonella enterica* in vesicles released by a soilborne *Tetrahymena* species. *Appl. Environ. Microbiol.*, **71**, 1562-1569.

Brodie, E.D., Jr., Ridenhour, B.J., & Brodie, E.D., III. 2002. The evolutionary response of predators to dangerous prey: hotspots and coldspots in the geographic mosaic of coevolution between newts and snakes. *Evolution Int. J. Org. Evolution,* **56**, 2067-2082.

Brüssow, H. 2007. Bacteria between protests and phages: from antipredation strategies to the evolution of pathogenicity. *Molecular Microbiol.,* **65**, 583–589.

Bruton, B.D., Mitchell, F., Fletcher, J., Pair, S.D., Wayadande, A., Melcher, U., Brady, J., Bextine, B., & Popham, T.W. 2003. *Serratia marcescens*, a phloem-colonizing, squash bug transmitted bacterium: Causal agent of cucurbit yellow vine disease. *Plant Dis.*, **87**, 937-944.

Buckling, A., Kassen, R., Bell, G. & Rainey, P.B. 2000. Disturbance and diversity in experimental microcosms. *Nature*, **408**, 961-964.

Buckling, A. *&* Rainey, P.B. 2002a. Antagonistic coevolution between a bacterium and a bacteriophage. *Proc. Roy. Soc. Lond. Ser. B*, **269**, 931-936.

Buckling, A. *&* Rainey, P.B. 2002b. The role of parasites in sympatric and allopatric host diversification. *Nature*, **420,** 496-499.

Bukharin O.V. & Nemtseva N.V. 2001. Investigation of lysozyme–antilysozyme interactions in a model *Tetrahymena–Escherichia* community, *Microbiology*, **70**, 564-569.

de Visser, A.J., Zeyl, C.V., Gerrish, P.J., Blanchard, J.L. & Lenski, R.E. 1999. Diminishing returns from mutation supply rate in asexual populations. *Science*, **283**, 404-406.

Cadotte, M. W. & T. Fukami. 2005. Dispersal, spatial scale, and species diversity in a hierarchically structured experimental landscape. *Ecology Letters*, **8**, 548–557.

Chao, L., Levin, B.R. *&* Stewart, F.M. 1977. A complex community in a simple habitat: Experimental study with bacteria and phage. *Ecology*, **58**, 369–378.

Chase, J. M., P. A. Abrams, J. P. Grover, S. Diehl, P. Chesson, R. D. Holt, S. A. Richards, R. M. Nisbet, *&* T. J. Case. 2002. The interaction between predation and competition: a review and synthesis. *Ecology Letters*, **5**, 302–315.

Chesson, P. & Kuang, J.J. 2008 The interaction between predation and competition, *Nature*, **456**, 235-238.

Chinery, M. 1993. *Collins guide to insects of Britain and Western Europe.* (3rd ed.), Harper-Collins, London.

Connell, J.H. 1978. Diversity in tropical rain forests and coral reefs. *Science*, **199**, 1302-1310.

Dallinger, W.D. 1887. The president's address. *J. R. Microsc. Soc.*, **7**, 184-199.

Dawkins, R. & Krebs, J.R. 1979. Arms races between and within species. *Proc. R. Soc. Lond. B.*, **205**, 489–511.

Deorukhkar, A.A., Chander, R., Ghosh, S.B. *&* Sainis, K.B.(2007. Identification of a red-pigmented bacterium producing a potent anti-tumor N-alkylated prodigiosin as *Serratia marcescens. Research in Microbiology*, **158**, 399-404.

Ding, M-J. & Williams, R.P. 1983. Biosynthesis of prodigiosin by white strains of *Serratia marcescens* isolated from patients. *J. Clinic. Microbiol.*, **17**, 476–480.

Drake, J.M. & Lodge, D.M. 2004. Effects of environmental variation on extinction and establishment. *Ecology Letters*, **7**, 26–30.

Edeline, E., Ari, T.B., Vollestad, L.A., Winfield, I.J., Fletcher, J.M., James, J.B. & Stenseth, N.C. 2008. Antagonistic selection from predators and pathogens alters food-web structure. *PNAS*, **105**, 19792–19796.

Elliott, A.M. 1974. *Biology of Tetrahymena.* Dowden, Hutchinson and Ross, Inc. Stroudsburg, PA.

Fenchel T. 1987. *Ecology of protozoa: the biology of free-living phagotrophic protists.* Science Tech Publishers, Madison, Wisconsin, 197 p.

