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Understanding the structure of interactions and the dynamics of spider populations in agricultural ecosystems

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Odile Bruggisser

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Der Dissertationsleiter:

Prof. Dr. Louis-Félix Bersier

Der Dekan:

Titus Jenny

Prof. Dr. Titus Jenny

CONTENTS

ZUSAMMENFASSUNG

Der gegenwärtige Klimawandel und der zunehmende Verlust an natürlichen Lebensräumen sind mitverantwortlich für den weltweiten Rückgang der Artenvielfalt. Untersuchungen zeigen, dass sich die Stärke und Vielfalt der Interaktionen zwischen einzelnen Arten wesentlich auf die Stabilität einer Lebensgemeinschaft auswirken. Ein erweitertes Verständnis der Struktur und Dynamik von Populationen ist daher für den Erhalt von Lebewesen von zentraler Bedeutung. Spinnen sind besonders geeignet zur Untersuchung der Beschaffenheit von Populationen. Als generalistische Räuber verbreiten sie sich auf effiziente Weise („ballooning“) mit Hilfe eines Seidenfadens durch die Luft, und sind in praktisch jedem terrestrischen Nahrungsnetz in hohen Dichten zu finden. Zudem zeichnet sich die Gruppe durch eine grosse Artenvielfalt aus, wobei einzelne Arten unterschiedlich stark auf Störungsfaktoren, Strukturen und physikalische Eigenschaften ihres Habitats reagieren. Diese Doktorarbeit gliedert sich in drei Teilstudien, in denen Spinnenpopulationen und deren Räuber und Beutetiere in naturnahen Lebensräumen untersucht wurden, um einen Einblick in die Struktur und Dynamik von Lebensgemeinschaften zu gewinnen.

Im ersten Experiment untersuchten wir verschiedener Faktoren, die das Vorkommen der Radnetzspinne *Argiope bruennichi* in naturnahen Lebensräumen beeinflussen. Getestet wurden der Einfluss von niedrigeren trophischen Ebenen (Struktur und Diversität der Pflanzen und die Dichte der Beutetiere), sowie von höheren trophischen Ebenen (das Vorkommen von Räubern). In einem Feldversuch manipulierten wir die Artenvielfalt von Pflanzen (2, 4, 6 oder 12 Arten) und somit die Struktur der Pflanzengemeinschaften innerhalb von 12 verschiedenen Buntbrachen. Die Abundanz von *A. bruennichi* wurde sowohl von höheren als auch niedrigere trophische Ebenen beeinflussen. Das Vorkommen ihres primären Feindes, der Hornisse (*Vespa crabro)*, hatte einen stark negativen Effekt auf die Abundanz der Spinnen, während sich die Vielfalt der Pflanzen und die Dichte der Beutetiere positiv auswirkten. Zudem bevorzugte *A. bruennichi* Lebensräume mit niedrigerer Vegetation. Eine erhöhte Artenvielfalt an Pflanzen wirkte sich positiv auf die Dichte der Beutetiere und somit indirekt auf die Dichte der Spinnen aus. Erstaunlicherweise zeigte unser Versuch nebst diesem indirekten Effekt aber auch einen direkten Effekt der Pflanzendiversität auf die Spinnen. Dies könnte darauf hindeuten, dass Spinnen die Fähigkeit entwickelt haben, ihr Habitat anhand der Pflanzendiversität, die indirekt die Beutedichte widerspiegelt auszuwählen.

Anhand eines Datensatzes über Spinnen, die sich durch die Luft verbreiten (ballooning), und die mit Hilfe einer Saugfalle zwischen 1994 und 2004 gefangen wurden, untersuchten wir in einem zweiten Teil den Effekt von Klimaveränderungen und Habitatverlust auf die Dynamik von Spinnenpopulationen. Hohe Dichten an fliegenden Spinnen wurden im Frühling sowie im Spätsommer beobachtet. Das Flugverhalten der insgesamt 103

Spinnenarten wurde stark von Temperatur, Sonneneinstrahlung und Feuchtigkeit beeinflusst. Auffallend war eine Veränderung des Flugverhaltens zwischen 1994 und 2004. Wir beobachteten eine Verschiebung der zweiten Hauptflugphase in Richtung Frühsommer. Die genauere Betrachtung der verschiedenen Spinnenarten zeigte einen generellen Rückgang epigäischer Spinnenarten, während die Dichte jener Arten, die in höheren Vegetationsschichten leben geringfügig zunahm. Diese Entwicklung spiegelte Veränderungen der Dichte günstiger Habitate im Untersuchungsgebiet wieder. Der extreme Sommer 2003 mit überdurchschnittlich hohen Temperaturen in ganz Europa hatte einen markanten Einfluss auf die Dynamik der fliegenden Spinnen. Während im Frühjahr überdurchschnittlich viele Spinnen beobachtet wurden, kollabierte die Population gänzlich gegen Ende des Sommers. Diese Studie zeigt, dass klimatische Extreme und der Verlust an Lebensräumen ein grösseres Risiko für Spinnenpopulationen darstellen als ein stetiger Anstieg der Temperatur.

Während Spinnen im ersten und zweiten Teil dieser Studie als Akteure im Zentrum standen, untersuchten wir in einem dritten Teil Spinnen als Beutetiere der solitären Wespe *Trypoxylon figulus*. Diese Wespenart fängt Spinnen als Nahrung für ihre Nachkommen. Ihre Larven fressen Spinnen meist bis auf wenige Überreste auf, was das Erfassen des Beutespektrums dieser Wespenart erschwert. Zudem bevorzugt *T. figulus* juvenile Spinnen, die aufgrund fehlender Merkmale grösstenteils nicht auf Artniveau bestimmt werden können. Das Ziel dieser Studie war die Erarbeitung einer molekularen Methode um trophische Beziehungen zwischen Räubern und Beutetieren zu erfassen. Dazu erstellten wir einen genetischen Barcode eines Abschnittes des mitochondrialen Zytochromoxidase I (COI) Gens von 104 Spinnen (46 verschiedene Arten), die zuvor anhand von morphologischen Merkmalen auf Artniveau identifiziert wurden. Wir versuchten Spinnen aus Wespennestern bis auf Artniveau zu bestimmen, indem wir solche COI Sequenzen aus Spinnenüberresten mit dem erarbeiteten Datensatz verglichen. Es gelang uns alle Testsequenzen aus Wespennestern einer bestimmten Spinnengattung oder Spinnenart zuzuordnen. Zudem erwies sich diese Methode ebenfalls nützlich zur Bestimmung von juvenilen Spinnen. Während der Gebrauch solcher COI Datensätze von verschiedenen Autoren kritisiert wird, möchten wir mit dieser Arbeit auf zwei praktische Vorteile hinweisen: 1. Die Bestimmung von Jungtieren, die nicht über geeignete morphologische Strukturen zur Artbestimmung verfügen, sowie 2. die Ermöglichung eines besseren Verständnisses von Räuber-Beute Beziehungen in Nahrungsnetzen.

SUMMARY

The maintenance of biodiversity is a major concern in the face of current drastic modifications of land use and climate change. An understanding of the structure and dynamics of communities is therefore an important long-term objective of ecology. Spiders as generalist predators with highly efficient dispersal abilities are among the most abundant terrestrial arthropods and play a role in food webs in many ecosystems. Their sensitivity to disturbance and to structural and physical aspects of their environment further emphasizes the suitability of this group to investigate basic questions in ecology. The global aim of this thesis was to investigate spiders and their natural prey and predator species in agricultural landscapes to gain insight into the structure, dynamics and stability of communities.

First we investigated experimentally the structure of a food web centered on the web-building spider *Argiope bruennichi*. We were interested in the bottom-up effect of vegetation structure and plant diversity and in the top-down effect of a predator, the hornet *Vespa crabro*, on the abundance of this spider species. We performed a semi-natural experiment in wild-flower-strips differing in plant diversity and hence in vegetation structure. Our study system showed evidence that the abundance of *A. bruennichi* is regulated by combined bottom-up and top-down effects, and by direct and indirect interactions between trophic levels. Two main factors were found to characterize this system: the strong negative effect of a single predator, namely hornets, and the positive effect of plant diversity, which affected spider abundance directly and indirectly via potential prey. Overall, this result may indicate that spiders have evolved the ability to distinguish between different habitats according to plant-diversity cues, which reflect prey availability.

The analysis of a time-series of 103 different ballooning spider species observed between 1994 and 2004, and their relation to climatic conditions and landscape modifications was the focus of our second study. The analysis revealed a tight relationship between spider population structure and temperature, global radiation and humidity, which confirms the observation of previous investigations. More interestingly, we found evidence for a significant shift in the ballooning phenology of most species, with the summer peak in ballooning activity shifted earlier in the year. In the long term, ground-living species decreased while tree-living spiders increased in abundance, which can be explained by concomitant landscape changes in the study area. We found no evidence that changes in meteorological parameters affected these trends. However, ballooning abundances decreased strongly in summer and fall 2003, when extreme meteorological conditions were prevailing in Europe. Interestingly, this collapse was preceded by very high ballooner abundances. The main results of this study show that spider populations appear to be more at risk from the expected intensification of extreme climatic events and changes in land use than from gradually rising temperatures.

In the third study, we aimed to develop a molecular method to track trophic links in food webs. This method was needed to identify the prey community of the wasp *Trypoxylon figulus* feeding in the larval stage on different spider species. Since larvae consume most of the spider prey, the identification of prey remains to the species level is very difficult. Furthermore, wasps prefer to trap immature spiders, which for a large majority cannot be identified because distinguishing morphological features are not developed. The study involved the establishment of a spider barcode of 104 individuals from 46 different spider species, previously identified to species level by morphological characteristics. The establishment of a maximum likelihood tree of mitochondrial cytochrome oxidase I (COI) fragments revealed terminal clades which corresponded to the morphologically identified species. With the help of the COI fragment database, we were able to identify the remains of spider tissue from *T. figulus* nests and immature spider individuals to the genus or species level. Despite criticism of the use of COI for the identification of species, our results thus highlight two practical advantages of this method: 1) the identification of species lacking clear morphological differentiation, like immature spiders, and 2) the better understanding of otherwise invisible trophic links in food webs by the identification of species from prey remains.

GENERAL INTRODUCTION

Understanding the structure and dynamics of communities is an important long-term objective of ecology, because it is fundamental in maintaining biodiversity, a major concern in the face of current drastic modifications of land use and climate change. Food web structure received particular attention in theoretic models aiming to predict the effect of structural food web complexity on the dynamics and stability of communities (MacArthur 1955, May 1974, De Angelis 1975, McCann 2000, reviewed in Bersier 2007). After the work of May (1974) showing that stability was not a mathematical consequence of complexity, many ecologists explored the question of how natural communities could persist. The first attempt to put biological realism in dynamical models was the work of De Angelis (1975) who proposed that the probability of food web stability increases with increasing connectance when the hierarchical nature of food web is taken into account. It has been shown that the extinction of single species in ecological communities can affect remaining species by causing a cascade of secondary extinctions (e.g., Borrvall *et al.* 2000; Koh *et al.* 2004; Petchey *et al.* 2008). For example the removal of predator sea star species (*Pisaster ochraceus*) from a rocky intertidal community leads to the uncontrolled dominance of mussels causing local extinction of many other species (Paine 1966). Such “secondary species extinctions” or “coextinctions” occur because a primary extinction creates a gap in the food web (a consumer may have no prey) or leads to a dynamically unstable community (Petchey *et al.* 2008). In order to understand trophic links within complex food webs, it is therefore crucial to understand interaction pathways among all organisms involved in the web of a given habitat and to evaluate the consequences of these interactions (Sheppard & Harwood 2005).

The following reasons make spiders a useful group of organisms to investigate community structure, dynamics and stability: 1) most spiders possess a highly efficient mode of dispersal, involving passive aerial movement by ‘ballooning’ on silk threads (Blackwall 1827, Bell *et al.* 2005). This powerful dispersal ability was proposed as a main reason why spiders are found in nearly every terrestrial ecosystem, ranging from the Arctic, to high altitudes, deserts or caves (Turnbull 1973); 2) As generalist predators, they evolved many different methods of prey catching, including hunting as well as the passive strategy by trapping prey in a web (Foelix 1979), which allow the colonization of a wide variety of ecological niches (Turnbull 1973); 3) they are among the most abundant terrestrial arthropods (Turnbull 1973) and can be considered as source (in the view of larger predators) as well as consumers of energy, which highlights their impact in many different food webs ; 4) with their sensitivity to structural and physical aspects of their environment and the narrow ecological tolerance of some species (Hänggi *et al.* 1995), spiders are known to be good indicators of the state of their habitat (Thaler 1985; Blick 1988; Hänggi 1991; 1993; Pozzi 1996). Under intensive culture spider diversity is impoverished (Nyffeler *et al.* 2004; but see also Bruggisser *et al.* 2010), but under favourable agricultural management they can even be more diverse and abundant than in natural habitats (Toft 1989).

It can be assumed that spiders, taking a centred place in food webs, are potentially regulated by both resources (bottom-up) as well as predators (top-down). Such a combined effect of top-down and bottom-up forces regulating species abundances have, to our knowledge rarely been demonstrated for arthropods (e.g., Spiller & Schoener 1994). There is ample evidence for the effect of vegetation structure on arthropod species composition (Lawton 1983, Morris 2000). The role of plant diversity is, however, less clear. Recent investigations demonstrated that higher plant diversity leads to better resource use and enhanced functioning – in terms of productivity – thanks to complementarity and positive interactions (Hooper *et al.* 2005; Spehn *et al.* 2005). It has been found that, on theoretical ground, the relationship between diversity and ecosystem functioning is not that straightforward when trophic aspects are taken into account (Paine 2002, Thébault & Loreau 2003). The effect of plant diversity on trophic interactions has, so far, less well been investigates. The general homogenisation of habitats, especially involving the loss of plants diversity, raises the question, how arthropod communities and their natural prey and predators will be influenced by the loss of plant diversity.

In a first part of my thesis the distribution and abundance patterns of the web-building spider *Argiope bruennichi* in relation to plant, prey and predator species were investigated. A field experiment was conducted in different semi-natural habitats, where plant diversity, and indirectly vegetation structure, was manipulated. The aim of this study was to test whether both bottom-up and top-down forces both affect the local distribution of the study species. Additionally, I wanted to know how spider abundances are influenced by plant diversity, whether through a direct relationship with plants or whether this effect is indirect, mediated through the abundance of prey.

In regard to the regional distribution of A*. bruennichi*, it has been observed that this species is expanding its range from southern into central Europe within the last decades (Guttmann 1979, Hänggi *et al.* 2001). The ability of dispersing by ballooning has been proposed as a major reason for their successful colonization of new habitats. It has been shown that ballooning depends on the occurrence of favourable conditions of temperature, wind speed and rainfall (Legel & van Wingerden 1980; Reynolds *et al.* 2007). Changes in temperature are known to influence the developmental rate of spiders (Li & Jackson 1996; Bonte *et al.* 2008), affecting their body size (Høye *et al.* 2009) and hence potentially affecting reproductive success. The influence of temperature on phenotypic traits and the dispersal behaviour of spiders raise the question, whether the colonisation of the northern hemisphere by *A. bruennichi* may be explained by climate change. Different studies demonstrated changes in distribution patterns of animal species to be a response to climate change (Walther *et al.* 2002). Alterations in climatic conditions leading to a range shift in some species can be expected to affect other species and hence to lead to changes in community structure. Beside climate change current drastic modifications in land use may additionally lead to structural changes in communities, since species which are more resistant to disturbance may outcompete the more sensitive ones (Sakai *et al.* 2001). Studies investigating the long-term effect of global change like climate change and habitat loss on the composition and dynamics of arthropod communities are rare.

In a second part, I tackled this research theme by analyzing a dataset of more than 15,000 ballooning spiders, which were caught at weekly intervals between 1994 and 2004 in south-western Switzerland using a 12 m high suction trap. With this dataset I aimed to study the effect of climatic conditions and landscape modifications on the dynamics of a ballooning spider assemblage.

Studies on interacting species within communities are rare, since the direct observations of predation events in food webs are difficult especially for highly mobile, underground, rare or cryptic species (Thies & Tscharntke 1999; Steffan-Dewenter *et al.* 2001, 2002). DNA-based species-identification tools have recently gained in importance and are promising tools to overcome the difficulties in tracking trophic links in food webs (Tautz *et al.* 2003; Herbert *et al.* 2003; Blaxter & Floyd 2003). In a third part of this study, I used a model food web of solitary living wasp species of the family Sphecidae, feeding their larvae with spider prey. The wasp larvae consume most of the provided prey, which complicates the identification of spider prey to the species level (Polidori *et al.* 2007; Buschini *et al.* 2008). The aim of the study was to establish a genetic barcode database of a spider community, to investigate the trophic link between trap-nesting wasps and their spider prey communities. With this experiment I emphasized on the potential of molecular techniques to answer basic questions in ecological research.

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Bottom-up and top-down control of *Argiope bruennichi* in semi-natural ecosystems

Odile T. Bruggisser, Nadine Sandau, Alex Aebi, Gilles Blandenier, Yvonne Fabian,
Patrik Kehrli, Russell E. Naisbit & Louis-Félix Bersier

Bottom-up and top-down control of *Argiope bruennichi* (Araneae: Araneidae) in semi-natural ecosystems

Odile T. Bruggisser[1*]; Nadine Sandau[1]; Alex Aebi[2]; Gilles Blandenier[3]; Yvonne Fabian[1]; Patrick Kehrli[4]; Russell E. Naisbit[5] & Louis-Félix Bersier[1]

1 University of Fribourg, Unit of Ecology & Evolution, ch. du Musée 10, CH-1700 Fribourg, Switzerland
2 Agroscope Reckenholz-Tänikon ART, Research Station ART, Reckenholzstrasse 191, CH-8046 Zürich, Switzerland
3 Centre Suisse de Cartographie de la Faune (CSCF), Passage Max-Meuron 6, CH-2000 Neuchâtel, Switzerland
4 Station de recherche Agroscope Changins-Wädenswil ACW, Nyon, Switzerland
5 University of Neuchâtel, Institute of Biology, Neuchâtel, Switzerland.

ABSTRACT:

Bottom-up and top-down forces have generally been recognized as important mechanisms regulating the abundance of arthropods. Only few studies emphasized, however, the importance of top-down forces regulating the abundance of intermediate sized arthropod predators. More attention has been paid to the bottom-up effect of the vegetation. Nevertheless, it remains unclear which aspect of the vegetation (vegetation structure, plant diversity, or plant species composition) plays the most important role.

In this study we were interested in the bottom-up and top-down mechanisms regulating the abundance and hunting success of the web-building spider *Argiope bruennichi* in semi-natural systems. We manipulated plant diversity (2, 6, 12, 20 species), and thereby indirectly habitat structure, in 12 wildflower strips in Swiss ecological compensation areas, situated in a region of intensive farmland. Within these wildflower strips, we studied the naturally occurring predator and prey community of *A. bruennichi* to investigate major interactions in this food web.

Our study system revealed that the abundance of *A. bruennichi* is regulated by combined bottom-up and top-down effects, and by direct and indirect interactions between trophic levels. Three main factors were found to characterize this system: the strong negative effect of a single predator, namely hornets, the positive direct effect of vegetation structure and the positive effect of plant diversity, which affected spider abundance directly and indirectly via potential prey. Overall, this result may indicate that spiders evolved the ability to distinguish between different habitats according

to plant-diversity cues, which reflect prey availability. In this case, we expect an indirect and a direct link, the latter occurring if plant diversity and prey availability are not perfectly correlated.

INTRODUCTION

Trophic regulation by either resources (bottom-up) or predators (top-down) has been recognized to be important in controlling the distribution and abundance of species (Hunter and Price 1992). The idea that a combination of bottom-up and top-down forces mediates the structure of communities has, however, found less support. Most attention has been paid to the bottom-up forces of plants as the prime determinants regulating species composition at different trophic levels (Root 1973, Hunter and Price 1992, Balvanera et al. 2006, Haddad et al. 2009). This can be explained by the fact that plants can be expected to directly affect consumer (Siemann et al. 1998, Knops et al. 1999, Haddad et al. 2001, Johnson et al. 2006) as well as predator (Pearson 2009) species because they are not only primary producers, but also architects, largely determining the physical structure of habitats. The top-down control of trophic interactions by predators has mostly been demonstrated for simply structured food-webs in terrestrial ecosystems (e.g. Finke and Denno 2003, 2004, Schmidt et al. 2003, Sanders et al. 2008). Only few experimental studies on top- and intermediate (medium-sized) predators found support for the less investigated combined effect of bottom-up and top-down forces regulating distribution patterns and abundance of species (Spiller and Schoener 1994; Elmhagen and Rushton 2007).

As intermediate predators, web-building spiders may be especially suited to study the possibility of a combination of bottom-up and top-down forces regulating their distribution and abundance. Spider species do not select their habitat randomly (Topping and Lövei 1997). Vegetation has been found to be more important for spiders than prey availability (Greenstone 1984, Bradley 1993). Web-building spiders in particular depend on specific spatial structures of their habitat for web construction (Greenstone 1984, Rypstra 1986, Uetz 1991), which makes them particularly sensitive to the vegetation structure of their habitat (Olive 1980, Rypstra 1986, Gunnarsson 1990, Bradley 1993, Lubin et al.1993, Petersson 1996, Balfour & Rypstra 1998). While structure can be expected to directly affect the distribution of spiders (e.g. Pearson 2009), other features of plant communities, like plant diversity or plant identity, have been less well investigated. It is not clear whether these characteristics might have a direct effect on distribution and abundance or an indirect effect via prey communities. Plant diversity (e.g. Siemann et al. 1998, Knops et al. 1999) and especially plant identity (Schaffers et al. 2008) has been shown to affect insect diversity and abundance, the main prey of spiders. Since spiders are not top predators, higher-order predators may also control spider abundance and diversity.

Argiope bruennichi (Scopoli 1972) is a generalist passive predator. This orb-weaving spider may play an important role in natural pest control (Rypstra and Carter 1995). Beside this potential beneficial effect, the presence of this species may, negatively affect ecosystem services like pollination, since hymenopterans are among their most important prey (Nyffeler and Benz 1981). *A. bruennichi* is very common in southern Europe and has been expanding northwards in recent decades (Hänggi et al. 2001, Guttmann 1979). In uncultivated margins and fallow land within central European agricultural landscape, *A. bruennichi* is one of the most abundant species (Barthel and Plachter 1995).

In this study, we were interested in the top-down and bottom-up mechanisms regulating the abundance and hunting success of the web-building spider *Argiope bruennichi* (Scopoli 1972) in semi-natural systems. We manipulated the plant diversity and identity of semi-natural habitats (wildflower strips) within an agricultural landscape in Switzerland and thereby indirectly diversified habitat structure. Within these wildflower strips, we studied the naturally occurring predator and prey community of *A. bruennichi* to investigate major interactions in this system. We considered the following factors: presence of predators, abundance of potential prey, and three characteristics of vegetation (diversity, species composition, and structure). Specifically, we tested the following hypotheses: 1) Vegetation structure has a direct effect on the abundance of spiders. 2) Plant species composition and diversity affect spiders indirectly through prey communities. 3) As intermediate predators, *A. bruennichi* abundance is mediated by a combination of top-down and bottom-up forces.

MATERIAL AND METHODS

To investigate interactions in the foodweb centered on *A. bruennichi* we made use of twelve wildflower strips with a specific study design, which had been established in purpose of a larger research project. In a first part, we will describe the study design of the field site, established for this larger research project. In a second part we will explain how *A. bruennichi* and its prey and predators were investigated within these fields; Finally, we will explain the statistical methods, which was adjusted to the specific design of the field site.

Study design of field site

The study took place in an intensively managed agricultural landscape around Grandcour (46° 52' 18'' N, 6° 55' 50'' W) in the lowlands of western Switzerland. In the context of a larger research project to explore the structure and functioning of metacommunities, 12 experimental wildflower strips (hereafter, fields) of ~870m^2 (9 x 96 m, or 6 x 144 m) were planted in spring 2007, of which nine were used in the present work. In purpose of the specific questions addressed in the larger research project two factors were manipulated: diversity of plants (4 levels) and fencing (3 levels). In contrast to the plant diversity treatment, the fencing treatment was not relevant for the study on *A. bruennichi*. The fields were subdivided into three fencing treatments, each of area 216m^2 (Fig. 1). The first treatment consisted of a fine-meshed (8 mm mesh size), the second of a wide meshed (25 mm mesh size) 1.2m high fence. The fences were installed ~20cm deep in the soil, with a ~10 cm long right-angled bend directed outwards from the plot and the fenced area was open on top. The fencing aimed at preventing the access to large herbivores and/or predators, which do not intervene in the present study; thus, the fencing factor was considered here as random blocks. The third treatment was, as a control, not fenced. The three fencing treatments were randomly distributed in each field. Within each fencing treatment, four plots (6 by 9 meters) differing in plant diversity (2, 6, 12, 20 sown species) were randomly assigned. Thus, in total each of the 12 fields (random factor) consisted of 12 plots (3 fencing x 4 diversity treatments). For the experiment, we seeded 20 herbaceous plant species belonging to the same functional group (tall herbs); they were chosen from the 24 plant species normally used in Swiss wildflower strip mixtures (wildflower strips are ecological compensation areas in Switzerland; see Pfiffner and Schaffner 2000, Haaland et al. 2010; Table 1). Plant species composition of each plant diversity plot was chosen by constrained random draw from the 20 plant species pool (obviously, testing all combinations is not achievable): 1) combinations of plants in the 3 fencing levels were the same within each field; 2) plants were chosen so that they appeared, within all 12 fields, the same number of times (with few exceptions) in the 2, 6 and 12 species levels; 3) within a field, there was no overlap in the species chosen in levels 2, 6 and 12 species. The experiment can be described as an incomplete (not all combinations of plants are considered) split block design, with fields as random factors, and diversity factor nested in fencing factor.

In contrast to other biodiversity experiments (e.g., Cedar Creek: Tilman et al. 1997, Tilman et al. 2006; BIODEPTH: Schmid and Hector 2004; The JENA Experiment: Roscher et al. 2005, Marqurd et al. 2009) our experimental plots were not weeded, with the exception of the problematic weeds *Cirsium arvense* and *Rumex obtusifolius*, and in the first year (2007) additionally *Chenopodium album* and *Amaranthus retroflexus* to prevent light competition during germination. In the following years, we allowed natural invasion and the establishment of seeds from the seed bank. Weeding was avoided to minimize perturbation of higher trophic levels, which were an important focus of the research project. Along the center of each field, a 30cm wide path was established before data acquisition to enable sampling in the centre of the fields and to reduce disturbance while walking through the fields.

Sampling of A. bruennichi and related trophic levels

Argiope bruennichi is a predominantly diurnal forager capturing mostly diurnal insects (Pasquet 1984, Baba and Miyashita 2006, Prokop 2006). Their web is characterized by a specific vertical zigzag silk band (stabilimentum) (Prokop and Grygláková 2005). In 2008 and 2009 all spider webs of adult female *A. bruennichi* were sampled once a year in each of 9 fields at the end of July and beginning of August, by transect observations. On sunny and calm days, from 10am until 6pm, webs were recorded along the central transect to a width of 50cm on each side, ignoring those built directly along the path and at the junction of plots. For each web, the following information was recorded: identity of support plants (only in 2009), number and identity of trapped prey (at the order level); indication of predator attack (remains of spider legs in the web, see below); web size (width and height), above ground height of the centre of the web, and web orientation. All prey species from the webs were collected for subsequent determination. If not too heavily damaged, prey volume was estimated from width and length, using the equation for a prolate spheroid.

Predators of *Argiope bruennichi* were investigated by the inspection of web damage or by direct observations during the same period as spider webs were counted. Observed predation acts were all due to a single species, the European hornet *Vespa crabro* L. (Hymenoptera: Vespidae). Hornet individuals were observed attacking spiders on their webs, in the majority of cases pinching off their legs to transport the spider to their nest. Due to their large foraging range, we recorded the presence of hornets at the field level (assuming they affected all plots equally, independently of the diversity and fencing treatment).

Prey availability was estimated using data from an independent experiment in six fields, four of which were included in the present work. Because the most abundant prey of *A. bruennichi* are dipterans and hymenopterans (Nyffeler and Benz 1981), we investigated insects visiting flowers. On calm and sunny days during July and August, insects were observed once per field and year between 10:00am and 16:00pm. In each plot, insects were counted during 15 minutes in two randomly positioned 1x1m plastic frames (placed carefully prior to observations). Species were identified directly in the field to the order level.

Vegetation sampling took place in 2008 and 2009 between July and October. In all plots the total number of plant species (2008: 22.17 ± 6.45 (mean ± s.d.), max: 42, min: 6; 2009: 19.31 ± 5.36, max: 35, min: 7) was determined and their individual percentage cover estimated (Perner et al. 2005, Woodcock et al. 2007). Vegetation structure was characterized with the following variables: average (2008: 1.39m ± 0.38, min: 0.3m, max: 2.1m; 2009: 0.99m ± 0.25, min: 0.3m, max: 1.8m) and maximum vegetation height (2008: 1.94 ± 0.45; 2009: 1.48 ± 0.41), and leaf area index LAI (2008: 1.86 ± 0.65; 2009: 3.77 ± 0.85). We had previously tested the relationship between LAI and above-ground biomass, and found that LAI is a good surrogate for vegetation biomass (correlation). We used LAI as an indirect measure of vegetation cover (hereafter, vegetation cover).

Statistical analysis

Plants used for web construction

We tested the null hypothesis that plant species were used in proportion to their availability with a X^2 test. The expected frequency with which a plant species was used for web construction was calculated by multiplying the percent cover of the plant species by the total number of plants used for web construction within the given plot (note that one web could be attached to several plant species).

We identified two plant species (see Results section) with the highest contributions to X^2, namely *Malva sp.* and *Dipsacus fullonum*. In subsequent analyses, we used the log-transformed cover of both plants separately to describe the effect of "plant composition" on the different measurements of spiders.

Effect of predators

Due to their high mobility, the influence of hornets on the abundance of *A. bruennichi* was analyzed at the field level. For each field, the percentage change (positive or negative) in spider web number between 2008 and 2009 was calculated. The nine fields were assigned to one of four categories: I) 0→0 during the sampling period no hornets were present in both years (*n* = 5); II) 1→1 hornets were present in both years (*n* = 1); III) 0→1 hornets were only present in 2009 (*n* = 2); IV) 1→0 hornets were only present in 2008 (*n* = 1). The effect of the presence of hornets on the percentage change in abundance of *A. bruennichi* webs was tested with a t-test assuming unequal variance between categories I and II (i.e., no change) versus III.

Pairewise interactions between trophic levels

We explored how vegetation could explain the distribution, abundance and trapping success of *A. bruennichi* in the 216 (nine fields and two years) plots with mixed effect models (Zuur et al. 2009). All dependent and indiependent variables used in this analysis are described in Table 3 (Analysis of parewise interactions). As dependent variables, we used "presence/absence of webs", "abundance of webs" (excluding plots without webs; the latter variable was Box-Cox transformed, Legendre and Legendre 1998), and "trapping success". The latter variable was composed of a single vector including a combination of the two vectors "number of webs with prey" and "number of webs without prey", created by the command "cbind" in R. We considered three aspects of vegetation as independent variables: plant diversity, composition and vegetation structure. To describe the diversity in each plot, we did not use the number of sown species (diversity treatment), but the actual species richness measured by the diversity number (or effective number of species, Jost 2006), based on Shannon diversity. The diversity number expresses species richness corrected for relative abundance – in our case, relative cover of plants

(Legendre and Legendre 1998). Note that diversity number was highly correlated with the number of sown species (Pearson $r = 0.42$, $t = 6.74$, $P < 0.01$). To describe plant compostion we considered the log transformed cover of *Malva sp.* and of *Dipsacus fullonum* as two independent variables. The structure of the vegetation in each plot we described by vegetation cover (LAI) and average vegetation height. The latter five independent variables were all standardized prior to analyses.

We used linear mixed-effect models assuming a random intercept, and using as random factors “fence” (fencing treatment), nested within “field”, nested within “year”. As explained above, fencing was used as a random factor since we did not expect this treatment to affect the abundance of *A. bruennichi* (indeed, including fencing as a fixed factor never yielded significant results). To select the best model, we started with a “full” model (all explanatory variables and their pairwise interactions; higher order interactions were not tested), and removed in turn variables least affecting the AIC of the model, using maximum likelihood; once the best model was found, its parameters were estimated with restricted maximum likelihood. For “abundance of webs”, we applied a linear mixed effect model with Gaussian family and identity link (function lme of package nlme in R; Pinheiro et al. 2008; R Development Core Team 2009). For the dependent variables “presence/absence of webs” (a binary variable) and “trapping success” (a proportion variable), we used a generalized linear mixed effect model with binomial family and logit link function (function glmer of package lme4 in R; Bates and Maechler 2009).

We performed two additional analyses, using the same method as for “abundance of webs”. First, to further explore the effects of vegetation on trapping success of spiders, we used as dependent variable “prey volume”, including the prey volume of the largest trapped prey observed in each spider web. Second, in the six fields where pollinators were sampled, we examined the relationship between “abundance of potential prey” and vegetation.

Direct and indirect interactions between all trophic levels

With the four fields (96 plots in two years) for which all information (abundance of spiders, presence of hornets, abundance of potential prey, and plant diversity, composition and vegetation structure) was available, we evaluated the relative importance of the explanatory variables on the abundance of *A. bruennichi*. The aim was to obtain a global view of the factors affecting spider abundance, as customarily achieved with path analysis. However, the structure of our experiment, with nested random factors (year, field and fence), made this approach impracticable. To solve this problem, we used a linear mixed effect model (of Gaussian family and random factors as above) after having standardized all variables. Standardization renders the variables dimensionless, and thus the slopes of the linear model can be compared and used as a measure of the importance of the “causal” paths. In the present analysis the vegetation was described by three independent variables. First, we included plant diversity expressed by plant "diversity number". As second independent variable we described plant species composition by a single variable: we performed a Correspondence Analysis (CA) of the plots described by the vegetation, and used the coordinates of the plots on the first ordination axis as summary variable. We used the same approach with vegetation structure, the third independent variable, but here used a Principal Component Analysis (PCA) to summarize the three variables average and maximum vegetation height and LAI. All ordinations were performed with the package vegan in R (Oksanen et al. 2009). We further included a first-order auto-regressive correlation structure (corAR1) in the linear mixed effect model to compound the correlation between observations from the two years.

RESULTS

Spider abundance, plant selection and diet

Argiope bruennichi abundances more than doubled between 2008 (917 webs) and 2009 (1926 webs). In 2008, a maximum of 4 spider webs per square meter were observed, while in 2009 densities could reach up to 7 webs per square meter. Web size and aboveground height did not significantly differ between plots or fields. During the sampling period, *A. bruennichi* was the dominant adult orb-weaving spider occurring in our fields and other species, like *Araneus diadematus* or *Nuctenea umbratica*, were only occasionally observed.

The frequency of use of plant species for web building is given in Figure 2. Among these 24 plant taxa, use of the *Malva sp.* group (*Malva moschata* and *Malva sylvestris*) was nearly double that than expected, while only one third of the available *Dipsacus fullonum* plants were used.

A total of 2082 (2008: 910; 2009: 1172) prey individuals belonging to 12 different orders were sampled in webs of *A. bruennichi* (Table 2). The prey community, representing prey individuals directly collected out of spider webs, was mostly composed of dipterans (2008: 45%, 2009: 26%) and hymenopterans (2008 and 2009: 35%). Around 50% of hymenopterans belonged to the family Apidae. In 2008, an average of 1.63 ± 1.04 (mean ± standard deviation) prey individuals were found per web, while in 2009 there were 1.49 ± 0.85. This decrease in trapping success in 2009 was further evidenced by a lower percentage of webs containing prey (44% in 2008; 36% in 2009; G-test of independence, $P < 0.001$). A maximum of 8 prey individuals were found in one spider web each year.

Simple interactions between trophic levels

Here, we present the results of analyses between *A. bruennichi* and 1) their predators, 2) their potential prey, 3) the vegetation, and 4) between the abundance of potential prey and the vegetation. Analysis 1) is performed at the field level, while other analyses are at the plot level (with one exception). Three aspects of vegetation are considered: diversity, structure, and composition.

1) Predators of *A. bruennichi*. Hornets were present in two fields in 2008, and in three fields in 2009.

Two fields were newly colonized in 2009 while one was abandoned (Fig. 3). It is apparent that the presence of hornets has a strong effect on *A. bruennichi* abundance, which is statistically significant despite the small sample size (2-tailed t-test assuming unequal variance between groups 0→0 and 1→1 versus 0→1 of Fig. 3: d.f. = 5, t = 3.97, *P* = 0.01).

2) Potential prey of *A. bruennichi*. There was a significant positive correlation between pollinator abundances and the presence/absence of the spider (mixed effect model with binomial response: n = 92, fixed effect = 0.98, *z* = 2.69; *P* = 0.007). The effect on spider abundance and on trapping success (number of webs with and without prey) was also positive, but not significant.

3) Vegetation effects on *A. bruennichi*. The effects of the different vegetation variables on the presence/absence, abundance and trapping success of *A. bruennichi* in the nine fields and two years (n = 216), are summarized in Table 4. The presence/absence of the spider was influenced by the diversity and composition of plants (*Malva*, *Dipsacus* cover) and by the structure of the vegetation. It was positively related to plant diversity and negatively affected by vegetation height. Significant interactions between explanatory variables revealed that high vegetation cover (LAI) decreased the negative effect of vegetation height, and that *Malva sp.* abundance reinforced the negative effect of vegetation height. Vegetation cover only had an effect in conjunction with *Malva sp.* abundance, by lowering its positive effect.

When considering *A. bruennichi* abundance, the only significant direct effect was that of *D. fullonum*, with fewer webs being found in plots with high abundances of *D. fullonum*. Interestingly, main effects (i.e., the effect of one variable when the other is equal to 0 – in our case to its mean since all variables were standardized) of *Malva sp.* and of vegetation height were of the same sign as those for *A. bruennichi* presence/absence, but were not significant.

Trapping success depended on vegetation structure and plant composition: success was increased in plots where the vegetation was tall and decreased where *Malva sp.* was abundant. Both relationships can be explained simply by the fact that plots with increased vegetation height and decreased *Malva sp.* abundance harbored fewer spider webs, and thus those present had a greater share of potential prey. Indeed, We also observed that trapping success was negatively correlated with spider density (Pearson *r* = -0.26, t = -3.59, *P* < 0.01). Plant diversity was found to have an effect in conjunction with vegetation cover (LAI): it was positive for low vegetation cover, but disappeared with high cover.

Prey items were significantly larger in plots with higher plant diversity, while in plots with high densities of *Malva sp.* significantly smaller prey were trapped (analysis performed for each web, d.f. = 818; estimate = 0.29, t = 2.14, *P* = 0.033, and estimate = -0.32, t = -2.28, *P* = 0.023, respectively). The structure of the vegetation did not affect prey volume.

4) Vegetation effects on the abundance potential prey. In the six fields where pollinator and plants were investigated, pollinator abundances were only affected by plant diversity (d.f. = 91, estimate = 0.45, t = 2.36, $P = 0.02$), while vegetation structure and the percent cover of *Malva sp.* and *D. fullonum* had no significant effect.

Direct and indirect interactions in the full system

All direct and indirect effects on spider abundance could be analyzed in a subset of 4 fields. The result is summarized in Figure 4. The correlation between both years was weak (Phi = 0.021). The observed interactions between single trophic levels were largely confirmed in this full model. The negative effect of hornets and the positive effect of the abundance of potential prey on *A. bruennichi* remained significant once the effect of all other explanatory variables had been excluded. Plant species composition (measured as the plot coordinate of the first axis of a CA on plant community) had no effect either on potential prey abundances or on spider abundance. Vegetation structure (measured as the plot coordinate of the first axis of a PCA on three variables) had a direct effect on spider abundance, but not on potential prey abundance. Plant diversity had a marginally significant effect on potential prey abundance and the latter a significant positive effect on spider abundance. This is an indication of an indirect effect of plant diversity on *A. bruennichi*. Surprisingly, however, plant diversity also directly affected spider abundance once all other effects were removed (the same result was found with diversity expressed as diversity number or as total number of species). Note that plant diversity was not correlated with vegetation structure or plant species composition, while both later variables were weakly and non-significantly correlated.

DISCUSSION

Our study system showed evidence for the combined importance of bottom-up and top-down effects and of direct and indirect interactions in order to understand the abundance of a focal species, in our case a web-building spider. Among the web of interactions found to affect *A. bruennichi*, three potential factors stood out: the strong negative effect of a single predator, namely hornets, the positive direct effect of vegetation structure and the positive effect of plant diversity, which affected spider abundance directly and indirectly via potential prey. The latter chain of interaction was expected; however, the observed direct effect of plant diversity in this system raises interesting questions about selection pressures underlying habitat choice in this spider.

Top-down effects

Although the distribution of *A. bruennichi* was mediated by the bottom-up effect of several vegetation variables, their abundances were strongly affected by the presence of hornets. Due to the observed drastic reduction in spider abundance in fields where hornets were present (Fig. 3), top-down forces are certainly a key factor determining *A. bruennichi* abundance. Compared to bottom-up effects of plants, this factor is most probably unpredictable by spiders during the habitat selection process, which occurs earlier (around May, Hänggi et al. 2001) than the main period of activity of hornets (late July to August, Tryjanowski et al. 2010). Thus, the effect of hornets should be primarily on spider abundance, and less on spider spatial distribution. It is known that *Argiope* as well as other web-building spider species add an irregular tangle of non-sticky silk threads, known as barrier webs around their web to prevent attacks from predators (Robinson and Mirick 1971, Lubin 1975). In our system, such an antipredator response was very commonly observed in a single field in 2009, where predation pressure by hornets was strongest.

Bottom-up effects

We observed a tight interaction between vegetation variables and *A. bruennichi* distribution patterns. Vegetation structure was, compared to plant diversity, identity or species composition, a better variable to predict distribution patterns of this spider. The importance of vegetation structure for spiders (Rypstra 1986, Halaj et al. 1998, McNett and Rypstra 2000) as well as for arthropods in general (Morris 2000, Perner et al. 2005) is well supported. Despite the interest in the relationship between spiders and vegetation structure, only few studies analyzed whether this relationship was direct or indirect through its influence on prey communities (Rypstra 1983, Pearson 2009). Here, we found that vegetation structure significantly influenced spider abundance once all other measured variables had been accounted for. Interestingly, such a direct effect of vegetation structure was not observed on the abundance of potential prey.