40

Flöder, S. & Sommer, U. 1999. Diversity in planktonic communities: an experimental test of the intermediate disturbance hypothesis. *Limnol. Oceanogr.*, **44**, 1114–1119.

Friman, V.-P., Hiltunen, T., Laakso J. & Kaitala, V. 2008. Prey resource availability drives evolution of predator prey interaction. *Proc. R. Soc. Lond. B*, **275**, 1625–1633.

Forde, S.E., Thompson, J.N. & Bohannan B.J.M. 2004. Adaptation varies through space and time in a coevolving host–parasitoid interaction. *Nature*, **431**, 841-844.

Forde, S.E., Thompson, J.N. & Bohannan B.J.M. 2007. Gene flow reverses an adaptive cline in a coevolving host-parasitoid interaction. *Am. Nat.*, **169**, 794-801.

Frankel, J. & Nelsen, E.M. 2001. The effects of supraoptimal temperatures on population growth and cortical patterning in *Tetrahymena pyriformis* and *Tetrahymena thermophila:* a Comparison. *J. Eukaryot. Microbiol.*, **48**, 135-146.

Fussmann, G. F., Loreau, M. & Abrams, P. A. 2007 Eco-evolutionary dynamics of communities and ecosystems. *Funct. Ecol.*, **21**, 465–477.

Fututyama, D.J. & Moreno, G. 1988. The evolution of ecological specialisation. *Annu. Rev. Ecol. Syst.*, **19**, 207–233.

Gao, Q., Parniak, M.A., Gu, Z. & Wainberg, M.A. 1992. Generation of nucleoside-resistant variants of HIV-1 by in vitro selection in the presence of AZT or DDI but not by combinations. *Leukemia*, **6**, 192-195.

Gause, G.F. 1934. *The Struggle for Existence*. Dover, New York, NY.

González, J. M., Sherr, E. B. & Sherr. B. F. 1993. Differential feeding by marine flagellates on growing versus starving, and on motile versus nonmotile, bacterial prey. *Mar. Ecol. Prog. Ser.*, **102**, 257–267.

Gorden, R.W., Beyers, R.J., Odum, E.P. & Eagon, R.G. 1979. Studies of a simple laboratory microecosystem: Bacterial activities in a heterotrophic succession. *Ecology*, **50**, 86–100.

Grimont, P. A. D. & Grimont, F. 1978. The genus Serratia. *Annu. Rev. Microbiol.*, **32**, 221–248.

Hairston, N.G., Allan, J.D., Colwell, R.K., Futuyma, D.J., Howell, J., Lubin, M.D., Mathias, J. & Vandermeer, J.H. 1968. The relationship between species diversity and stability: An experimental approach with protozoa and bacteria. *Ecology*, **49**, 1091–1101.

Hairston, N.G. Jr & Dillon, T.A. 1990. Fluctuating selection and response in a population of freshwater copepods. *Evolution*, **44**, 1796–1805.

Hall, A.R. & Colegrave, N. 2007. How does resource supply affect evolutionary diversification. *Proc. R. Soc. Lond. B*, **274**: 73-78.

Hall, A.R., Meyer, J.R. & Kassen, R. 2008. Selection for predator resistance varies with resource supply in a model adaptive radiation. *Evolutionary ecology Research*, **10**, 735-746.

Harb, O.S., Gao, L.-Y. & Kwaik, Y.A. 2000. From protozoa to mammalian cells: a new paradigm in the life cycle of intracellular bacterial pathogens. *Environ. Microbiol.*, **2**, 251–265.

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

Hedges, S. B., Parker, P. H., Sibley, C. G. & Kumar, S. 1996. Continental breakup and the ordinal diversification of birds and mammals. *Nature*, **381,** 226–229.

Hill D.L. 1972. *The biochemistry and physiology of Tetrahymena.* Academic Press, New York and London, 230 p.

Hillesland, K.L., Velicer, G.J. & Lenski, R.E. 2008. Experimental evolution of microbial predator's ability to find prey. *Proc. R. Soc. B*, **276**, 459–467.

Hochberg, M.E., & van Baalen, M.V. 1998. Antagonistic coevolution along environmental gradients. *Am. Nat.*, **152**, 620–634.

Hochberg, M.E. & Holt, R.D. 1995. Refuge evolution and the population-dynamics of coupled host–parasitoid associations. *Evol. Ecol.*, **9**, 633–661.