Contrary to other studies (e.g., Perner et al. 2005, Schaffers et al. 2008) global plant species composition did not predict *A. bruennichi* abundance. This discrepancy can be simply explained by the fact that variability of plant species composition was comparatively low in our experimental system, where sown plants belonged to a single functional group. This does not, however, mean that plant identity is neutral for habitat selection. The presence of *A. bruennichi* was found to depend positively on *Malva sp*. and the abundance of the spider negatively on *Dipsacus fullonum*. This result is in line with experiments of Pearson (2009), who observed that the presence of a single invasive plant species enhanced abundances of the web-building spider *Dictyna* spp. In their experiement the observed spider species seemded to show a preference for a certain plant species, as it was observed in our experiment with Malva sp.

Plant species composition and vegetation structure were found to have no significant effects on potential prey community, and thus could not be ascribed any indirect effect on spider abundance, unlike plant diversity.

Direct and Indirect effects of plant diversity

Several biodiversity studies observed

a positive relationship between plant diversity and total predator abundance (Siemann et la. 1998, Haddad et al. 2001, Haddad et al. 2009). For spider abundance, however, this response is less clear. In a grassland experiment by Perner et al. (2005), total web-building spider abundance was positively but not significantly related to plant diversity. In another grassland experiment, Koricheva et al. (2000) even found a negative effect of plant diversity. This study however considered epigeic spiders, which can be expected to show a different response to plants than web-building spiders, since they do not need plants for web construction. In our system, we found an effect of plant diversity which could be decomposed into a direct and an indirect component.

A positive effect of plant diversity on pollinators – the main prey of *A. bruennichi* – is seen in habitats dominated by flowering plants (Heithaus 1974, Banaszak 1996, Ghazoul 2006). Thus, we can expect plant diversity to affect spider abundance indirectly through its effect on potential prey. In our system, this chain of interactions was substantiated by the positive effect of plant diversity on the abundance of potential prey, and in turn of potential prey abundance on *A. bruennichi* abundance.

Our experiment also revealed a direct effect of plant diversity on spider abundance. Three potential explanations have been suggested for the direct effect of plant diversity on predator diversity (Siemann et al. 1998), which we also expect to apply for predator abundance. Firstly, additional resources from a variety of plants may directly increase predator abundance (e.g. pollen for parasitoids). This is not likely to be the case for web-building spiders. Secondly, the effect may be apparent through a change in the interaction between pollinators and predators in more diverse habitats (Wooton 1993). The observation that prey size in webs was larger in plots with higher plant diversity may support this hypothesis. Thirdly, some form of habitat selection by predators may be a simple and widespread cause for such a direct effect. If a predator selects habitats with high plant diversity because they generally harbor more prey, a direct effect between plant and predator is expected when plant diversity and prey abundance are not "perfectly" correlated (Siemann et al. 1998). Habitats may be selected for other reasons, for example to enhance trapping efficiency, to obtain protection from larger predators (Price et al. 1980), or to avoid negative effects of meteorological conditions like wind (Enders 1975). In any case, the observed direct link between plant diversity and *A. bruennichi* abundance suggests that the spider has evolved the ability to select more diverse habitats. Such an ability of spiders to discriminate between different habitat types has been proposed by several authors (Greenstone 1984, Lubin et al.1993, McNett and Rypstra 2000, Pearson 2009), and studies have shown that web-building spiders respond to habitat diversity and complexity (Rypstra 1986, Greenstone 1984, Halaj et al. 1998, Rypstra et al. 1999).

Caveats

Our study is a semi-natural experiment where the diversity of sown plant species was manipulated. A strict experimental approach to our questions would have required independent manipulations of all other factors. This may be feasible for the abundance of potential prey and of hornets, but would involve closed experiments, and possibly artificial prey communities. Independently manipulating diversity, composition and structure of the vegetation would be even more challenging. Thus, we cannot exclude that our observed relationships are the result of artifactual correlations. However, this uncertainty is at the benefit of experimental conditions being closer to natural situations.

Conclusions

The significant results of vegetation variables, of prey and of hornets support the hypothesis that our system is mediated by bottom-up and top-down forces. Although the importance of combined bottom-up and top-down effects have been invoked in several models of community organization (Oksanen et al.1981, Leibold 1989, Hunter and Price 1992, Elmhagen and Rushton 2007), they have so far rarely been considered together in studies of arthropods (e.g., Spiller and Schoener 1994). Spiders are often viewed as the dominant invertebrate predator of most ecosystems (Wise 1993), and their predators have often been neglected. Our study highlights the need to consider the effects of both top-down and bottom-up forces to understand the distribution patterns of these arthropod predators. To understand the role of spiders in agricultural food-webs and to maximize their potential as biological control agent, spiders have thus to be considered as intermediate predators in food-webs where plants, prey species and larger predators interact.

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TABLE 1. List of species sown in the wildflower strips used presenting this study.

Plant family	Plant species	Seed density (g/ha)	Life history	Months of flowering
Apiaceae	*Daucus carota*	150	Biannual	6-8
	Pastinaca sativa	80	Biannual	7-8
Asteraceae	*Achillea millefolium*	20	Perennial	6-9
	Anthemis tinctoria	20	Perennial	6-8
	Centaurea cyanus	500	Biannual	6-10
	Centaurea jacea	200	Perennial	6-9
	Cichorium intybus	120	Perennial	7-9
	Leucanthemum vulgare	80	Perennial	5-10
	Tanacetum vulgare	3	Perennial	6-9
Boragninaceae	*Echium vulgare*	200	Perennial	5-10
Caryophyllaceae	*Agrostemma githago*	600	Biannual	6-8
	Silene alba	100	Biannual	6-9
Clusiaceae	*Hypericum perforatum*	60	Perennial	6-9
Dipsacaceae	*Dipsacus fullonum*	2	Biannual	7-8
Lamiaceae	*Origanum vulgare*	60	Perennial	7-9
Malvaceae	*Malva moschata*	20	Perennial	6-9
	Malva sylvestris	60	Biannual	4-9
Papaveraceae	*Papaver rhoeas*	150	Biannual	5-9
Scrophulariaceae	*Verbascum thapsus ssp.*	50	Biannual	6-9
	Verbascum lychnitis	30	Perennial	6-9

TABLE 2. Percentage of observed prey individuals in *Argiope bruennichi* spider webs in 2008 and 2009.

order	2008	2009
Diptera	45	26
Hymenoptera	35	35
Hemiptera	7	11
Coleoptera	6	17
Lepidoptera	3	4
Thysanoptera	1	1
Mecoptera	1	0
Araneae	1	2
Orthoptera	1	0
Acari	0	1
Caelifera	0	2
Neuroptera	0	1
Total	910	1172

TABLE 3. Independent and dependent variables used in the analysis of pairwise interactions between trophic levels (see Table 4) and in the global analysis of direct and indirect effects in all trophic levels (see Figure 4).

Analyses of pairewise interactions		
Variable type	Variable name	Comment
Dependent variable		
	presence/absence of webs	binary variable
	abundance of webs	excluding plots without webs; Box-Cox transformed
	trapping success	number of webs with and number of webs without prey (proportion variable)
	prey volume	prey volume of largest prey observed in each web; Box-Cox transformed
	abundance of potential prey	number of flower visiting insects in each plot; Box-Cox transformed
Independent variable		
Vegetation structure:		
	vegetation cover measured as leaf area index (LAI)	quantitative variable
	average vegetation heigth	quantitative variable
Plant composition:		
	cover of *Malva sp*	log-transformed percentage (1 to 100) cover
	cover of *Dipsacus fullonum*	log-transformed percentage (1 to 100) cover
Plant diversity:		
	diversity number	quantitative variable
Global analysis of all interactions		
Dependent variable		
	abundance of webs	quantitative variable, Box-Cox transformed
Independent variable		
Vegetation structure:		
	1^{st} PCA axis based on the plots described by the variables of vegetation structure	quantitative variable
Plant composition:		
	1^{st} CA axis based on the plots described by the cover of individual plant species	quantitative variable
Plant diversity:		
	diversity number	quantitative variable

Table 4. Effect of vegetation variables on the presence/absence (nb. of observations: 206), abundance (d.f. = 112) and trapping success (d.f. = 111) of *Argiope bruennichi* analyzed by generalized linear mixed effect models (SE standing for standard error). "Trapping success" represents a single vector including a combination of the two vectors "number of webs with prey" and "number of webs without prey". The results shown here represent the best models obtained by backward elimination. Significant effects are shown in bold.

	presence/absence of webs				**abundance of webs**				**trapping success**			
	Estimate	SE	*Z*	*P*	Estimate	SE	*T*	*P*	Estimate	SE	*Z*	*P*
Plant diversity	0.789	0.318	2.485	**0.013**								
Average vegetation height	-0.928	0.351	-2.649	**0.008**	-0.035	0.109	-0.325	0.746	0.178	0.059	3.014	**0.003**
LAI (vegetation cover)					0.185	0.145	1.292	0.199	-0.139	0.086	-1.625	0.104
Malva cover	0.616	0.347	1.776	0.076	0.031	0.088	0.349	0.728	-0.138	0.053	-2.592	**0.010**
Dipsacus cover					-0.221	0.095	-2.317	**0.022**				
Plant Diversity x LAI									-0.092	0.045	-2.044	**0.041**
Veg. height x LAI	0.829	0.379	2.185	**0.029**	0.183	0.094	1.939	0.054				
Veg. height x *Malva* cover	-0.917	0.372	-2.462	**0.014**	0.194	0.092	2.105	**0.037**				
LAI x *Malva* cover	-0.810	0.346	-2.340	**0.019**								
Malva cover x *Dipsacus* cover	0.583	0.301	1.937	0.053	-0.124	0.081	-1.535	0.127				

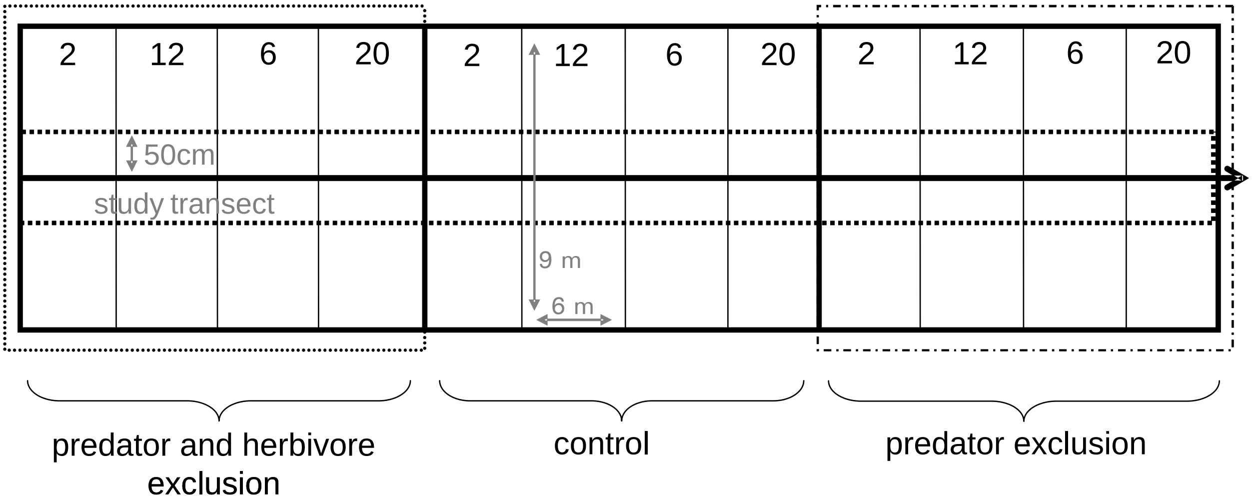

FIG. 1. Experimental setup of one of the twelve wildflower strips. Each field consisted of twelve plots including 3 fencing and 4 plant diversity treatments. The number of plant species sown is indicated by the numbers (2, 6, 12, 20) at the top of each plot. A fine meshed fence (represented by the dotted line around the first four plots) was established in order to exclude major predator and herbivore species. A wide-meshed fence (here represented by the dashed line surrounding the last four plots) was used to exclude the main predators only; four plots were not fenced, serving as a control. The dotted line through the centre of the field represents the sampling area where spider webs were investigated.

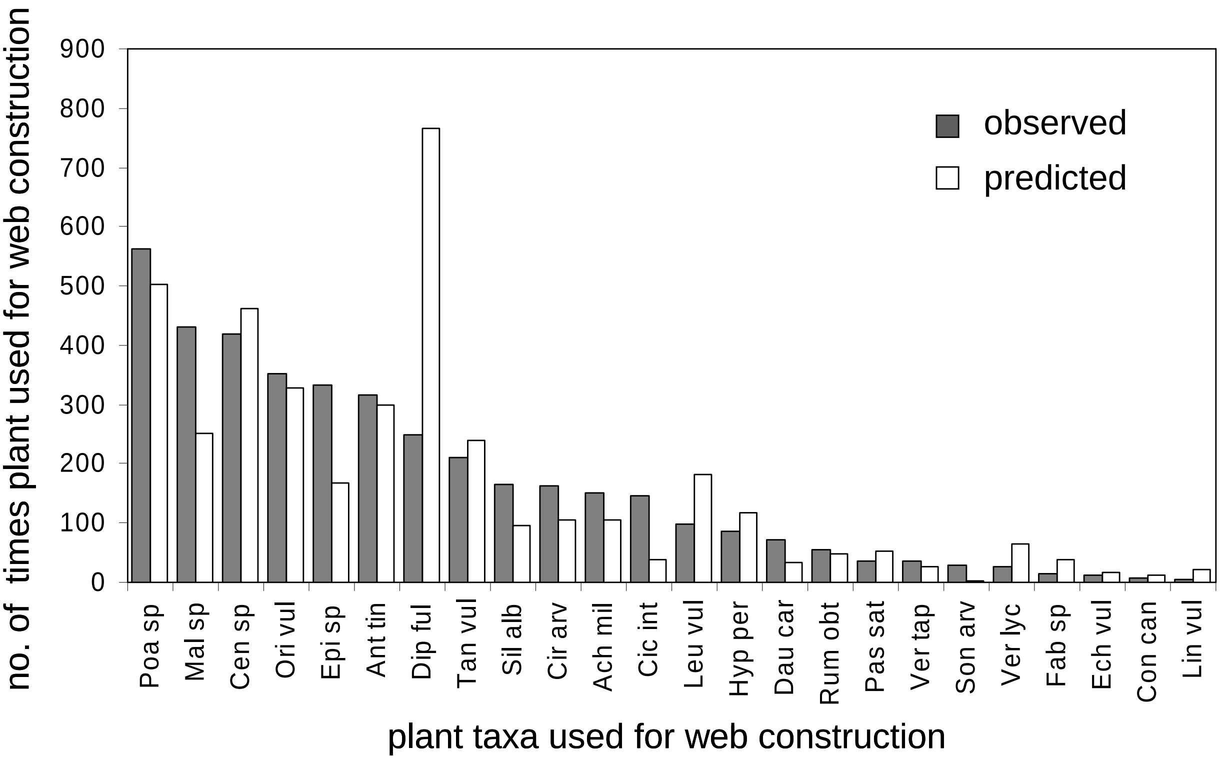

FIG. 2. Twenty four plant taxa most often used for web construction in nine fields in 2009. Gray boxes indicate the observed number of times a spider web was attached to a given plant species. White boxes represent the predicted number of times the plant species would be used for web construction if chosen in proportion to their abundance. Name of plant species or groups of species: Poa sp: Poacea sp.; Mal sp: *Malva moschata* or *M. sylvestris*; Cen sp: *Centaurea cyanus* or *C. jacea*; Ori vul: *Origanum vulgare*; Epi sp: *Epilobium* sp.; Ant tin: *Anthemis tinctoria*; Dip ful: *Dipsacus fullonum*; Tan vul: *Tanacetum vulgare*; Sil alb: *Silene alba*; Cir arv: *Cirsium arvense*; Ach mil: *Achillea millefolium*; Cic int: *Cichorium intybus*; Leu vul: *Leucanthemum vulgare*; Hyp per: *Hypericum perforatum*; Dau car: *Daucus carota*; Rum obt: *Rumex obtusifolius*; Pas sat: *Pastinaca sativa*; Ver tap: *Verbascum thapsus* spp.; Son arv: *Sonchus arvensis*; Ver lyc: *Verbascum lychnitis*; Fab sp: *Fabaceae* sp., Ech vul: *Echium vulgare*; Con can; *Conyza canadensis*; Lin vul: *Linaria vulgaris*.

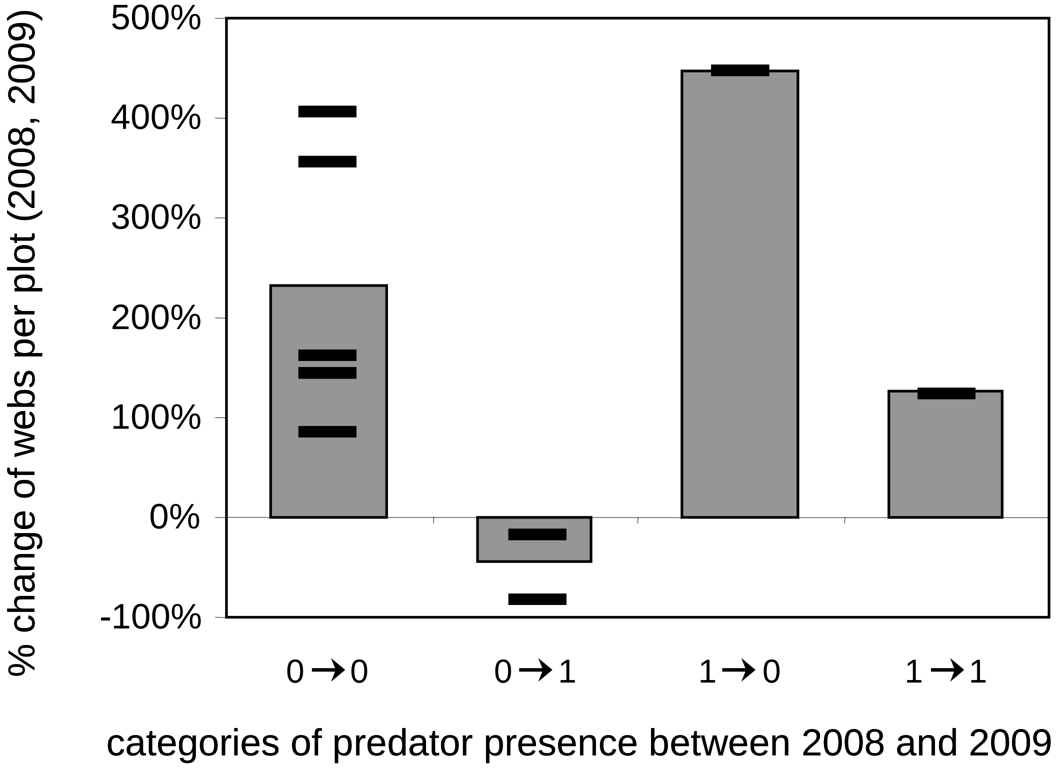

FIG. 3. Percentage change in the total number of *Argiope bruennichi* webs between 2008 and 2009 recorded in nine different fields, belonging to four categories related to the occurrence of the hornet species *Vespa crabro*. Black bars indicate the observed and gray boxes the average percentage change between 2008 and 2009. Category 0→0 represents fields where *V. crabro* was absent in both years; 0→1 fields where hornets were only present in 2009; 1→0 fields where hornets were present in 2008 but not in 2009; 1→1 hornets present in both years.

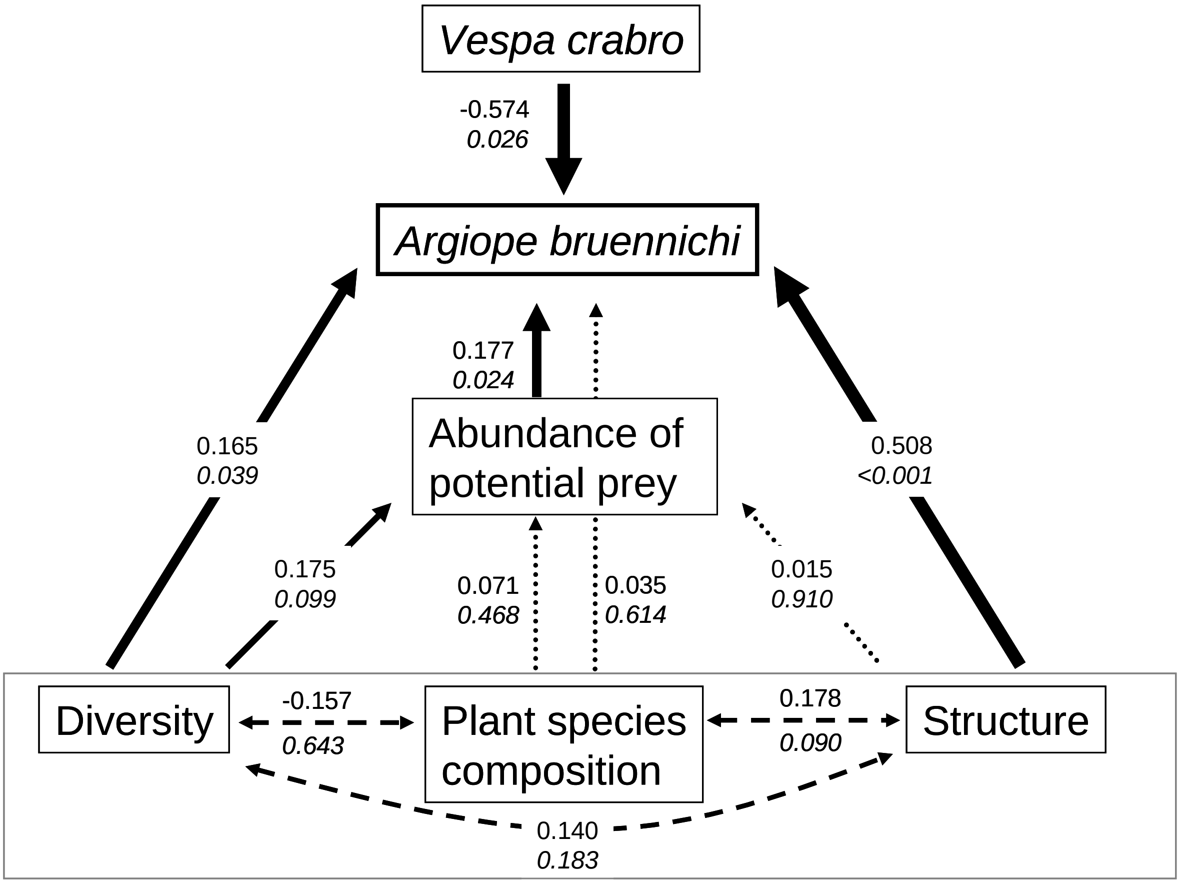

FIG. 4. Significant (continuous arrows) and non-significant (dotted arrows) interactions observed between the different trophic levels. Values indicate estimates of standardized slopes, with p-values (italic) obtained from linear mixed effect models. Dashed arrows indicate correlations (Pearson *r*, p-value in italic) between the different measurements of the plant community. Plant diversity is expressed as plant "diversity number", plant species composition rerpresents the coordinates of the plots on the first ordination axis of a Correspondence Analysis (CA) of the plots described by the vegetation, vegetation structure includes the first ordination axis of a Principal Component Analysis (PCA) on the three variables average and maximum vegetation height and LAI. The abundance of potential prey speices was established by direct observations of flower visiting insects at the plot level.

Trends in climate, land use and the dynamics of a ballooning spider assemblage

Odile T. Bruggisser, Gilles Blandenier & Louis-Félix Bersier

Trends in climate, land use and the dynamics of a ballooning spider assemblage

Odile T. Bruggisser[1*]; Gilles Blandenier[2] & Louis-Félix Bersier[1]

1 University of Fribourg, Unit of Ecology & Evolution, ch. du Musée 10, CH-1700 Fribourg, Switzerland
2 Centre Suisse de Cartographie de la Faune (CSCF), Passage Max-Meuron 6, CH-2000 Neuchâtel, Switzerland

Abstract

1. Long-term observations provide growing evidence that global change, notably habitat loss and climate change, has an impact on population dynamics. A decrease in abundances and shifts in distribution patterns and phenology have been observed in plant, vertebrate and aquatic invertebrate populations. The effect of global change on terrestrial invertebrate populations is, however, less well investigated.

2. We used a unique long-term data set to study the effect of climatic conditions and landscape modifications on the dynamics of a ballooning spider assemblage. Ballooning in spiders is a passive dispersal behaviour involving the use of their silk threads. More than 15,000 ballooning spiders were caught at weekly intervals between 1994 and 2004 in south-western Switzerland using a 12 m high suction trap.

3. First, we hypothesized that meteorological conditions influence short term changes in spider abundances and long term shifts in phenology. Second, for long term trends in spider abundance, land use changes should affect species differently and in proportion to changes in their specific habitat availability, whereas climatic effects should influence species irrespectively of habitat.

4. We found a strong relationship between spider assemblage structure and meteorological parameters. On a weekly basis, the assemblage responded to temperature, global radiation and humidity. Over the eleven years, a significant shift in ballooning phenology of most species was observed, with the summer peak in ballooning activity shifted earlier in the year.

5. In the long term, ground-living species decreased while tree-living spiders increased in abundance, which can be explained by concomitant landscape changes in the study area. We found no evidence that changes in meteorological parameters affected these trends. However, ballooning abundances decreased strongly in summer and fall 2003, when extreme meteorological conditions were prevailing in Europe. Interestingly, this collapse was preceded by very high ballooner abundances, a pattern that we explain with a "climatic trap" hypothesis.

6. Spider populations appear to be more at risk from the expected intensification of extreme climatic events and changes in land use than from gradually rising temperatures.

Introduction

Long-term observations of the development of population dynamics are essential to improve our understanding of the life history and ecology of populations as well as for management and conservation purposes (Caughley 1977; Tuljapurkar & Caswell 1997). This is especially important in the face of current drastic modifications of land use and climate change. The 20th century was reported to be the warmest since 1500 (Luterbacher *et al.* 2004). Furthermore, a general increase in extreme climatic events, like heat waves, extreme precipitation and windstorms, has been observed in the present and predicted for the future (Beniston 2007).

European land use change over the last decade was mostly characterized by the growth of urban areas, leading to a displacement of agricultural and natural ecosystems (Houghton 1994). Land use change has been shown to be a major cause of reduced biodiversity globally (Stoate *et al.* 2001; Collins & Storfer 2003). Furthermore, there is evidence that climate change is influencing the ecology and behaviour of many plant and animal taxa (Parmesan & Yohe 2003; Root *et al.* 2003). Plants change their flowering phenology (Alsos *et al.* 2007; Franks, Sim & Weis 2007), birds tend to migrate earlier (Gordo & Sanz 2006; Jonzén *et al.* 2006), butterflies and zooplankton species shift their distribution pattern (Walther *et al.* 2002), and the effect of rising temperatures is especially pronounced in fresh water ecosystems affecting macroinvertebrate assemblages (Chessman 2009). To investigate the long-term effect of global change on population dynamics, mostly plant, vertebrate or aquatic invertebrate populations have been examined. Similar studies on terrestrial invertebrate populations are however rare (e.g., Aebischer 1991; Parmesan *et al.* 1999).

Here we make use of an unusual dataset which allows us to investigate the long-term dynamics of a whole invertebrate community. A ballooning spider assemblage has been monitored over 11 years (1994-2004) in an agricultural landscape in Switzerland (Changins). More than 15’000 spider individuals belonging to 103 different spider species were collected in weekly samples. Ballooning in spiders is a dispersing behaviour involving passive aerial movement carried by silk threads (Blackwall 1827; Bell *et al.* 2005). Ballooning has been demonstrated to be induced by food limitation and habitat crowding (Weyman & Jepson 1994) but little is known about the proximate stimuli that trigger this behaviour. In addition to the spider’s physiological state, the occurrence of favourable conditions of temperature, wind speed and rainfall are definitely important (Legel & van Wingerden 1980; Reynolds, Bohan & Bell 2007).

It has been shown that temperature affects the developmental rate of spiders as well as their dispersal behaviour (Li & Jackson 1996; Bonte *et al.* 2008). Furthermore there is ample evidence that dispersal behaviour strategies are plastic and condition-dependent rather than fixed. Therefore the strong selection pressure, emerging from climate change and habitat fragmentation, is expected to influence the evolution of dispersal rate (Bowler & Benton 2005; Ronce 2007). This raises the question of the future dynamics of ballooning spiders under the current drastic global change.

The interpretation of the effect of global change on the number of migrating individuals is not straightforward, since global change can influence ballooning behaviour and/or population abundance. Ballooning spiders represent the migrating fraction of a spider population, and several authors provided evidence that ballooning abundance is linked to overall population density (Weyman, Jepson & Sunderland 1995; Thomas & Jepson 1999; Blandenier 2009). Furthermore, an increase or decrease in the number of migrating individuals will obviously affect population size in the next generation. Therefore, we assume that changes in ballooning abundance reflect an overall trend of population density. For this reason we focus on the short- and long term dynamics of ballooning spiders and their phenology and analyze how spider abundances are related to the trend of meteorological and landscape variables. First, because ballooning propensity depends on meteorological conditions, we hypothesize that the latter influence both short term changes in spider abundance and long term shifts in phenology. Second, for long term trends in spider abundance, we hypothesize that land use changes affect spiders differently and in proportion to changes in their specific habitat availability, but that climatic effects should generally influence spiders irrespectively of habitat.

Material and Methods

Study site

Ballooning spiders were trapped in the western region of the Swiss Plateau at the Station de Recherches en Production Végétales de Changins (6°14'0'' E/46°24'8'' N, 440m a.s.l.) (see Blandenier 2009). The landscape surrounding the sampling site is mainly characterized by agriculture, woodland or housing and infrastructure. Between 1992 and 2004 in a radius of five kilometres (excluding lake and forests of the Jura Mountains), agricultural surface area decreased by 3% (1992: 30.5 km^2; 2004: 29.6 km^2), housing and infrastructure increased by 7% (1992: 11.3 km^2; 2004: 12.1 km^2), and forest surface did not change (10.8 km^2) (data from the Swiss Federal Statistical Office).

Sampling of ballooning spiders

Ballooning spiders were sampled by a Rothamsted Insect Survey suction trap (Taylor & Palmer 1972; Derron & Goy 1987) between 16 April 1994 and 31 December 2004 (except for the following periods: 17 December 1994 to 18 March 1995, 3 December 1995 to 17 March 1996 and 12 February to 22 April 1998). The trap absorbed 42-43 m^3 air per minute at 12.2 m altitude. Trapping was continuous, with samples removed on a weekly basis (see Blandenier 2009).

Adult spiders were determined to species; immatures to family or genus level (see Appendix 1 for a list of trapped spiders). We obtained a database of 519 weekly samples containing 10587 immature and 4811 adult spider individuals from 103 species and 16 families. Spider taxa were separated into 5 different ecological groups, with regard to their distribution in the vegetation. According to Blandenier (2009) we distinguished generalists (21% of observed species) from ground (39%), herb (4.5%), tree (10.5%) and herb and tree (24%) living taxa (see Appendix 1).

Meteorological parameters

Meteorological data were provided on a daily basis by the meteorological station of Changins (MeteoSwiss) located around 300 m southwest of the spider sampling suction trap. To be consistent with the spider sampling regime, measures were considered for each week. Environmental parameters considered were average, absolute minimum and absolute maximum temperature [°C]; absolute minimum temperature at five centimetres above herb layer [°C]; average relative humidity [%]; the sum of precipitation [mm]; and the sum of global radiation [MJ/m^2]. Additionally, daily average wind velocities [m/s] were measured at 12.2 m height, 10 m northwest of the chimney of the suction trap.

Data analysis

We first examined changes in species composition and in phenology based on weekly and fortnightly data, respectively. Secondly, we concentrated on yearly trends in ballooning spider abundance and in meteorological parameters, and analyzed the relationship between the two. Finally, we gave special attention to the year 2003, which exhibited extreme meteorological conditions.

The weekly response of the ballooning assemblage to meteorological parameters was analyzed by ordination (Canonical Correspondence Analysis CCA; Jongman, ter Braak & Van Tongeren 1995). Total spider abundance in each week was square-root transformed prior to analysis to reduce skewness, and rare species were downweighted (option available in Canoco, version 4.5; ter Braak 1997-2002) to reduce their influence. Singletons were excluded. The influence of meteorological parameters was tested by forward selection and their relative significance by Monte Carlo Permutation for time series (cyclical shift permutations under the reduced model with 9’999 permutations; ter Braak & Smilauer 1998).

The evolution of the long-term phenological pattern of ballooning spiders was first examined graphically by plotting total spider abundance against time (fortnight windows of one year). The detection of statistically significant changes in phenology was carried out with an original Monte Carlo test where whole years are the permutable units. The procedure is as follows:

Starting with the raw abundances N_{ty} of the species or group of species of interest for each fortnight t in year y, we compute the proportional abundance within each year: $p_{ty} = N_{ty} \big/ \sum_t N_{ty}$. In this way we give the same weight to each year irrespective of their total abundance. For each fortnight, we then compute the correlation r_t between years y and proportions p_{ty}. A positive r_t indicates that the abundances increased for that fortnight, and vice versa. The two-tailed statistical significance of each r_t was evaluated by permuting whole years and computing randomized r_t to build the reference distribution (2000 simulations). To evaluate if a change in phenology was consistent at the assemblage and taxon level, we first performed the test with all immature and adult taxa pooled, and then separately with the 26 most abundant taxa (i.e., with total abundance > 50 individuals; see Appendix 1). For the latter we checked with a binomial test for each fortnight if the majority of these 26 taxa behaved similarly to the whole assemblage, i.e. if correlation coefficients went in the same direction.

We used mixed effect models to test for yearly trends in the meteorological parameters and spider abundances of the 26 most abundant taxa. We followed the approach of Crawley (2007) and used R Package. The method compares the performance of two mixed effect models (function glmer of the library lme4), one with a linear series as explanatory variable, and one without. In both models weekly data were used, year was a random factor and seasonality was described by a sinus-cosinus function. We used the Gaussian family for meteorological data and the Poisson family for spider abundances. The two models, with and without the linear series as explanatory variable, are compared using the function ANOVA; significantly better performance of the former model is evidence of a yearly trend. To test the relationship between ballooner abundance (response variable) and meteorological data (explanatory variable) at a yearly time scale, we used a similar approach as above, but replaced the linear series with the trend of the meteorological variable extracted with the function STL. The latter stands for Seasonal Decomposition of Time Series by Loess (Cleveland *et al.* 1990), which decomposes an observed time series into three parts: a seasonal component, a trend, and a remainder (with the options s.window = "periodic" and robust = TRUE).

Finally, we examined five additional relationships at the yearly time scale: 1) the existence of a trend in the weekly meteorological parameters; 2) the existence of a trend in the following descriptors of weekly community composition: species richness, Shannon diversity, beta diversity measured as compositional change (Tokeshi 1990), and evenness, estimated with Hurlbert's (1971) probability of interspecific encounter; 2) the relationship between community descriptors and meteorological data; 4) the existence of a trend in the coefficients of variation (CVs) of meteorological parameters; 5) the relationship between the CVs of spider abundances and those of meteorological parameters. In cases 1 to 3, analyses were similar to those with spider abundance (mixed effect models, with Poisson family for species richness and Gaussian family for the other dependent variables; we considered only weeks where 20 or more individuals were sampled). For cases 4 and 5, CVs were computed over the whole year based on weekly measures of environmental parameters, and the relationships analyzed with Spearman rank correlation. In the case of multiple tests, we used the conservative Holm correction of P-values (Legendre & Legendre 1998).

To test whether temperatures and spider abundances were significantly different in 2003 and 2004 compared to all other years, we performed a Dixon's Q-test for outliers. The test was computed for the period of peak spider abundances in June, August and October. Also, we evaluated the effects of both years on the results of all analyses described above, simply by excluding them. We report only instances where the exclusion of these years yielded different results.

Results

Meteorological trends

Table 1 summarizes the yearly evolution of meteorological parameters for the periods 1994 until 2004 at the study site. During this period, no significant change in the investigated meteorological parameters was observed, but the variability of minimum temperature significantly increased while the variability of global radiation significantly decreased. The year 2003 was characterized by extremely high temperatures and low precipitation levels during summer. For 82 of the 365 days, average temperature exceeded 20C° (average between 1994 and 2004 excluding 2003: 37.6 days ± 7.8 s.d.); global radiation was greater than 20Mj/m^2 for 97 days (average: 76.3 ± 7.1 s.d.); humidity was above 80% for only 82 days (average: 117.6 ± 14.2 s.d.); and during the summer months (June to August), 65 days were observed without precipitation (average: 54 ± 4.5s.d.).

Short-term response of the spider assemblage to meteorological parameters

Figure 1 displays an ordination biplot of a Canonical Correspondence Analysis (CCA) relating spider species and meteorological parameters. It summarizes the individual response of spiders at a weekly time scale. Forward selection of explanatory variables revealed the following statistically significant parameters: average temperature ($F = 22.1$, $P \leq 0.001$), global radiation ($F = 8.0$, $P = 0.002$), and humidity ($F = 3.5$, $P = 0.008$). Maximum temperature, minimum temperature, minimum temperature above the herb layer, precipitation and wind velocities had no significant influence on the assemblage composition (all P-values ≥ 0.05). The eigenvalues of the first and second canonical axes were low compared to the trace (0.127, 0.044, and 2.83, respectively), which can be explained by the large number of samples (519 weeks). Despite these low values, the three canonical axes were significantly related to the distribution of ballooner abundances (F = 22.4, P = 0.001), the first axis was significant (F = 11.4, P = 0.001), and the second only marginally (F = 7.9, P = 0.084). When examining the CCA biplot (Fig. 1) for the spider species classified in ecological groups, no clear pattern emerges. Only tree-living spiders are globally correlated with increased temperature and global radiation.

Long-term trends of spider abundances

We first examined if a change in phenology was apparent during the study period; second, we tested for trends in ballooning spider abundance and in descriptors of the community; and finally, we tested if yearly change in spider abundance could be explained by meteorological parameters.

Phenology. Figure 2 illustrates the weekly dynamics of ballooning spider abundances between 1994 and 2004. The phenological pattern is characterized by two main peaks of abundances, a "summer peak" between May and August and an "autumn peak" between October and November. The second peak is mainly attributed to high abundances of adults of the most frequently trapped Linyphiid species. During the study period, a significant change in the phenology of ballooning activities was observed for the entire dataset (Fig. 3). The analyses for the 26 most abundant ballooning taxa were in line with the global results (plus and minus signs in Fig. 3). The timing of the first peak was brought forwards, which is apparent as increased abundances in the beginning of July followed by decreased abundances in the end of September.

Species trends. At the species level, significant increases were found for *Nuctenea umbratica*, mainly living in upper vegetation like bushes and trees (Hänggi, Stöckli & Nentwig 1995) and for *Mermessus trilobatus (Eperigone trilobata*), described as an expanding alien species from North America (Wittenberg 2005). Four ground-living species common in agricultural habitats, *Araeoncus humilis*, *Bathyphantes gracilis*, *Erigone atra*, and *Oedothorax apicatus*, decreased significantly (Table 2).

At the assemblage level, the total abundance of adult ballooning spiders (*coeff* = -0.722, *P* = 0.152) and immatures (*coeff* = -0.873, *P* = 0.051) showed non-significant negative trends (mixed effect model, with *coeff* the slope of the linear term representing time). Although these trends are not statistically significant, a decline is evidenced by the observation that 19 of the 26 most abundant taxa (Table 2) decreased (binomial test, *P* = 0.029). For ground-living taxa, a significant negative trend was observed (*coeff* = -0.370, *P* = 0.044). Tree-living species, however, significantly increased during the study period (*coeff* = 0.325, *P* = 0.047). When excluding the years 2003 and 2004 from the analyses, only the increase of all tree-living species remained significant, but the signs of the other trends remained the same. For community descriptors, we observed no yearly trend in species richness, Shannon diversity, evenness or beta-diversity.

Response to long-term meteorological trends. Using trends in meteorological parameters as explanatory variable, mixed effect models revealed no or weak relationships with the abundances of the 26 most common spider taxa. Linyphiidae sp (*coeff* = -4.552, $P = 0.014$) and *O. apicatus* (*coeff* = -3.882, $P < 0.01$) were negatively related to trends in global radiation, while *N. umbratica* showed a positive response (*coeff* = 5.757, $P < 0.01$) to this parameter. *N. umbratica* was also positively correlated with average temperature (*coeff* = 3.930, $P < 0.01$), but negatively with average humidity (*coeff* = -5.350, $P < 0.01$). For *Araneidae sp.*, a positive response to average humidity was observed (*coeff* = 2.679, $P = 0.048$).

We also analyzed if the variability of spider abundance was correlated with the variability of meteorological parameters with Spearman rank correlation. We found a strong relationship between the coefficient of variation (C.V.) of average temperature ($\rho = 0.845$, $P < 0.01$), minimum temperature ($\rho = 0.827$, $P < 0.01$) and minimum temperature 5cm above herb layer ($\rho = 0.646$, $P = 0.036$) the coefficient of variation of total spider abundance. For maximum temperature, humidity, precipitation, global radiation and wind velocities no significant correlation was found. At the yearly time scale, none of the community descriptors (species number, diversity, evenness, beta diversity) evidenced a trend or a relation to meteorological parameters.

Extreme climatic event

In Figure 2, the distribution of spider abundance in 2003 and 2004 is clearly different from the preceding years. In these two years ballooning abundances were significantly lower during the usual second peak of ballooning activity in September until November. This pattern is illustrated in Figure 4. Temperatures in 2003 were significantly higher than the preceding nine years in June ($Q = 0.58$, $P = 0.05$) and August ($Q = 0.62$, $P = 0.03$) and somewhat lower in October ($Q = 0.44$, $P = 0.23$) (Fig. 4a). While the abundance in the “summer peak” was significantly higher compared to all other years ($Q = 0.62$, $P = 0.02$), it crashed in the “autumn peak” ($Q = 0.57$, $P = 0.052$) (Fig. 4b).