Holt, R. D., J. Grover, & D. Tilman. 1994. Simple rules for interspecific dominance in systems with exploitative and apparent competition. *Am. Nat.*, **144**, 741–771.

Holt, R.D. & Roy, M. 2007. Predation can increase the prevalence of infectious disease. *Am. Nat.*, **169**, 690–699.

Huston, M.A. 1994. *Biological Diversity*. Cambridge Univ. Press, Cambridge.

Jessup, C.M., Forde, S. & Bohannan, B.J.M. 2005. Microbial experimental systems in ecology. *Advances in ecological research*, **37**, 276-307.

Johnson, M.T.J. & Agrawal, A.A. 2003. The ecological play of predator - prey dynamics in an evolutionary theatre. *TREE*, **18**, 549–551.

Josenhans C. & Suerbaum, S. 2002. The role of motility as a virulence factor in bacteria. *Int. J. Med. Microbiol.*, **291**, 605–614.

Kassen, R. & Bell, G. 1998. Experimental evolution in *Chlamydomonas*. IV. Selection in environments that vary through time at different scales. *Heredity*, **80**, 732-741.

Kassen, R., Buckling, A., Bell, G. & Rainey, P.B. 2000. Diversity peaks at intermediate productivity in laboratory microcosms. *Nature*, **406**, 508–512.

Kassen, R. 2002. The experimental evolution of specialists, generalists, and the maintenance of diversity. *J. Evol. Biol.*, **15**, 173-190.

Kaunzinger, C.M.K. & Morin, P.J. 1998. Productivity controls food-chain properties in microbial communities. *Nature*, **395**, 495–497.

Ketola, T., Laakso, J., Kaitala, V. & Airaksinen, S. 2004. Evolution of HSP90 expression in *Tetrahymena thermophila* (Protozoa, Ciliata) populations exposed to thermally variable environments, *Evolution*, **58**, 741-748.

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

Kondoh, M. 2007. Anti-predator defence and the complexity–stability relationship of food webs. *Proc. R. Soc. Lond. B,* **274**, 1617–1624.

Kondoh, M. 2001. Unifying the relationships of species richness to productivity and disturbance. *Proc. R. Soc. Lond. B*, **268**, 269–271.

Laakso J., Löytynoja K. & Kaitala V. 2003. Environmental noise and population dynamics of the ciliated protozoa *Tetrahymena thermophila* in aquatic microcosms. *Oikos*, **102**, 663-671.

Leibold, M.A. 1996. A graphical model of keystone predators in food webs: trophic regulation of abundance, incidence, and diversity patterns in communities. *Am. Nat.*, **147**, 784–812.

Lennon, J.T. & Cottingham, K.L. 2008. Microbial productivity in variable resource environments. *Ecology*, **89**, 1001–1014.

Lenski, R.E. 1988. Dynamics of interactions between bacteria and virulent bacteriophage. *Adv. Microb. Ecol.*, **10**, 1–44.

Levin, B.R., Stewart, F.M. & Chao, L. 1977. Resource-limited growth, competition, and predation: A model and experimental studies with bacteria and bacteriophage. *Am. Nat.*, **111**, 3–24.

Levin, B.R. & Lenski, R.E. 1985. Bacteria and phage: A model system for the study of the ecology and co-evolution of hosts and parasites. In: *Ecology and Genetics of Host-Parasite Interactions* (Ed. by D. Rollinson and R.M. Anderson), pp. 227–242. Academic Press, London, UK.

Levins, R. 1968. *Evolution in Changing Environments*. Princeton, University Press, New Jersey.

Lindstedt, C., Lindström, L. & Mappes, J. 2008. Hairiness and warning colours as components of antipredator defence: additive or interactive benefits? *Anim. Behav.*, **75**, 1703–1713.

Lindstedt, C. 2008. Maintenance of variation in warning signals under opposing selection pressures. *Jyväskylä studies in biological and environmental sciences*, **193**, 57p. (http://dissertations.jyu.fi/studbiol/9789513934446.pdf)

Lindstedt, C., Lindström, L. & Mappes J. 2009. Thermoregulation constrains warning signal expression. *Evolution*, **63**, 469-478.

Lively, C.M. & Dybdahl, M.F. 2000. Parasite adaptation to locally common host genotypes. *Nature*, **405**, 679–681.