Discussion

Our analysis of a ballooning spider assemblage revealed evidence of an interaction between spider dispersal patterns and current global change, namely landscape modification and climate change. This conclusion is supported by the following observations: 1) on a weekly basis, the ballooning assemblage responded to temperature, global radiation and humidity. 2) The phenological pattern changed, in the form of increased ballooning spider abundances in July followed by decreased abundances in September. 3) Tree-living species increased, while most ground-living spider species decreased in abundances over the study period. 4) The extreme climatic event of 2003 had a strong impact on ballooning abundances, which may be indicative of a "climatic trap" (see below).

Relationship to meteorological parameters

At weekly timescales the ballooning assemblage showed a clear response to temperature, global radiation and humidity (CCA, Fig. 3). The importance of meteorology has already been demonstrated by several authors: clear sky, warm ambient temperatures and light winds have been shown to favour ballooning (Richter 1970; Bishop 1990; Reynolds, Bohan & Bell 2007). A direct link between ballooning activity and global radiation or humidity has not been observed before, but is not unexpected because they are closely associated with the other conditions. Wind speed has been shown to be a very important factor in ballooning action: wind speed at 2m altitude exceeding 3m/s reduces rising air movements and hence ballooning success (Richter 1970; Reynolds, Bohan & Bell 2007). In our investigation, we failed to detect this relationship, but this may be explained simply because wind speed data were averaged over weekly intervals to match the spider sampling periods. In general, the tight link between ballooning spider abundances and meteorological parameters at a short-time scale implies that this dispersal behaviour should be affected by long-term changes in climatic conditions.

Phenology

The detection of a shift in the phenological pattern can be a difficult task for multiple time series with several modes, and we provide here a new approach to tackle this problem. To our knowledge, we are the first to provide statistical evidence of a change in the phenology of ballooning activities of a spider assemblage. This observed shift was consistent for many species, suggesting a general mechanism. Developmental rates of arthropods are strongly dependent on temperature (Li & Jackson 1996). Increasing temperatures lead to accelerated development rates of spiders (Li & Jackson 1996; Bonte *et al.* 2008) and would thereby move forward the timing of their ballooning activity. Spiders can thus be added to the list of taxonomic groups for which changing phenological patterns can be attributed to climate change (Walther *et al.* 2002).

Trends in ballooning abundances

Ballooning abundances of ground-living species, accounting for more than one third of the ballooning assemblage (39%), decreased during the study period, while the abundances of tree-living species (11%) showed a positive trend. Trends in ballooning abundances can imply changes in population densities on the ground and/or behavioural changes of dispersal strategy. In our system, the following factors can be considered: change in habitat availability and quality, and change in meteorological conditions.

Spider population size will clearly be affected by a decline in habitat surface area and quality. For example, intensification of agriculture, including increased pesticide use and soil cultivation, has been shown to reduce spider densities (Aebischer 1991; Topping & Sunderland 1994). The effect of meteorological parameters on abundances is more difficult to predict. One can generally assume that the response of species abundance to meteorological parameters follows a unimodal function, with the optima of the response curves differing between species. Thus, positive or negative trends can be expected depending on the position of the species on the curve and on the magnitude of change of the variable of interest.

Ballooning behaviour has been found to be affected by temperature during development: in an experimental study involving the Linyphiid spider *Erigone atra*, long distance migration was found to be negatively associated to elevated temperatures during development (Bonte *et al.* 2008). Interestingly, fragmentation resulting from habitat loss can also affect dispersal behaviour: isolated spider populations may evolve decreased ballooning propensities, presumably as a consequence of the higher mortality risk involved in finding suitable habitats (Bonte *et al.* 2003). Finally, habitat quality is also expected to influence spider behaviour, as ballooning propensity has been observed to increase when food availability decreases (Weyman & Jepson 1994).

Over the period of our observations, there was to our knowledge no change in farming and silvicultural practices (Jacques Derron, comm. pers.). We did, however, observe changes in habitat availability in the study region: while the surface of woodland did not change, a marked increase in housing and infrastructure (+7%) together with a decrease of arable land (-3%) was observed. Consequently, the increase in tree-living spider abundances can be interpreted as a response to habitat availability, since some species from this group (e.g. *Nuctenea umbratica*, *Zygiella x-notata*, *Keijia tincta*, *Theridion mystaceum*) are able to live in trees within urban areas and even on buildings (Hänggi, Stöckli & Nentwig 1995). As the decrease of ground-living spiders mirrors the loss of habitat availability, a causal link is reasonable. To summarize, it is not possible to assign a clear role of climatic variables on the population trends for both groups of species. This does not, however, exclude the possibility that changes in land use and climate act synergistically.

Increased variability

At yearly timescales, ballooning abundances were unrelated or weakly related to meteorological parameters. However, yearly ballooning variability was strongly and positively related to the variability of average temperature. As a consequence, the stability of ballooning populations may depend on the stability of meteorological conditions. Besides increasing temperature and precipitation, current climate change is characterized by an increase in the variability of meteorological conditions (Schär *et al.* 2004; Beniston & Goyette 2007). The variability of the dynamics of ballooning spiders can thus be expected to increase in the future, potentially exposing populations to stochastic loss.

Extreme climatic events

In 2003, meteorological conditions in Europe were extremely warm and dry from May until late August, and very cold in October. Models suggest that such extreme heat waves will become more intense, frequent, and longer lasting over the coming century (Beniston 2004; Meehl & Tebaldi 2004).

All observed ballooning spider species witnessed an abrupt decrease in August and an almost complete collapse in fall. One possible explanation for this crash is the death of many individuals, especially those living on the ground (most Linyphiids). Spiders are able to regulate their body temperature by retreating to cooler places in their habitat (Foelix 1979). Thermoregulation in general involves an increase in transpiration, which leads to death of spiders if water loss exceeds 20% of their body weight (Cloudsley-Thompson 1957).

We observed only one extreme climatic event in our dataset, which makes it difficult to draw general conclusions. However, we can hypothesize that our observations typify a "climatic trap" (Fig. 5): a situation where, during development, climate firstly has a positive effect on reproduction and abundances, but secondly reaches the physiological limits for an organism and strongly impacts their populations when they are unusually large (Fig. 4b).

Conclusions

Our data confirms the tight link between ballooning abundance and meteorological parameters at short timescales. It is thus not surprising to find a shift in ballooning phenology over the 11 years of the study. However, climatic effects are not necessary to explain the long-term change in abundance, as this mirrors the pattern of habitat modification in the study region. Climate can, however, have a huge impact, as exemplified by the extreme conditions of 2003. The combination of changes in land use by human populations and the predicted increase in abnormal climatic events may prefigure a difficult future for spider populations.

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Table 1: Yearly trends in meteorological parameters during the period 1994-2004 at the study site. Trends in averages were tested using mixed effect models; *coeff* is the linear relationship with time. Trends in coefficients of variation were tested using Spearman rank correlation ρ. Significant values are shown in boldface.

	average		coefficient of variation	
Meteorological parameters	*coeff*	*P-value*	ρ	*P-value*
Average temp. [°C]	0.029	0.517	0.164	0.624
Maximum temp. [°C]	0.040	0.440	-0.518	0.107
Minimum temp. [°C]	0.005	0.918	0.755	**0.010**
Min. temp. 5cm above herb layer [°C]	0.036	0.477	0.2	0.548
Humidity [%]	-0.250	0.138	0.624	0.164
Precipitation [mm]	-0.194	0.528	-0.155	0.653
Global radiation [MJ/m^2]	0.585	0.056	-0.636	**0.040**
Wind velocity [m/s]	0.009	0.482	-0.009	0.989

Table 2: Yearly trends in abundances of the 26 most common taxa tested by mixed effect models. Adult taxa are in boldface type, immatures in normal font; stratum indicates the position in the vegetation: GEN = no defined stratum, G = ground, H = herb, T = tree, HT = herb and tree; n gives the total abundance.

taxon	stratum	n	trend (coeff.)	p-value
Linyphiidae sp.	GEN	4878	- 0.542	0.042*
Araneidae sp.	GEN	1571	- 0.196	0.534
Philodromus sp.	HT	1209	- 0.023	0.537
Meioneta rurestris	G	1228	0.040	0.581
Araeoncus humilis	G	706	- 0.126	0.001***
Nuctenea umbratica	T	666	0.325	0.034*
Porrhomma microphthalmum	G	541	0.019	0.720
Erigone dentipalpis	G	533	- 0.062	0.071
Theridion sp.	GEN	434	0.051	0.015*
Pardosa sp.	G	350	- 0.073	0.040*
Erigone atra	G	358	- 0.064	0.011*
Araniella sp.	HT	332	- 0.013	0.348
Tenuiphantes tenuis	G	245	- 0.016	0.149
Mermessus trilobatus	G	219	0.083	0.025*
Bathyphantes gracilis	G	159	- 0.036	0.009**
Zygiella sp.	T	138	- 0.013	0.526
Mangora acalypha	HT	126	0.008	0.320
Oedothorax apicatus	G	121	- 0.039	<0.001***
Tetragnatha sp.	GEN	125	- 0.019	0.105
Diaea sp.	HT	108	0.004	0.619
Thomisidae sp.	GEN	106	- 0.007	0.420
Philodromus rufus	HT	67	- 0.007	0.271
Salticidae sp.	GEN	67	- 0.006	0.445
Xysticus sp.	G	66	- 0.005	0.348
Pachygnatha sp.	GEN	64	- 0.029	0.060
Microlinyphia sp.	H	60	- 0.0003	0.962

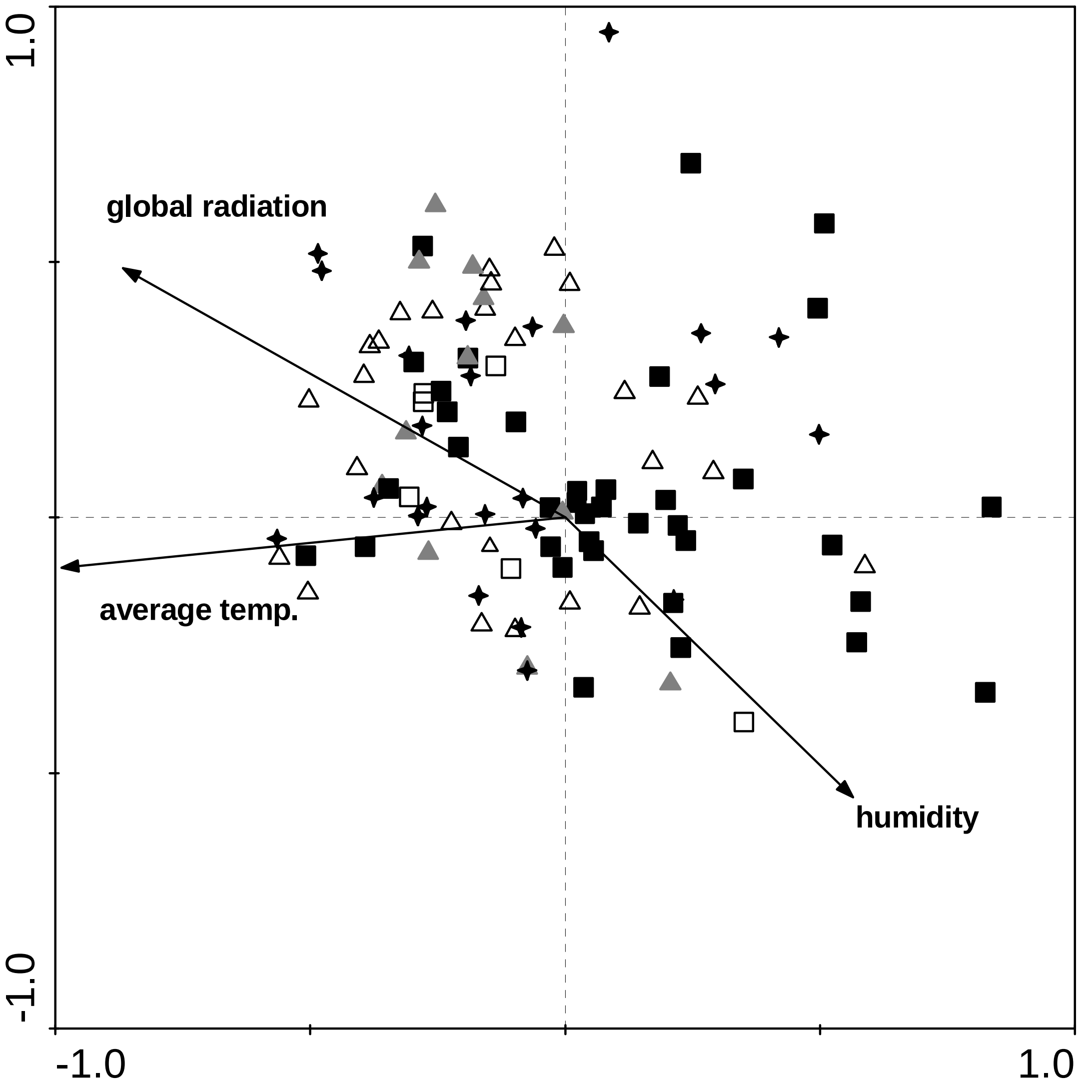

Figure 1:
Canonical correspondence analysis (CCA) of ballooning spider abundances in relation to meteorological conditions in the 519 weeks of observation. The biplot shows taxa and the significant meteorological parameters: weekly average temperature [°C], humidity [%] and sum of global radiation [MJ/m^2]. Filled squares represent ballooners living on the ground, grey triangles in trees, open squares on herbs, and open triangles in trees and on herbs. Stars represent taxa with no defined habitat preference.

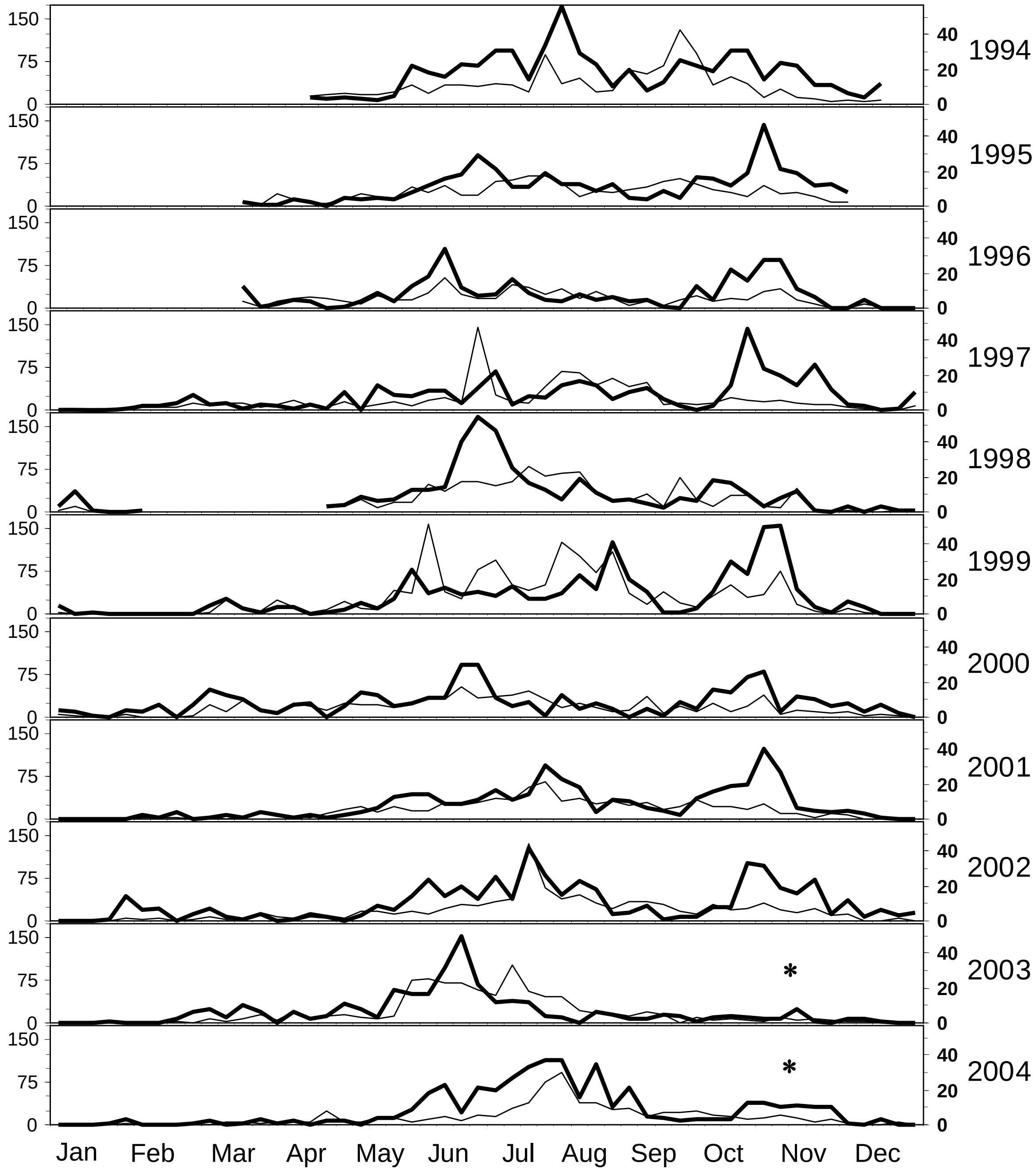

Figure 2:
Weekly patterns of ballooning spider abundances between 1994 and 2004. The bold line represents the phenology of adult total abundances (right axis), the thin line that of immatures (left axis). The two asterisks in the last two graphs highlight the abnormal absence of the second peak of ballooners in 2003 and 2004.

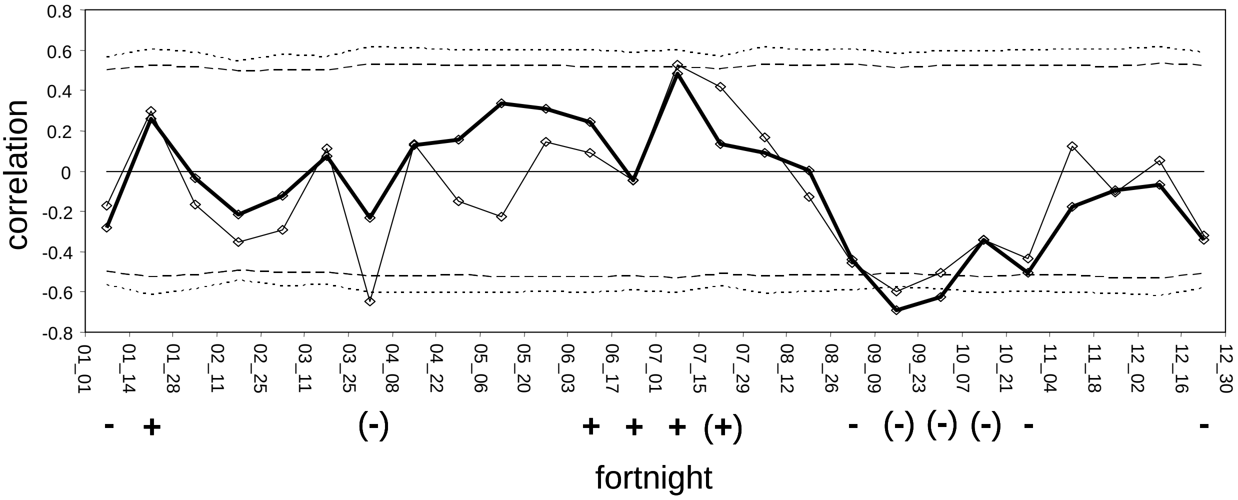

Figure 3:
Evolution of the phenological pattern of adult and immature ballooning abundances between 1994 and 2004. The x-axis represents fortnights, the y-axis shows the correlation coefficients with year (bold line: adults; thin line: immatures). A positive correlation indicates that abundances increased during that fortnight. The dotted horizontal lines represent the 2.5% and 97.5%, the dashed lines the 5% and 95% significance thresholds, computed with a permutation test. Plus and minus signs below the x-axis indicate that a higher than expected proportion of the 26 most abundant taxa increased or decreased, respectively (binomial test; $P\leq0.05$, except for signs in brackets: $0.05<P\leq0.1$).

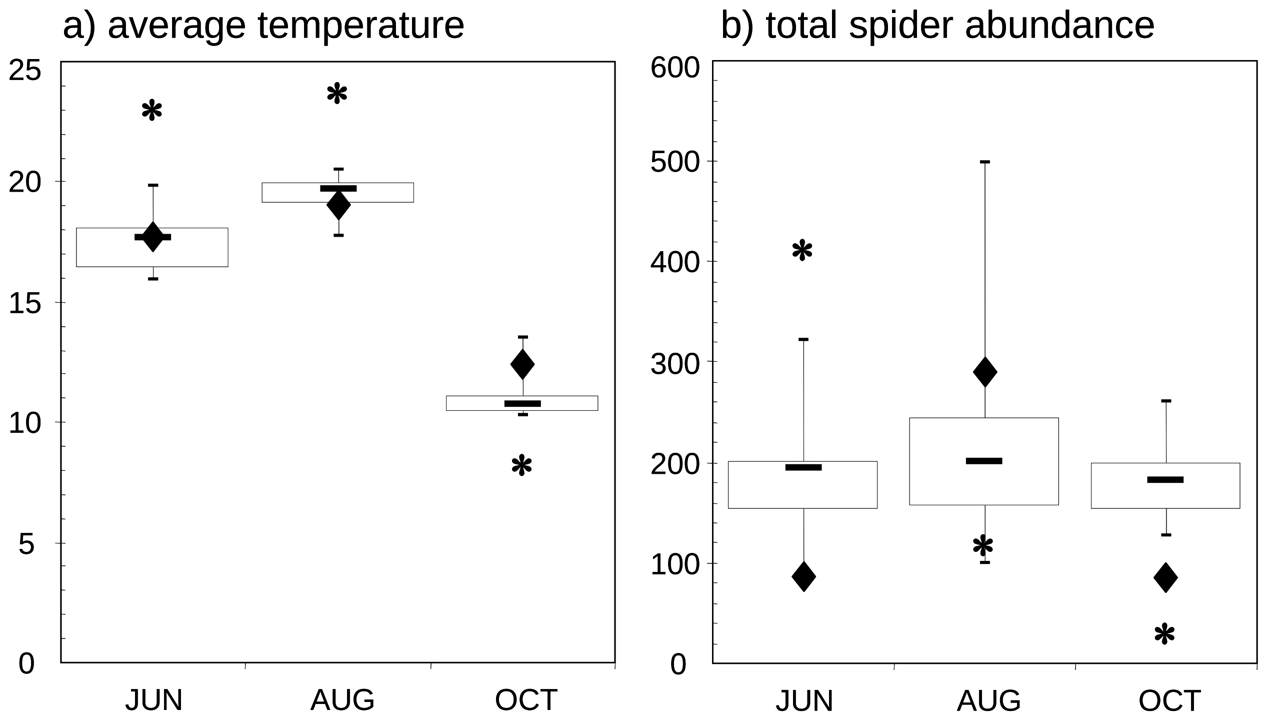

Figure 4:
Boxplots showing a) median temperatures in June, August and October between 1994 and 2002; and b) median spider abundances in June, August and October between 1994 and 2002. The average values for 2003 (asterisks) and 2004 (diamonds) are superimposed on the graphs.

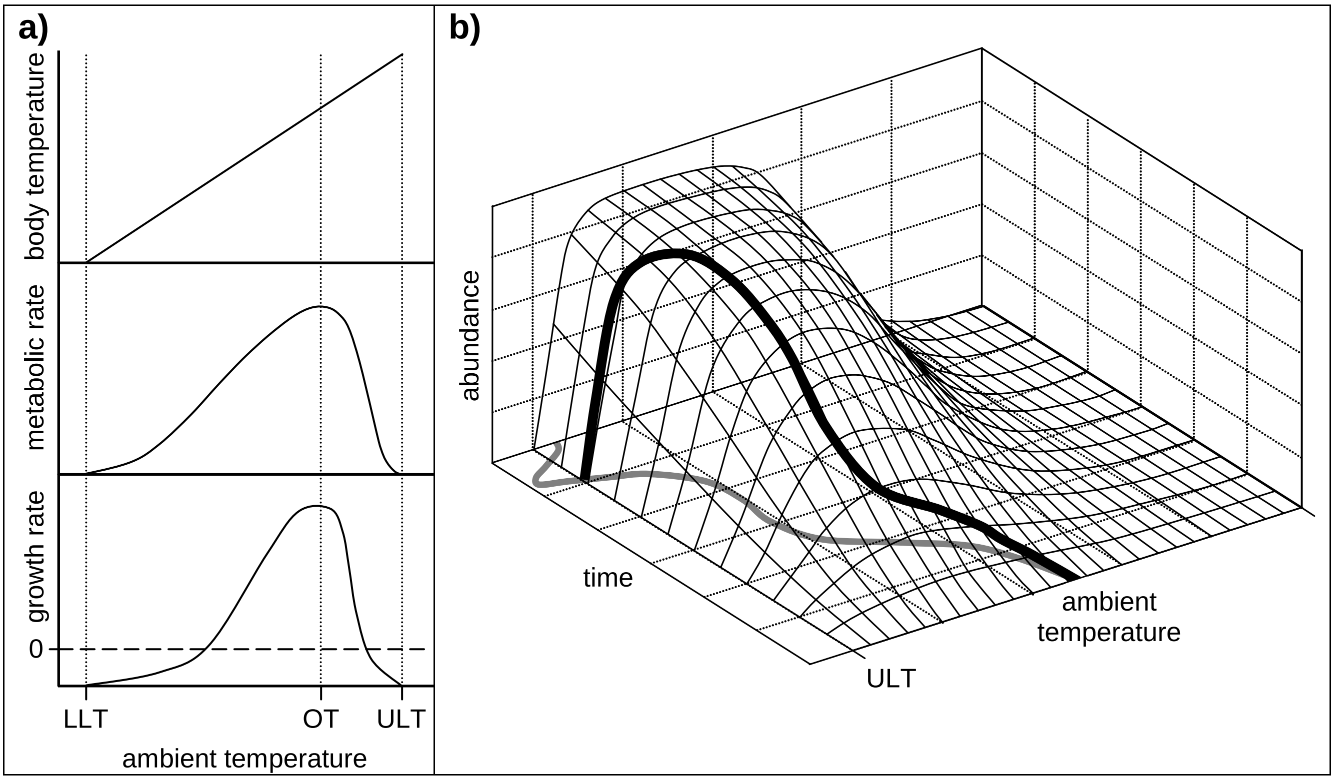

Figure 5:
Illustration of the climatic trap hypothesis. **a**) Relation of ambient temperature to body temperature and metabolic rate for ectoderms (from Randall, Burggren & French 2002), and putative effect on population growth rate. LLT, lower lethal temperature; OT, optimal temperature; ULT, upper lethal temperature **b**) A logistic growth with $K=1$ and r a function of ambient temperature, modelled by a beta distribution, similar to the curve in the lower left panel. Trajectory of species abundance (black line) and temperature (grey line) for an extreme climatic event akin to that observed in Fig. 4.

Spider molecular barcode to track trophic links in food webs

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Spider molecular barcode to track trophic links in food webs

Odile T. Bruggisser[1*]; Yvonne Fabian[1]; Nadine Sandau[1]; Gilles Blandenier[2], Louis-Félix Bersier[1] & Alexandre Aebi[3]

1 University of Fribourg, Unit of Ecology & Evolution, ch. du Musée 10, CH-1700 Fribourg, Switzerland
2 Centre Suisse de Cartographie de la Faune (CSCF), Passage Max-Meuron 6, CH-2000 Neuchâtel, Switzerland Forschungsanstalt Agroscope Reckenholz-Tänikon ART, Zürich, Switzerland
3 Agroscope Reckenholz-Tänikon ART, Research Station ART, Reckenholzstrasse 191, CH-8046 Zürich, Switzerland

Abstract:
The investigation of trophic links in food webs is crucial to understand the structure and stability of communities. Due to inherent difficulties to track trophic links in food webs, field observations on the quantitative structure of food webs remain rare. The observation of predation events in the trap-nesting wasp *Trypoxylon figulus* is difficult because wasp larvae consume most of the spider prey provided by the female when she lays the egg. Furthermore wasps prefer to trap immature spiders, which for a large majority can not be identified on the base of morphological features. The aim of this study was to develop and test a molecular barcode approach to fully characterize the trophic links among the mud-dauber wasp *T. figulus* and its spider prey community and to test whether immature spiders can be molecularly identified to the species level. Maternally-inherited bacterial endosymbionts were screened to consider the potential effect of maternally inherited symbionts on mitochondrial haplotype diversity. The 104 mitochondrial cytochrome oxidase I (COI) fragments of 46 spider species (12 families) and the 47 ribosomal 28S RNA segments of 37 species (11 families) clustered in terminal clades, corresponding to their classification based on morphological traits. In most cases we were able to confidently assign immature spiders and prey remains to the species level.

With this study we emphasize on two practical advantages of COI sequence databases for species identification, which somehow counter the criticism on this method: 1) the identification of species, lacking a clear morphological differentiation, like immature spiders, and 2) the better understanding of otherwise invisible trophic links in food webs by the identification of species from prey remains.

Introduction

Understanding how ongoing reductions in natural surface area and complexity will affect species diversity and trophic links in food webs is paramount in face of the current biodiversity crisis. The extinction of single species in ecological communities, characterized by trophic and competitive species interactions, can affect remaining species by causing a cascade of secondary extinctions (e.g., Paine 1966; Borrvall *et al.* 2000; Koh *et al.* 2004; Petchey *et al.* 2004, 2008). For example the removal of predator sea star species (*Pisaster ochraceus*) from a rocky intertidal community leads to the uncontrolled dominance of mussels causing local extinction of many predator species (Paine 1966). Such "secondary species extinctions" or "coextinctions" occur because a primary extinction creates a gap in the food web (a consumer may have no prey) or leads to a dynamically unstable community (Petchey *et al.* 2008). In order to understand trophic links within complex food webs, it is thus crucial to understand interaction pathways among all organisms involved in the web of a given habitat and to evaluate the importance of these interactions (Sheppard & Harwood 2005). Most studies investigating the effect of environmental change concentrated on single species groups (e.g., McNett & Rypstra 2000; Lassau & Hochuli 2004) thereby ignoring trophic systems, mostly due to inherent difficulties to track trophic links (Thies & Tscharntke 1999; Steffan-Dewenter *et al.* 2001, 2002).

The direct observations of trophic links, like predation events in food webs is in general relatively straightforward for large vertebrate or invertebrate predators, but can be difficult in highly mobile, underground, rare or cryptic species. Traditional ecological sampling methodology like faeces analyses can in some cases allow the visual identification of prey remains, provided that identification characters were not destroyed by digestion (e.g. Green & Tylere 1989; Burger *et al.* 1999). The sound morphological identification of solid prey remains is, however, often imprecise at the species level for many taxonomic groups. A general lack of simple identification keys for small or cryptic species may cause additional problems. For example, the morphological determination of the genetically well distinct, cryptic bug species from the family Adelgidae (Insecta: Hemiptera: Aphidoidea) is difficult because of their small size and morphological plasticity caused by environmental and host plant effects (Foottit *et al.* 2009). Even in well known groups like spiders, sexual dimorphism and intra-specific genitalia polymorphism pose problems for a sound morphological identification (Barrett & Hebert 2005, Coddington & Levi 1991). Furthermore, the vast majority of immature spiders can not be determined to the species level on the basis of morphological characters. A problem enhanced by the fact that immatures occur at high abundances and play an important ecological role.

DNA-based species identification tools have recently gained in importance and are promising techniques to overcome the difficulties in the morphological identification of cryptic species and in tracking trophic links in food webs (Tautz *et al.* 2002, 2003; Herbert *et al.* 2003b; Blaxter & Floyd 2003). Due to its high evolutionary rate Cytochrome Oxydase subunit I (COI) of the mitochondrial DNA (mtDNA) was used for the identification of a very large number of taxonomic groups (Lepidoptera: Hebert *et al.* 2003a, 2004a, birds: 2004b; collembola: Hogg & Herbert 2004) and recently for spider species (Barrett & Herbert 2005). Sequence database for several groups of species are already established, with a specific diagnostic mtDNA sequence tag or barcode (e.g. Theron & Cloete 2000).

Wasp species, using holes of a suitable diameter to build their nests, and their association to natural prey and predator species have recently gained in interest as a model system or "indicator food web" to investigate the effect of environmental change on trophic systems (Tscharntke *et al.* 1998; Gathmann & Tscharntke 1999; Tylianakis et al. 2007). These so called trap-nesting wasp species reflect environmental changes through their species richness and ecological functions or interactions like pollination, predation, and the mortality due to natural enemies (Tscharntke *et al.* 1998). Solitary living wasp species of the family Sphecidae can easily be trapped and monitored by offering artificial nesting sites. In particular species of the mud-dauber wasp genus *Trypoxylon* are known to preferentially use man-made provided nests (Kruess & Tscharntke 2002; Tormos *et al.* 2005; Polidori *et al.* 2007). In an experiment by Kruess & Tscharntke (2002) *Trypoxylon figulus* (Linnaeus 1758) was the most abundant species (43%) found in trap-nests, placed in different grassland habitats in Schleswig-Holstein, Germany. While adult *T. figulus* wasps preferentially feed on floral and extra-floral nectar (Kevan & Baker 1983, 1999) their larvae feed on paralyzed web-building (Linyphiidae, Araneidae, Tetragnathidae, and Theridiidae) or wandering (Thomisidae, Oxyopidae, and Salticidae) spiders (Blackledge *et al.* 2003; Araújo & Gonzaga 2007; Polidori *et al.* 2007). The spider prey is trapped by the female and transferred to the nest, which consist of a linear series of cells, with partitions of mud or other material. Immature spiders represent the most favoured prey (Polidori *et al.* 2007; Santoni & Del Lama 2007; Buschini *et al.* 2008). Little has however been reported on the spider species range used by *T. figulus*. A single Sphecid wasp may have a considerable effect on a spider population, as one female can catch 100-300 spiders in the course of one summer (Bristowe, 1941). One single egg is laid on the trapped spiders and, after hatching, larvae consume most of the provided prey, which complicates the identification of preys to the species level. In most studies investigating the prey community of solitary living wasps, nests were inspected before the end of the larval development was complete (Araújo & Gonzaga 2007; Polidori *et al.* 2007; Buschini *et al.* 2008). The disruption of larval development to investigate the wasp's prey community hinders the investigation of other members of the community centered on trap-nesting wasps, such as parasitic wasps, which attack larvae in brood cells (e.g. Evans & O'Neill 1988; Strohm 2001; Taki *et al.* 2008). Several hymenopteran parasitoids from the family Chrysididae, Pimplinae or Eurytomidae inflict ca. 20% mortality by attacking T*. figulus* larvae in brood cells (Gathmann &Tscharntke 1999; Asís *et al.* 1994).

In this study we established the genetic barcode database of a spider community, to investigate the trophic link between trap-nesting wasps and their spider prey communities. The study was performed in ecological compensation zones (wildflower strips) in Switzerland's agricultural landscape. Over the last decade, these ecological compensations zones have been established in the matrix of European intensive agriculture landscapes to conserve biodiversity. Wildflower strips are perfectly suited to investigate such an "indicator food web" centered on trap-nesting wasp species, because they are relatively simple habitats, and their study has strong theoretical and practical value for conservation. The objective of this work was to develop and test a molecular barcoding approach to fully characterize the trophic links among the mud-dauber wasp *T. figulus* and its spider prey.

Material and Methods

Spider sampling:

The composition of spider communities of wildflower strips was investigated between 2006 and 2008 by pitfall trapping and vacuum sampling in 15 wildflower strips of an average size of 900 m^2 around Grandcour (46°52'18'' N, 6°55'50'' O) and 14 wildflower strips in the surroundings of Fribourg (46°48'0'' N, 7°9'0'' E) in Switzerland. A few species from two additional regions, close to Bern (46°57'4'' N, 7°26'19'' O) and Gruyère (46°29'11'' N, 7°12'35'' O), collected in previous studies, were added to the database. The distance between the two sampling areas is ca. 20 kilometers. Pitfall traps consisted of plastic cups (6.5 cm diameter, 8 cm depth) filled to one third with Gault's solution (Walker & Crosby, 1988) and some drops of detergent to break the surface tension and to accelerate the wetting and killing of arthropods (Topping & Luff 1995). Traps were protected against rainwater with a 15 × 15 cm plastic roof. Three traps were placed in the fields around Fribourg for two week in June 2006 and fourteen traps in each field around Grandcour in August and September 2007. Traps were placed along a transect line, leading through the centre of the field with a minimal distance of 5 meters between traps. A foliage hoover type SH 85C (Stihl, Dieburg, Germany) with a suction tube of 0.11 m diameter was used to collect two vacuum samples (D-Vac) per wildflower strip between 10.00h and 16.00h once during warm and sunny days around Fribourg during October 2006 and around Grandcour during July and September 2007 and 2008. For this purpose, a metal frame was used to delimit an area of 1x1 meter in the centre of each field. The apparatus was lowered to just above the ground to sample the ground and vegetation within the sample area for one minute. All trapped spiders were conserved in 80% ethanol, to prevent hardening of spider's cuticle which is crucial for morphological identification. Samples were stored at 4° Celsius. Spiders were identified to the species level by using taxonomic keys based on external morphology (Nentwig *et al.* 2003).

Trap-nesting wasps sampling:
Fourteen trap-nests were placed along a transect leading through the centre of each field between May and October 2008 in 10 different fields, located in the surroundings of Grandcour. Traps, consisted of grey plastic tubes (20cm long, 10 cm diameter), fixed by an elastic band on a wooden pole (1.5m long) and protected from rain by a wooden board (25x25cm). Each plastic tube was filled with 150-180, 15-20 cm long, internodes of the common reed *Phragmites australis* (diameter in the range of 2 and 10mm) (Gathmann *et al.* 1994). In October all traps were transferred to a cooling chamber (4°C; 5% RH) in the laboratory to simulate winter conditions. After 16 weeks traps were removed from the cooling chamber and each reed internode was dissected under a binocular. Adult wasps and parasitoids were reared from larvae and pupae in glass tubes sealed with cotton wool. Emerging adults of *T. figulus* and of potential prey species were counted and identified to the species level by experts on the base of external morphology characters. Remains of spiders consumed by the wasps were transferred into individual Eppendorf tubes in 100% Et-OH for further genetic analysis.

mtDNA extraction, amplification and sequencing:
Cytochrome Oxydase I (COI) haplotype were obtained from three groups of spiders: a) identified species based on morphological characteristics, b) immatures and c) spiders consumed by *T. figulus* (Table S1). Both latter groups were used as test samples for our barcode database. To avoid potential contamination from gut content material, genomic DNA was extracted from a single leg of one to six individuals per species. DNA was extracted using Dneasy tissue kit (Quiagen, Hilden, Germany), following the manufactures' instructions or by recently established method using hot sodium hydroxide and Tris (HotSHOT, Montero-Pau *et al.* 2008).

Polymerase chain reaction (PCR) was used to amplify a 579 bp segment of the mitochondrial COI gene (see Table 1 for a list of primers used). PCR reactions were carried out in 25 μl containing 2 μl of DNA extract, 0.5 μl of dNTPs (10 mM), 0.5 μl of each primer (20 uM), 0.5 μl of $MgCl_2$ (25 mM), 18.375 μl of sterile water, 2.5 μl of 10x buffer solution (Quiagen) and 0.125 μl Top*Taq* DNA polymerase (5000 U ml^{-1}, Quiagen, Hilden, Germany). PCR reactions were performed on a Biometra T-Gradient Thermocycler (Biolabo Scientific Instruments). The following cycling conditions were used for COI: initial step of 3min at 94C°, followed by 40 cycles of 30s at 94C°, 30s at specific annealing temperature and 1min at 72C° and a final step of 10min at 72C° (see Table 1). One negative control (2 μl of distilled water) was run with every set of PCR reactions. Bands were visualized by gel electrophoresis using 1.5% agarose gel, stained with ethidium bromide. Gels were run for 45 minutes at 120 V and photographed under UV light. Remaining primers and nucleotides were removed by incubating 20 μl of PCR product with 0.8 μl of Shrimp Alkaline Phosphatase (SAP, 5000 U ml^{-1}) and 1.2 μl of Exonuclease I (10 mM, with dilution buffer) for 1 hour at 37 C°. Purified PCR products were sequenced in both directions on an Automatic Sequencer 3730xl under BigDyeTM terminator cycling conditions with appropriate primers.

rRNA extraction, amplification and sequencing:

To rule out biases due to demographic processes such as founder effect or bottlenecks, we additionally considered a gene fragment of the nuclear DNA to confirm the observed pattern in mitochondrial haplotype diversity between species. Therefore, we sequenced the ribosomal RNA sub-unit 28S region D1-D3 of representative samples of every spider family or genus included in our study. The 583-bp segment of rRNA was amplified by polymerase chain reaction (PCR). Cycling conditions and PCR reactions were as above. For some species of the family Gnaphosidae, Linyphiidae, Lycosidae, Tetragnathidae, Theridiidae and Thomisidae, a nested PCR using 2 μl of a 1/100–1/1000 dilution of the PCR product obtained with the O/C primers was needed to obtain a good quality sequence (see Table S1).

Endosymbiont screening:

Maternally-inherited endosymbionts such as *Wolbachia* and *Cardinium* infect 66% and 6-7%, respectively, of arthropod species (Hurst & Jiggins 2000; Zchori-Fein & Perlman 2004; Hilgenboecker *et al.* 2008). Such endosymbionts and mitochondria are in linkage disequilibrium and by causing cytoplasmic incompatibility between hosts with different infection status (infected versus non-infected or infected with different bacteria strains) they can select a given mitochondrial haplotype in a process named “selective sweep” (Werren 1997; Bourtzis *et al.* 1998). Such phenomenon can have a dramatic influence on their host haplotype diversity and lead to interpretation mistakes when using only mitochondrial sequence data to infer genetic similarity among species or populations (Hurst & Jiggins 2005). To evaluate the endosymbiont incidence and prevalence in the spider community and to control for endosymbiont-mediated bias in our dataset, the majority of individuals used in this study were molecularly screened for the following endosymbionts: *Arsenophonus*, *Cardinium*, *Flavobacteria*, *Spiroplasma poulsoni*, *Rickettsia* spp. and *Wolbachia* (Table 2). One negative (2 μl of autoclaved MiliQ water) and one positive control consisting of the DNA extract of a specimen known to be infected with an endosymbiont (Table 1) was run with every set of PCR reactions. Visualization, purification and direct sequencing were performed as above.