Lopez-Pascua, L.D.C. & Buckling, A. 2008. Increasing productivity accelerates host-parasite co-evolution, *J. Evol. Biol.*, **21**, 853-860.

Luckinbill, L.S. 1973. Coexistence in laboratory populations of Paramecium Aurelia and its predator Didinium nasutum. *Ecology*, **54**, 1320–1327.

Luckinbill, L.S. 1974. The effects of space and enrichment on a predator‑prey system. *Ecology*, **55**, 1142–1147.

Luckinbill, L.S. & Fenton, M.M. 1978. Regulation and Environmental Variability in Experimental Populations of Protozoa, *Ecology*, **59**, 1271-1276.

Luckinbill, L.S. 1979. Regulation, stability, and diversity in an model experimental microcosm. *Ecology*, **60**, 1098–1102.

MacLean, R.C., Bell, G. & Rainey, P.B. 2004. The evolution of a pleiotropic fitness tradeoff in *Pseudomonas fluorescens. PNAS*, **101,** 8072–8077.

Malik-Kale, P., Raphael, B.H., Parker, C.T., Joens, L.A., Klena, J.D., Quinones, B., Keech, A.M. & Konkel, M.E. 2007. Characterization of genetically matched isolates of *Campylobacter jejuni* reveals that mutations in genes involved in flagellar biosynthesis alter the organism's virulence potential. *Appl. Environ. Microbiol.* **73**, 3123–3136.

Matz, C., Bergfeld, T., Rice, S.A., & Kjelleberg, S. 2004. Microcolonies, quorum sensing and cytotoxicity determine the survival of *Pseudomonas aeruginosa* biofilms exposed the protozoan grazing. *Environ. Microbiol.*, **6**, 218–226.

Matz, C. & Kjelleberg, S. 2005. Off the hook – how bacteria survive protozoan grazing. *TREM*, **13**, 302–307.

Matz, C., McDougald, D., Moreno, A.M., Yung, P.Y., & Yildiz, F.H. 2005. Biofilm formation and phenotypic variation enhance predation-driven persistence of *Vibrio cholerae*. *PNAS*, **102**, 16819–16824.

Matz, C., Webb, J.S., Schupp, P.J., Phang, S.Y., Penesyan, A., Egan, S., Steinberg, P. & Kjelleberg, S. 2008. Marine biofilm bacteria evade eukaryotic predation by targeted chemical defense., *Plos ONE*, **3**, e2744.

Meyer, J.R. & Kassen R. 2007. The effects of competition and predation on diversification in a model adaptive system. *Nature*, **446**, 432–435.

Mole, S. 1994. Trade-offs and constraints in plant-herbivore defence theory: A life-history perspective. *Oikos*, **71**, 3–12.

Monger, B. C., & Landry, M. R. 1992. Size-selective grazing by heterotrophic nanoflagellates: an analysis using live-stained bacteria and dual-beam flow cytometry. *Arch. Hydrobiol. Beih. Ergebn. Limnol.*, **37**, 173–185

Morgan, A.D., Gandon, S. & Buckling, A. 2005. The effect of migration on local adaptation in a coevolving host-parasite system. *Nature*, **43**, 253-256.

Montaner, B., Navarro, S., Pique, M., Vilaseca, M., Martinell, M., Giralt, E., Gil, J., Perez-Tomas, R. 2000. Prodigiosin from the supernatant of Serratia marcescens induces apoptosis in haematopoietic cancer cell lines, *Br. J. Pharmacol.*, **131**, 585-593.

Mougi, A. & Nishimura, K. 2008. The paradox of enrichment in an adaptive world. *Proc. R. Soc. Lond. B*, **275**, 2563–2568.

Nakajima T. & Kurihara Y. 1994. Evolutionary changes of ecological traits of bacterial populations through predator-mediated competition 1. Experimental analysis. *Oikos*, **71**, 24-34.

Navas. A., Cobas, G., Talavera, M., Ayala, J.A., López, J.A. & Martínez, J.L. 2007. Experimental validation of Haldane's hypothesis on the role of infection as an evolutionary force for Metazoans. *PNAS*, **104**, 13728–13731.

Nielsen, J.K., & de Jong, P.W. 2005.Temporal and host-related variation in frequencies of genes that enable *Phyllotreta nemorum* to utilize a novel host plant, *Barbarea vulgaris. Entomol. Experiment. Appl.*, **115**, 265–270.