Sequence analysis:
Forward and reverse sequences were aligned using Bioedit sequence editor (Hall 1999) and further adjusted by eye. Sequence data were transformed in a distance matrix by using the uncorrected distance option (maximum likelihood) available in the version 4.0b10 of PAUP (Swofford 2002). The distance matrix was then used to produce a phenogram via the neighbor joining clustering method implemented in PAUP. Bootstrap values for branches support were calculated in PAUP (Swofford 2002). The haplogyn spider species *Pholcus phalangioides* (Pholcidae) and *Scytodes thoracica* can be considered as sister group of the entelegyn spiders and were, therefore, used as outgroups in this study, (Coddington 2005; Kral *et al.* 2006). All COI and 28S sequences were confronted with the nucleotide database in GenBank (BLAST) as a quality check and to avoid the introduction of wrongly assigned sequences (miss-identification or laboratory contamination) in our database.

Results

Spider community:
DNA was extracted from a total of 129 spider individuals belonging to 49 species from 12 families (Table S1). Up to 130 spider species were described in Swiss ecological compensation areas such as extensified grassland, traditional orchards, hedges and wild flower strips (Jeanneret et al. 2003). Considering the spider diversity exclusively found in wildflower strips around 70 species can be expected (Schmidt-Entling & Döbeli 2009). The diversity of spiders found in different experiments largely depended on the sampling effort, the period of measurements and the sampling area. An estimation of the expected spider diversity in a given habitat is difficult. We are aware that the number of species used in this experiment is an underestimation of the real diversity. Comparing the list of spider species in our dataset with the ones observed in wildflower strips in other studies (Schmidt-Entling & Döbeli 2009), some families like Corinnidae, Hahniidae or Liocranidae are missing, as well as some species from the family Linyphiidae or Theridiidae. However, most of the common spider species found in wildflower strips in the Plateau of Switzerland are included in our analysis. Voucher specimens from all spider species used in this experiment are preserved at the Department of Ecology and Evolution, University of Fribourg, Switzerland. Derived COI sequences were deposited in GenBank (Accession numbers XX) and specific information on the geographical provenance of samples can be found in Table S1.

Trap-nesting wasp community:
A total of 403 *T. figulus* nests were found in ten different fields and 58 different trap-nests. The density of *T. figulus* in the different fields was highly variable. While in some fields only two nests were observed, others had up to 140 nests (mean 33 ± 48.4 s.d.). Predation on wasp larvae was observed in 30% of the investigated nests. The most important parasitoid was the parasitic wasp species *Melittobia acasta* (Hymenoptera: Eulophidae). Further

natural enemies of *T. figulus* such as *Chrysis cyanea* (Hymenoptera: Chrysididae) (Villers, 1789), *Trichodes apiarius* (Coleoptera: Cleridae) and *Megatoma undata* (Linnaeus, 1758) (Coleoptera: Dermestidae) were found.

Phenogram of COI sequences:
Parts of the COI gene (579 bp) from 104 distinct COI sequences (haplotypes) belonging to 46 spider species from 12 families were successfully sequenced and used in phylogenetic analysis (Table S1). The latter sequences included three individuals from two outgroup species *Pholcus phalangioides* (Pholcidae) and *Scytodes thoracica* (Scytodidae), 10 immature and 19 test samples of spider remains out of *T. figulus* nests. Phylogenies based on COI genes were constructed using PAUP v. 4.0b10 software with strict maximum likelihood (ML) inference. The appropriate model of evolution was estimated with ModelTest v. 3.7 (Posada & Crandall, 1998), and the best likelihood score was evaluated with the Akaike Information Criterion (Posada & Buckley, 2004). The best model of nucleotide substitution was the transversional model with invariant sites and rates at variable sites following a Gamma distribution (TVM+I+G). The latter model recognizes four separate transversion rates and a single transition rate. The full neighbor-joining tree revealed evidence for the clustering of conspecifics and congeners in a pattern we expect from morphological identification (Fig.1). At the species level, the monophyly of different taxa was confirmed with a high bootstrap support. Especially in the family of Linyphiids, where multiple individuals were tested, the monophyly of different species was confirmed with a high bootstrap support (e.g., *Tenuiphantes tenuis*, *Meioneta rurestris*, *Centromerita bicolor* or *Porrhomma microphthalmum*). In other families, the monophyly of different species was also observed with high bootstrap support (e.g., *Neottiura bimaculata* (Theridiidae), *Alopecosa pulverulenta* (Lycosidae) or *Misumena vatia* (Thomisidae)). At the genus level, the monophyly of different taxa was confirmed with a high bootstrap support in most cases. With the exception of the genus *Pardosa*, all the genera with multiple species representatives (*Erigone*, *Pachygnatha*, *Enoplognatha*, *Xysticus* and *Drassodes*) clustered in clearly distinctive clades. At the family level, we also found a moderate level of associations, with the monophyly confirmed in eight of the 12 families. From the three non-monophyletic families (Linyphiidae, Theridiidae and Gnaphosidae) no more than two separated groups were found. This separation was most often due to one genus not clustering with the rest of the genera within this family.

Test samples obtained from the DNA of unknown immature spiders (represented by the numbers 1 to 10 in Fig. 1) could be molecularly identified to the species or the genera level. Two samples (8 and 9) clustered with a high bootstrap support with the spider species *Tetragnatha montana* or *Pachygnatha clercki* (Tetragnathidae) respectively. For the remaining samples (1-7 and 10) we found high similarity at the genera level.

The majority of the 19 other test samples (11-29) obtained from spider tissue out of the nest of *T. figulus* were assigned to known species. Eleven test samples (16, 17, 19, 20, 22-25, 27-29) from different traps formed a highly supported clade with *Theridion impressum* (Theridiidae). Six samples (11, 12, 14, 15, 21, 26) formed a highly supported clade with *Larinioides cornutus* (Araneidae). A high bootstrap value supported the grouping of the test sample 18 with *Mangora acalypha* (Araneidae). The test sample 13 grouped within the family Araneidae, could, however, not be assigned to a specific species. A comparison of the obtained sequence with a nucleotide database in GenBank confirmed the similarity of this sample with samples obtained from spiders of the family Araneidae.

Phenogram of 28S sequences:

A total of 47 distinct sequences from parts of the more slowly evolving 28S gene (spanning the D1-D3 regions) (583 bp) were obtained, belonging to 37 spider species from 11 families, whereof 3 sequences belonged to the outgroup species *Pholcus phalangioides* or *Scytodes thoracica* respectively (Table S1). To construct the 28S phylogenetic tree we used the same criteria for the construction of the models like in the phylogenetic analysis of the COI sequences (see above). The best substitution model was the General Time Reversible plus Invariant sites plus Gamma distributed model (GTR+I+G). In this model each possible nucleotide change includes separate transition probabilities, which are scaled to the nucleotide frequencies in the datat set. The gamma + invariable sites model further accommodates site-to-site variability of evolutionary rate using eight site-rate categories and an estimated proportion of invariant sites.

With the exception of the genus *Pardosa*, which did not form a monophyletic clade, the examination of the full neighbor-joining tree confirmed the clustering of congeners and conspecifics as it was found in the COI tree (Fig. 2). In comparison to the COI tree, the phylogenetic analysis of the 28S sequences revealed the clustering of clearly distinctive familial clades.

Endosymbionts:

A total of 99 spider individuals belonging to 44 spider species (12 families) and 10 immatures were tested for the presence of the following endosymbiont species; *Arsenophonus*, *Cardinium*, *Flavobacteria*, *Rickettsia*, *Spiroplasma poulsoni* and *Wolbachia*. Ten individuals (10%) belonging to 6 species from 4 families were infected by either *Cardinium* or *Wolbachia* (Table 2, Fig. 1). We did not observe any co-infected individuals, hosting two different bacterial species. *Cardinium* infection was found in 4.5% of the species and in 4% of all tested individuals, while *Wolbachia* was found in 9% of all species and in 6% of all individuals tested. High infection rates for males and females were found in *Pachygnatha degeeri* (113, 174 and 175) and for females in the outgroup species *Pholcus phalangioides* (262, 263 and 265). In *Bathyphantes gracilis* (148) and *Oedothorax apicatus* (215) only females were infected. From the infected individuals, only seven were considered in the analysis of COI sequences. The tow COI sequences from *P. phalangioides* (262 and 163) considered in the phylogenetic analysis were highly similar, which was not the case for the sequences of the two *P. degeeri* (113, 174) individuals. Although the number of screened individuals is low, there is no strong evidence of a selective sweep (correlation between endosymbiont infection and low or null haplotype diversity).

Discussion

Spider identification through molecular markers (COI, 28S):
Our phylogenetic analysis provides evidence that the identification of spider species based on molecular markers (COI, 28S) is accurate. The high potential of COI sequences for the identification of a large number of different spider species has already been reported (Barrett & Herbert 2005; Greenstone *et al.* 2005). The latter studies were, however, exclusively based on the use of one molecular marker of the mitochondrial DNA (COI). Demographic processes such as founder effect or bottlenecks can affect the intraspecific mitochondrial haplotype diversity. Therefore the sole use of mitochondrial DNA for molecular barcoding studies may be prone to data misinterpretations (Tautz *et al.* 2002, 2003; Mallet & Willmott 2003; Moritz & Cicero 2004; Wheeler *et al.* 2004). To account for this problem, we incorporated ribosomal RNA sequence (28S) data in our investigation. Almost all species represented in the dataset of COI sequences were found to be monophyletic with strong bootstrap support. COI only failed to clearly distinguish 6 out of 46 spiders to the species level, species previously identified based on morphological characters (Table S1; Fig. 1). The absence of monophyly in the species *Drassodes lapidosus*, *Drassodes pubescens*, *Drassyllus pusillus* and *Zelotes latreillei* which all belong to the family Gnaphosidae, is most likely due to demographic processes like founder effects or bottlenecks. The use of the COI gene does however not provide accurate deep phylogenetic resolution to answer this question. A detailed analysis of more species from this family may help to clarify these inconsistencies between genetic and morphological identification.

According to several authors, the ability of barcodes to precisely assign individuals to species is not assured for individuals from different geographical origin (Sperling 2003; Moritz & Cicero 2004; Prendini 2005). The relatively high bootstrap values between individuals from the monophyletic species *Oedothorax apicatus*, *Theridion impressum* (Linyphiidae) and *Neottiura bimaculata* (Theridiidae) may be indeed due to their difference in sampling site (see Table S1). Since the monophyly of these three species was nevertheless confirmed by the analysis, we can assume that the two populations in the different sampling areas of this study are not strictly genetically isolated from each other. In favor of this assumption is the relatively small distance between the two sampling sites (20 km) and the fact that they were not separated by any geographic barrier like mountains or large water bodies. Furthermore nearly all spider species investigated are known to disperse over large distances by ballooning (Blandenier 2009) enabling genetic exchanges between remote populations. In some species, like *Pardosa proxima* (Lycosidae), COI sequences were even found to be identical between individuals coming from either study site.

The lack of support for the clade of the genus *Pardosa* (Lycosidae) corresponds to previous observations (Murphy *et al.* 2006). While the monophyly of the Lycosidae is well supported (Dondale 1986; Griswold 1993) the relationship within the family are poorly understood and a stable subfamilial classification does not exist. Discrepancies between gene trees and species trees (Maddison 1997; Nichols 2001) can further lead to not strictly correlated distinctions between morphology and genetics in lycosid spiders (Vink & Paterson 2003). The timing of speciation event in morphological trades used for species classification may differ from the timing of the evolution of COI gene sequences. To consider different gene sequences in several individuals from the same species are, therefore, important. As suggested by Murphy *et al.* (2006) the position of some genera within their respective subfamilies may be in doubt and more work is needed to clarify these inconsistencies between genetic and morphological identification.

The analysis of the slower evolving and thereby more conserved 28S sequences confirmed the classification of species recognized through previous morphological identification, and highly agreed in the monophyly of the different clades found in the COI tree. This further emphasizes the accuracy of the phylogeny of COI generated in our analysis and supports the use of a COI-based identification system to distinguish spider species.

The effect of inherited symbionts in phylogenetic studies:

Goodacre *et al.* (2006), who investigated the infection with endosymbionts in a large variety of spider species, found *Wolbachia*, *Cardinium*, *Rickettsia* and *Spiroplasma* to be the most important ones. In our experiment we detected *Wolbachia* or *Cardinium* but not *Rickettsia* nor *Spiroplasma*. The observed infection rate with *Wolbachia* (9%) was lower than could be expected from literature (66% in arthropods), while *Cardinium* infection (4.5%) was in the range of infection rates of other arthropods (6-7%) (Hurst & Jiggins 2000; Zchori-Fein & Perlman 2004; Hilgenboecker *et al.* 2008). The observed endosymbiont incidence may, however, be underestimated, because the infection within some species may not have been detected due to small sample sizes. Furthermore, bacterial titer differs depending on tissue types and with age (Ijichi *et al.* 2002). Since DNA was extracted from spider legs, we may have failed to detect endosymbionts in some spiders.

COI sequences used for phylogenetic analysis were not biased by correlations between endosymbiont infections and haplotype diversity. We can, therefore, assume that the observed clustering of species based on COI sequences was not misinterpreted due to the presence of endosymbionts. In the case of *Pholcus phalangioides*, two individuals had identical COI sequences, which might be the indication of mitochondrial haplotype selection by endosymbionts (selective sweep) for more similar sequences. The high genetic similarity of these two individuals might thus have been due to the presence of *Wolbachia*. To be able to know whether the infections of endosymbionts or demographic processes are responsible for these results, more detailed analyses are needed. This could for example be achieved by mating experiments between infected females and uninfected males.

The potential use of COI to track immatures and trophic links in food webs:

Although the use of COI for the identification of species has largely been criticised (Lipscomb *et al.* 2003; Tautz *et al.* 2003; Scotland *et al.* 2003; Scoble 2004; Prendini 2005), our analysis highlights the great potential of this genetic marker for the identification of immature spiders, where the development of genital structures, crucial for species identification, is not complete. In line with the observation of Greenstone *et al.* (2005), COI sequences of this study proved to be useful for the identification of immatures in most cases to the species level. Immature spider individuals may occur, depending on the season, in identical or higher densities as adults in the field, which highlights the need of a sound method for their identification in ecological studies (Greenstone *et al.* 2005). Although our dataset did not include COI sequences from all species that might be trapped by *T. figulus* in this habitat type, a surprisingly high number of spider test samples out of wasp nests could be assigned to a specific spider species. To be able to use COI sequences to track trophic links in food webs, we emphasize the importance of large COI sequence databases of many species from different communities and geographic regions.

Our investigation highlights another major advantage of COI sequence databases: the identification of trophic links in food webs. By the use of this genetic marker, we were able to assign spider remains found in nests of the mud-dauber wasp *T. figulus* to the family Theridiidae and Araneidae. This is in line with the current knowledge of the prey spectrum of *Trypoxylon* wasps in general, which have been found to show a preference for spiders from the family Araneidae, Theridiidae and/or Linyphiidae, while spiders from other families like Lycosidae, Salticidae or Thomisidae are less often trapped (Asís *et al.* 1994; Polidori *et al.* 2007; Buschini *et al.*2008). Furthermore, there is good evidence that most of the test samples taken from the *T. figulus* traps belonged to the three spider species *T. impressum*, *Mangora acalypha* and *L. cornutus*. The latter species has been found in the prey spectrum of another *Trypoxylon* species *T. albonigrum* (Eberhard 1970) and all three spider species have been observed in nests of other mud-dauber wasp species from the family Sphecidae (*Sceliphron spirifex* and *S. caementarium*, Drury) in an experiment performed in northern Italy (Polidori *et al.* 2007). To our knowledge the prey spectrum of *T. figulus* has not been fully investigated so far.

The two parasitoid species of *T. figulus* found in this experiment corresponded to observations of the predator spectrum of previous studies (Tscharntke et al. 1998; Gathmann & Tscharntke 1999). However, the two beetle species *T. apiarius* and *M. undata* have, to our knowledge, not been reported as natural enemies of *T. figulus* so far. The investigated system of a wasp species and its prey spectrum, as presented here, is only one example to illustrate the potential use of COI sequences for tracking trophic links in food webs.

Conclusion

The use of a COI sequence database for the identification of spiders demonstrated by Barrett and Herbert (2005) was largely criticised because they only considered one mitochondrial gene which can be subject to demographic processes like bottlenecks, founder effects, or genetic drift and selective sweeps through endosymbiont infection. In this study the latter potential bias were taken into account by the consideration of an additional nuclear gene and by a survey of the endosymbionts infecting spiders. With this experiment, we emphasize the major advantage of genetic barcodes for the detection of otherwise invisible trophic links in food webs, which is crucial for the study of important ecological questions on the stability of communities.

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Table 1: Genes and primers used in polymerase chain reaction (PCR) for the construction of spider phylogenies and for the molecular screen of maternally-inherited endosymbionts.

Organism	Gene	Primer (5′ – 3′)	Annealing temperature	Positive control	Size	Reference
Araneae	COI B cDNA	LCO-1628 –ATAATGTAATTGTTACTGCTCATGC HCO-2396 –ATTGTAGCTGAGGTAAAATAAGCTCG	47 C°	NA	768 bp	Gillespie (2002)
Araneae	COI B cDNA	ChelF1 -TACTCTACTAATCATAAAGACATTGG ChelR1 –CCTCCTCCTGAAGGGTCAAAAAATGA	47 C°	NA	660 bp	Barrett & Hebert (2005)
Araneae	28S rDNA	28S ‘‘O’’ -GAA ACTGCTCAAAGGTAAACGG 28S ‘‘C’’ –GGTTCGATTAGTCTTTCGCC	55 C°	NA	800 bp	Hedin & Maddison (2001)
Araneae	28S rDNA	G700 -TGCGGACCTCCACCAGAGTTTCT G701 -ACTGCTCAGAGGTAA ACGGGAGG	50 C°	NA	800 bp	Murphy *et al.* (2006)
Arsenophonus	16S rDNA	ArsF -GGGTTGTAAAGTACTTTCAGTCGT ArsR2 -GTAGCCCTRCTCGTAAGGGCC	52C°	*Nasonia vitripennis*	581-804 bp	Duron *et al.* (2008)
Cardinium	16S rDNA	CHR -TACTGTAAGAATAAGCACCGGC CHR -GTGGATCACTTAACGCTTTCG	60-50 C°	*Holocnemus pluchei*	450 bp	Zchori-Fein & Perlman (2004)
Flavobacterium	16S rDNA	FL1 -AATGTTAAAGTTCCGGCG-3 FL2 -CTGTTTCCAGCTTATTCGTAGTAC	60-50 C°	*Adalia decempuntata*	800 bp	Hurst *et al.* (1997)
Rickettsia spp.	16S rDNA	RSSUF -CGGCTTTCAAAACTACTAATCTA RSSUR -GAAAGCATCTCTGCGATCCG	60-50 C°	*Bemisia tabachi*	450 bp	von der Schulenburg *et al.* (2001)
Spiroplasma	16S rDNA	SpoulF -GCTTAACTCCAGTTCGCC SpoulR -CCTGTCTCAATGTTAACCTC	55 C°	*Drosophila melanogaster*	421 bp	Duron *et al.* (2008)
Wolbachia	wsp	WSP 81F -TGGTCCAATAAGTGATGAAGAAAC WSP 691R -AAAAATTAAACGCTACTCCA	60-50 C°	*Culex pipiens*	540 bp	Zhou *et al.* (1998)

Table 2: Incidence and prevalence of maternally inherited endosymbiotic bacteria infecting wild flower strip spider species. N indicating the total number of individuals tested; f: females, m: males, im: immature (male or female).