Nosil, P. & Crespi, B.J. 2006. Experimental evidence that predation promotes divergence in adaptive radiation. *PNAS*, **103**, 9090–9095

Ojala, K., Julkunen-Tiitto, R., Lindström, L. & Mappes, J. 2005. Diet affects the immune defence and life-history traits of an Arctiid moth *Parasemia plantaginis. Evol. Ecol. Res.*, **7**, 1153-1170.

Ojala. K., Lindström, L. & Mappes, J. 2007. Life-history constraints and warning signal expression in an arctiid moth. *Functional Ecology*, **21**, 1162–1167.

Oksanen, L., Fretwell, S. D., Arruda, J. & Niemela, P. 1981. Exploitation ecosystems in gradients of primary productivity. *Am. Nat.*, **118,** 240–261.

Ostfeld, R. S., & F. Keesing. 2000. Pulsed resources and community dynamics of consumers in terrestrial ecosystems. *TREE*, **15**, 232–237.

O'Toole, G.A. & Kolter, R. 1998. The initiation of biofilm formation in *Pseudomonas aeruginosa* WCS365 proceeds via multiple, convergent signaling pathways: a genetic analysis. *Mol. Microbiol.,* **28**, 449–61

Paine, R. T. 1966. Food web complexity and species diversity. *Am. Nat.*, **100**, 65–75.

Paine, R. T. 1969*a*. A note on trophic complexity and community stability. *Am. Nat.*, **103**, 91–93.

Paine, R. T. 1969*b*. *Pisaster-Tegula* interaction: prey patches, predator food preference, and intertidal community structure. *Ecology*, **50**, 950–961.

Paruchuri, D.K. & Harshey, R.M. 1987. Flagellar variation in *Serratia marcescens* is associated with color variation. *J. Bacteriology*, **169**, 61–65.

Pujol, N., Link, E.M., Liu, L.X., Kurz, C.L., Alloing, G., Tan, M-W, Ray, K.P., Solari, R., Johnson, C.D. & Ewbank, J.J. 2001. A reverse genetic analysis of components of the Toll signaling pathway in *Caenorhabditis elegans. Current Biology*, **11**, 809–821.

Ranta, E., Lundberg, P. & Kaitala, V. 2006. *Ecology of populations*. Cambridge University Press, Cambridge.

Reboud, X. & Bell, G. 1997. Experimental evolution of *Chlamydomonas*. III. Evolution of specialists and generalists types in environments that vary in space and time. *Heredity*, **78,** 507–514.

Rigby, M.C. & Jokela, J. 2000. Predator avoidance and immune defence: costs and trade-offs in snails. *Proc. R. Soc. Lond. B*, **267**, 171–176.

Ripa, J., Lundberg, P. & Kaitala V. 1998. A general theory of environmental noise in ecological food webs. *Am. Nat.*, **151**, 256–263.

Rosenzweig, M.L. 1971. Paradox of enrichment: destabilization of exploitation ecosystems in ecological time. *Science*, **171**, 385–87.

Rosenzweig, M.L. 1995. *Species diversity in space and time*. Cambridge University Press, Cambridge.

Ruxton, G. D., Sherratt, T. N. & Speed, M. P. 2004. *Avoiding Attack: Evolutionary Ecology of Crypsis, Warning Signals & Mimicry*. Oxford University Press, New York.

Schluter, D. 2000. *The Ecology of Adaptive Radiation* (Oxford Univ. Press, Oxford, UK).

Schmid-Hempel, P. 2003. Variation in immune defence as a question of evolutionary ecology. *Proc. R. Soc. Lond. B*, **270**, 357–366.

Schulenburg, H., Kurtz, J., Moret, Y. & Siva-Jothy, M.T. 2008. Introduction. Ecological immunology. *Proc. R. Soc. Lond. B*, **364**, 3–14.

Sih, A., P. Crowley, M. McPeek, J. Petranka, & K. Strohmeier. 1985. Predation, competition, and prey communities: a review of field experiments. *Annu. Rev. Ecol. Syst.*, **16**, 269–311.

Someya, N., Nakajima, M., Hirayae, K., Hibi, T. & Akutsu, K. 2001. Synergistic antifungal activity of chitinolytic enzymes and prodigiosin produced by biocontrol bacterium, *Serratia marcescens* strain B2 against gray mould pathogen, Botrytis cinerea. *J. Gen. Plant. Pathol.*, **67**, 312–317.

Sommer, U. 1984. The paradox of the plankton: Fluctuations of phosphorus availability maintain diversity of phytoplankton in flow‑through cultures. *Limnology and Oceanography*, **29**, 633–636.

Spiers, A.J., Bohannan, J., Gehrig, S.M. & Rainey, P.B. 2003. Biofilm formation at the air–liquid interface by the *Pseudomonas fluorescens* SBW25 wrinkly spreader requires an acetylated form of cellulose. *Mol. Microbiol.*, **50**, 15–27.

Takagi, T., Kisumi, M. & Chibata, I. 1985. Construction of an arginine-producing strain of *Serratia marcescens*. *Appl. Microbiol. Biotechnol.*, **21**, 378-382.

Tan, M-W. 2002. Cross-species infections and their analysis. *Annu. Rev. Microbiol.*, **56**, 539–565.

Thompson, J.N. 1998. Rapid evolution as an ecological process. *TREE*, **13**, 329–332.

Thompson, J.N., & Cunningham, B.M. 2002. Geographic structure and dynamics of coevolutionary selection. *Nature*, **417**, 735–738.

Thompson, J.N. 2005. *The Geographic Mosaic of Coevolution*. University of Chicago Press, Chicago, IL.

Thrall, P.H., Burdon, J.J., & Bever, J.D. 2002. Local adaptation in the *Linum marginale—Melampsora lini* hostpathogen interaction. *Evolution Int. J. Org. Evolution*, **56**, 1340–1351.

Tilman, D. 1977. Resource competition between planktonic algae: Experimental and theoretical approach. *Ecology*, **58**, 338–348.

Tilman, D. 1982. *Resource Competition and Community Structure* (Princeton Univ. Press, Princeton).

Tilman, D. & Sterner, R.W. 1984. Invasions of equilibria: Tests of resource competition using two species of algae. *Oecologia*, **61**, 197–200.

Vamosi, S.M. 2005. On the role of enemies in divergence and diversification of prey: a review and synthesis. *Can. J. Zool.*, **83**, 894–910.

van den Ende, P. 1973, Predator‑prey interactions in continuous culture. *Science*, **181**, 562–564.

Vandermeer, J.H. 1969. The competitive structure of communities: An experimental approach with protozoa. *Ecology*, **50**, 362–371.

Van Valen, L. 1974. Predation and species diversity. *J. Theor. Biol.*, **44**, 19–21.

Van Voris, P., O'Neill, R.V., Emanuel, W.R. & Shugart, H.H. 1980. Functional complexity and ecosystem stability. *Ecology*, **61**, 1352–1360.

Vermeij G.J. 1987. *Escalation and Evolution.*, MA: Harvard Univ. Press, Cambridge, 544 p.

Vermeij, G.J. 1994. The evolutionary interaction among species: selection, escalation, and coevolution. *Annu. Rev. Ecol. Syst.*, **25**, 219–236.

Wei, J.-R. & Lai, H.-C. 2006 N-Acylhomoserine lactone-dependent cell-to-cell communication and social behavior in the genus *Serratia. Int. J. Med. Microbiol.*, **296**, 117–124.

Wildschutte, H., Wolfe, D.M., Tamewitz, A. & Lawrence J.G. 2004. Protozoan predation, diversifying selection, and the evolution of antigenic diversity. *PNAS*, **101**, 10644–10649.

Yamauchi, A. & Yamamura, N. 2005. Effect of defence evolution and diet choice on population dynamics in a one-predator-two-prey system. *Ecology*, **86**, 2513–2524.

Yoshida, T., Jones, L.E., Ellner, S.P., Fussmann, G.F. & Hairston Jr, N.G. 2003. Rapid evolution drives ecological dynamics in a predator-prey system. *Nature*, **424**, 303–306.

Yoshida, T., Hairston Jr, N.G. & Ellner, S.P. 2004. Evolutionary trade-off between defence against grazing and competitive ability in a simple unicellular alga, *Chlorella vulgaris. Proc. R. Soc. Lond. B*, **271**, 1947–1953.

Yoshida, T., Ellner, S.P., Jones, L.E., Bohannan, B.J.M., Lenski, R.E. & Hairston Jr, N.G. &. 2007. Cryptic population dynamics: rapid evolution masks trophic interactions, *PLOS Biology*, **e235**, 1868-1879.

Zangerl, A.R., & Berenbaum, M.R. 2003. Phenotypic matching in wild parsnip and parsnip webworms: causes and consequences. *Evolution Int. J. Org. Evolution*, **57**, 806–815.