						Prevalence																	
		Total number of individuals				*Arsenophonus*			*Cardinium*			*Flavobacteria*			*Rickettsia*			*Spiroplasma poulsoni*			*Wolbachia*		
Family	Name	N	f	m	im	f	m	im	f	m	im	f	m	im	f	m	im	f	m	im	f	m	im
Araneidae	*Aculepeira ceropegia*	2	0	0	2			0			0			0			0			0			0
	Argiope bruennichi	2	0	2	0	0			0			0			0			0			0		
	Larinioides cornutus	2	2	0	0	0			0			0			0			0			0		
	Mangora acalypha	2	0	0	2			0			0			0			0			0			0
	sp.	2	0	0	2			0			0			0			0			0			0
Gnaphosidae	*Drassodes lapidosus*	1	0	1	0		0			0			0			0			0			0	
	Drassodes pubescens	1	0	1	0		0			0			0			0			0			0	
	Drassyllus praeficus	1	0	1	0		0			0			0			0			0			0	
	Zelotes latreillei	1	0	1	0		0			0			0			0			0			0	
Linyphiidae	*Bathyphantes gracilis*	4	3	1	0	0	0		0	0		0	0		0	0		0	0		0.33	0	
	Centromerita bicolor	5	3	2	0	0	0		0	0		0	0		0	0		0	0		0	0	
	Dicymbium nigrum	1	0	1	0		0			0			0			0			0			0	
	Erigone atra	3	3	0	0	0			0			0			0			0			0		
	Erigone dentipalpis	5	3	2	0	0	0		0	0		0	0		0	0		0	0		0	0	
	Linyphia triangularis	1	1	0	0							0			0			0			0		
	Meioneta rurestris	6	3	3	0	0	0		0	0		0	0		0	0		0	0		0	0	
	Microlinyphia pusilla	1	0	1	0		0			0			0			0			0			0	
	Oedothorax apicatus	4	2	2	0	0	0		0	0		0	0		0	0		0	0		0.5	0	
	Pelecopsis parallela	1	1	0	0	0			1			0			0			0			0		
	Porrhomma microphthalmum	2	1	1	0	0	0		0	0		0	0		0	0		0	0		0	0	
	Tenuiphantes tenuis	5	2	3	0	0	0		0	0		0	0		0	0		0	0		0	0	

						Prevalence															
		Total number of individuals				*Arsenophonus*			*Cardinium*			*Flavobacteria*			*Rickettsia*			*Spiroplasma poulsoni*			*Wolbachia*
Family	Name	N	f	m	im	f	m	im	f	m	im	f	m	im	f	m	im	f	m	im	f	m	im
Linyphiidae	*Walckenaeria nudipalpis*	1	1	0	0	0			0			0			0			0			0		
Lycosidae	*Alopecosa pulverulenta*	2	1	1	0	0	0		0	0		0	0		0	0		0	0		0	0	
	Arctosa lutetiana	2	0	2	0		0			0			0			0			0			0	
	Aulonia albimana	1	0	1	0		0			0			0			0			0			0	
	Pardosa agrestis	2	2	0	0	0			0			0			0			0			0		
	Pardosa amentata	1	1	0	0	0			0			0			0			0			0		
	Pardosa hortensis	2	2	0	0	0			0			0			0			0			0		
	Pardosa proxima	3	3	0	0	0			0			0			0			0			0.33		
	Pardosa pullata	1	1	0	0	0			0			0			0			0			0		
	Pirata uliginasus	1	1	0	0	0			0			0			0			0			0		
Pholcidae	*Pholcus phalangioides*	3	3	0	0	0			0			0			0			0			1		
Pisauridae	*Pisaura mirabilis*	1	1	0	0	0			0			0			0			0			0		
	sp.	5	0	0	5			0			0			0			0			0			0
Salticidae	*Marpissa muscosa*	1	0	1	0		0			0			0			0			0			0	
Scytodidae	*Scytodes thoracica*	1	0	0	1			0			0			0			0			0			0
Tetragnathidae	*Pachygnatha clercki*	2	0	2	0		0			0			0			0			0			0	
	Pachygnatha degeeri	3	2	1	0	0	0		1	1		0	0		0	0		0	0		0	0	
	sp.	2	0	0	2			0			0			0			0			0			0
Theridiidae																							
	Enoplognatha latimana	3	0	3	0		0			0			0			0			0			0	
	Enoplognatha ovata	2	2	0	0	0			0			0			0			0			0		
	Neottiura bimaculata	1	1	0	0	0			0			0			0			0			0		
	Theridion impressum	2	2	0	0	0			0			0			0			0			0		

		Total number of individuals				Prevalence																	
						Arsenophonus			*Cardinium*			*Flavobacteria*			*Rickettsia*			*Spiroplasma poulsoni*			*Wolbachia*		
Family	Name	N	f	m	im	f	m	im	f	m	im	f	m	im	f	m	im	f	m	im	f	m	im
Thomisidae	*Xysticus cristatus*	1	0	1	0		0			0			0			0			0			0	
	Xysticus kochi	1	0	1	0		0			0			0			0			0			0	
	Ozyptila simplex	1	0	1	0		0			0			0			0			0			0	
	Sp.	1	0	0	1			0			0			0			0			0			0
Zoridae	*Zora spinimana*	1	0	1	0		0			0			0			0			0			0	

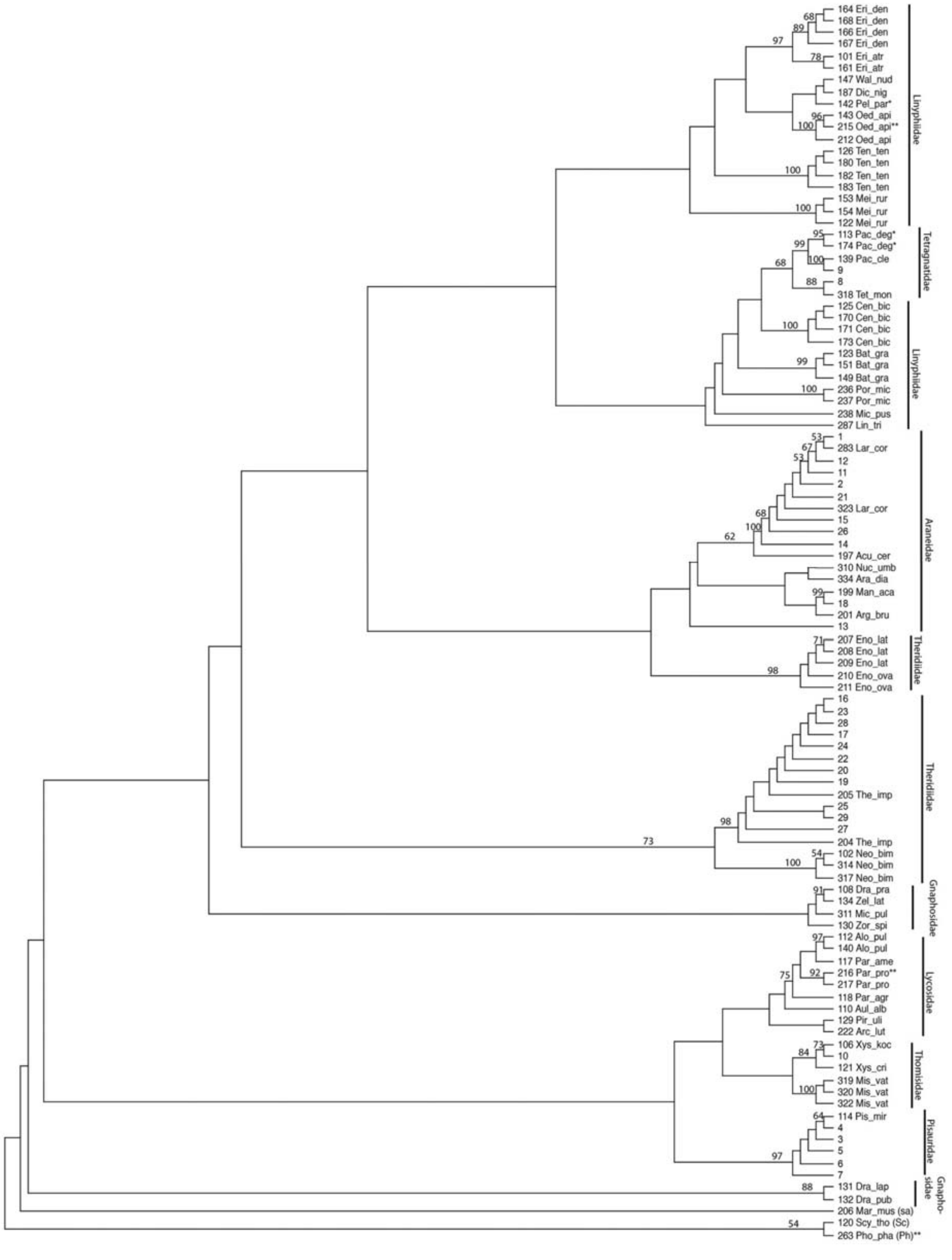

Figure 1: Neighbour-joining tree of COI mtDNA haplotypes from 46 spider species (2 outgroup species) of semi-natural habitats in Switzerland. Number 1-10 indicating immature spider test samples, 11-29 representing test samples of spider tissue from unknown species collected in nests of the wasp species *Trypoxylon figulus*. Endosymbiont infection: * *Cardinium*, ** *Wolbachia*.

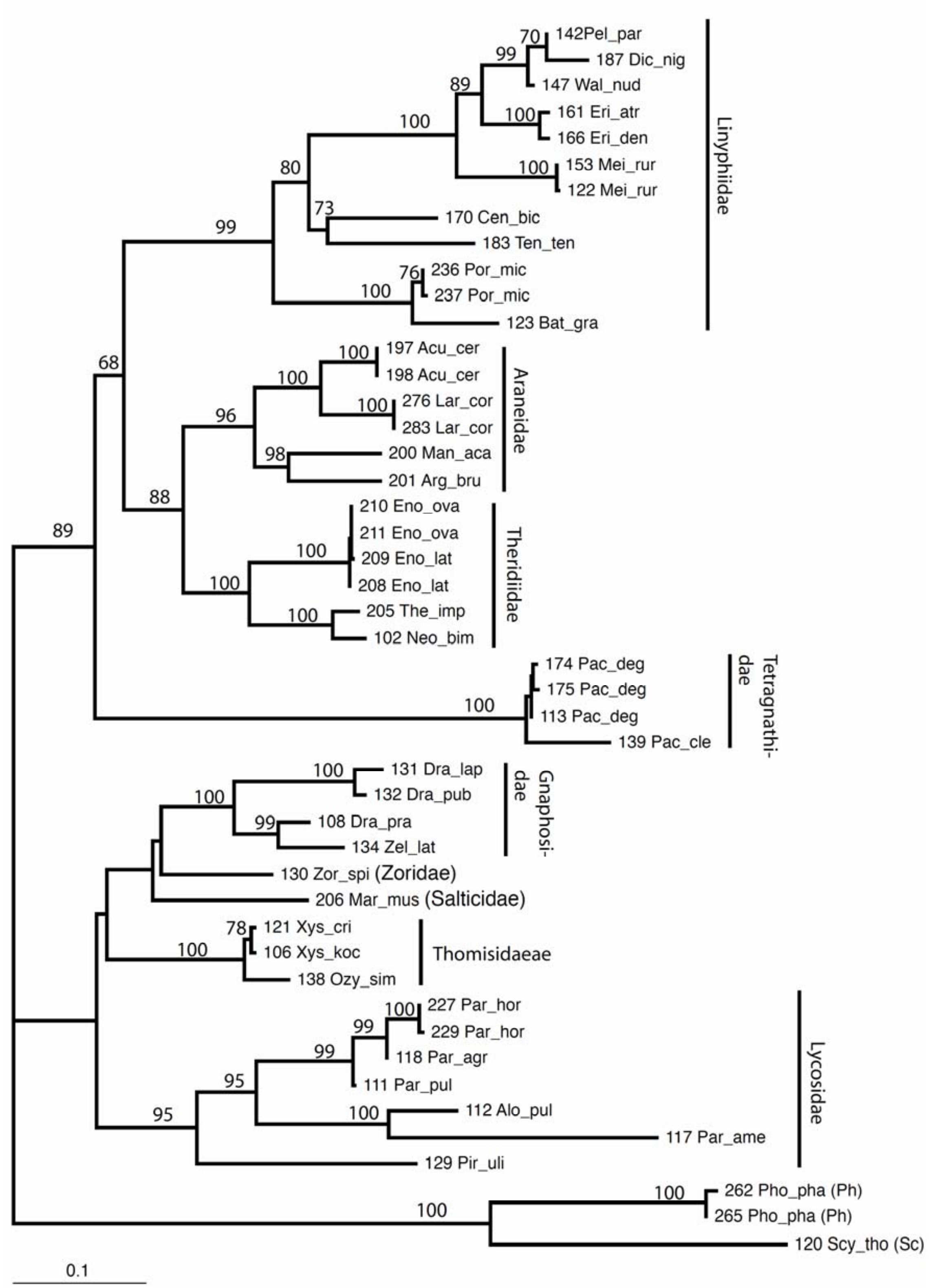

Figure 2: Neighbour-joining tree of 28S rRNA haplotypes from 37 spider species (2 outgroup species) of semi-natural habitats in Switzerland.

Table S1: Spider specimens used for this study. Individuals not tested for endosymbionts or not represented in the COI or 28S tree are indicated by na.

		COI mtDNA		28S rRNA					
Family	Species	Primers HCO/LCO	Primers Chel F1/R1	Primers O/C	Primers G700/G701	Endosymbiont	Label in COI tree	Label in 28S tree	Study site
Araneidae	*Aculepeira ceropegia*		1	1			197 Acu_cer	197 Acu_cer	FR
	Aculepeira ceropegia		1	1			= 197 Acu_cer	198 Acu_cer	FR
	Araneus diadematus	1				na	334 Ara_dia	na	BE
	Argiope bruennichi		1	1			201 Arg_bru	201 Arg_bru	GR
	Argiope bruennichi		1				= 201 Arg_bru	na	GR
	Larinioides cornutus	1				na	323 Lar_cor	na	GR
	Larinioides cornutus		1	1			= 323 Lar_cor	276 Lar_cor	GR
	Larinioides cornutus		1	1			283 Lar_cor	283 Lar_cor	GR
	Mangora acalypha	1					199 Man_aca	na	FR
	Mangora acalypha		1	1			= 199 Man_aca	200 Man_aca	FR
	Nuctenea umbratica	1				na	310 Nuc_umb	na	Gru
	sp.	1					**2**	na	GR
	sp.		1				**1**	na	GR
Gnaphosidae	*Drassodes lapidosus*	1		1			131 Dra_lap	131 Dra_lap	FR
	Drassodes pubescens	1		1			132 Dra_pub	132 Dra_pub	FR
	Drassyllus praeficus	1		1			108 Dra_pra	108 Dra_pra	FR
	Micaria pulicaria	1				na	311 Mic_pul	na	GR
	Zelotes latreillei	1			1		134 Zel_lat	134 Zel_lat	FR
Linyphiidae	*Bathyphantes gracilis*	1				*Wolbachia*	= 134 Zel_lat	na	FR
	Bathyphantes gracilis	1		1			123 Bat_gra	123 Bat_gra	FR
	Bathyphantes gracilis	1					149 Bat_gra	na	FR
	Bathyphantes gracilis		1				151 Bat_gra	na	FR
	Centromerita bicolor	1					125 Cen_bic	na	FR
	Centromerita bicolor	1					= 125 Cen_bic	na	FR
	Centromerita bicolor	1		1			170 Cen_bic	170 Cen_bic	FR
	Centromerita bicolor	1					171 Cen_bic	na	FR

		COI mtDNA		28S rRNA					
Family	Species	Primers HCO/LCO	Primers Chel F1/R1	Primers O/C	Primers G700/G701	Endosymbiont	Label in COI tree	Label in 28S tree	Study site
Linyphiidae	*Centromerita bicolor*		1				173 Cen_bic	na	FR
	Dicymbium nigrum		1		1		187 Dic_nig	187 Dic_nig	FR
	Erigone atra		1				101 Eri_atr	na	FR
	Erigone atra		1				= 101 Eri_atr	na	FR
	Erigone atra		1	1			161 Eri_atr	161 Eri_atr	FR
	Erigone dentipalpis		1				164 Eri_den	na	FR
	Erigone dentipalpis		1				= 164 Eri_den	na	FR
	Erigone dentipalpis		1	1			166 Eri_den	166 Eri_den	FR
	Erigone dentipalpis		1				167 Eri_den	na	FR
	Erigone dentipalpis		1				168 Eri_den	na	FR
	Linyphia triangularis		1				287 Lin_tri	na	GR
	Meioneta rurestris	1		1			122 Mei_rur	122 Mei_rur	FR
	Meioneta rurestris	1		1			153 Mei_rur	153 Mei_rur	FR
	Meioneta rurestris	1					154 Mei_rur	na	FR
	Meioneta rurestris	1					= 154 Mei_rur	na	FR
	Meioneta rurestris	1					= 154 Mei_rur	na	FR
	Meioneta rurestris	1					= 154 Mei_rur	na	FR
	Microlinyphia pusilla	1					238 Mic_pus	na	GR
	Oedothorax apicatus		1			*Wolbachia*	215 Oed_api	na	GR
	Oedothorax apicatus	1					143 Oed_api	na	FR
	Oedothorax apicatus	1					212 Oed_api	na	GR
	Oedothorax apicatus	1					= 212 Oed_api	na	GR
	Pelecopsis parallela	1			1	*Cardinium*	142 Pel_par	142 Pel_par	FR
	Porrhomma microphthalmum		1	1			236 Por_mic	236 Por_mic	GR
	Porrhomma microphthalmum		1	1			237 Por_mic	237 Por_mic	GR
	Tenuiphantes tenuis	1					126 Ten_ten	na	FR
	Tenuiphantes tenuis	1					= 126 Ten_ten	na	FR

		COI mtDNA		28S rRNA					
Family	Species	Primers HCO/LCO	Primers Chel F1/R1	Primers O/C	Primers G700/G701	Endosymbiont	Label in COI tree	Label in 28S tree	Study site
Linyphiidae	*Tenuiphantes tenuis*	1					180 Ten_ten	na	FR
	Tenuiphantes tenuis	1		1			182 Ten_ten	id	FR
	Tenuiphantes tenuis	1		1			183 Ten_ten	183 Ten_ten	FR
	Walckenaeria nudipalpis	1			1		147 Wal_nud	147 Wal_nud	FR
Lycosidae	*Alopecosa pulverulenta*	1		1			112 Alo_pul	112 Alo_pul	FR
	Alopecosa pulverulenta	1					140 Alo_pul	na	FR
	Arctosa lutetiana	1					222 Arc_lut	na	FR
	Aulonia albimana	1					110 Aul_alb	na	FR
	Pardosa agrestis	1			1		118 Par_agr	118 Par_agr	FR
	Pardosa agrestis	1					= 118 Par_agr	na	GR
	Pardosa amentata	1			1		117 Par_ame	117 Par_ame	FR
	Pardosa hortensis				1		na	227 Par_hor	FR
	Pardosa hortensis				1		na	229 Par_hor	FR
	Pardosa proxima		1			*Wolbachia*	216 Par_pro	na	GR
	Pardosa proxima		1				217 Par_pro	na	GR
	Pardosa proxima		1				= 217 Par_pro	na	FR
	Pardosa pullata				1		na	111 Par_pul	FR
	Pirata uliginosus	1		1			129 Pir_uli	129 Pir_uli	FR
Pholcidae	*Pholcus phalangioides*	1		1		*Wolbachia*	= 129 Pir_uli	262 Pho_pha	FR
	Pholcus phalangioides	1				*Wolbachia*	263 Pho_pha	na	FR
	Pholcus phalangioides			1		*Wolbachia*	na	265 Pho_pha	FR
Pisauridae	*Pisaura mirabilis*	1					114 Pis_mir	na	FR
	sp.	1					3	na	FR
	sp.	1					4	na	FR
	sp.	1					5	na	FR
	sp.	1					6	na	FR
	sp.	1					7	na	FR
Salticidae	*Marpissa muscosa*		1	1			206 Mar_mus	206 Mar_mus	FR

		COI mtDNA		28S rRNA					
Family	Species	Primers HCO/LCO	Primers Chel F1/R1	Primers O/C	Primers G700/G701	Endosymbiont	Label in COI tree	Label in 28S tree	Study site
Scytodidae	*Scytodes thoracica*	1		1			120 Scy_tho	120 Scy_tho	GR
Tetragnathidae	*Pachygnatha clercki*		1		1		139 Pac_cle	139 Pac_cle	FR
	Pachygnatha clercki		1				= 139 Pac_cle	na	GR
	Pachygnatha degeeri		1		1	*Cardinium*	113 Pac_deg	113 Pac_deg	FR
	Pachygnatha degeeri	1		1		*Cardinium*	174 Pac_deg	174 Pac_deg	FR
	Pachygnatha degeeri	1		1		*Cardinium*	= 174 Pac_deg	175 Pac_deg	FR
	Tetragnatha montana	1				na	318 Tet_mon	na	GR
	sp.		1				8	na	GR
	sp.		1				9	na	GR
Theridiidae	*Enoplognatha latimana*		1				207 Eno_lat	na	GR
	Enoplognatha latimana	1		1			208 Eno_lat	208 Eno_lat	GR
	Enoplognatha latimana	1		1			209 Eno_lat	209 Eno_lat	GR
	Enoplognatha ovata	1		1			210 Eno_ova	210 Eno_ova	GR
	Enoplognatha ovata		1	1			211 Eno_ova	211 Eno_ova	GR
	Neottiura bimaculata	1				na	314 Neo_bim	na	GR
	Neottiura bimaculata	1				na	= 314 Neo_bim	na	GR
	Neottiura bimaculata	1				na	317 Neo_bim	na	GR
	Neottiura bimaculata	1			1		102 Neo_bim	102 Neo_bim	FR
	Theridion impressum	1					204 The_imp	na	FR
	Theridion impressum		1	1			205 The_imp	205 The_imp	GR
Thomisidae	*Misumena vatia*	1				na	319 Mis_vat	na	BE
	Misumena vatia	1				na	320 Mis_vat	na	BE
	Misumena vatia	1				na	= 320 Mis_vat	na	BE
	Misumena vatia	1				na	322 Mis_vat	na	BE
	Ozyptila simplex				1		na	138 Ozy_sim	FR
	Xysticus cristatus	1		1			121 Xys_cri	121 Xys_cri	FR
	Xysticus kochi	1			1		106 Xys_koc	106 Xys_koc	FR
	sp.		1				10	na	GR
Zoridae	*Zora spinimana*	1		1			130 Zor_spi	130 Zor_spi	FR

Family	Species	COI mtDNA		28S rRNA		Endosymbiont	Label in COI tree	Label in 28S tree	Study site
		Primers HCO/LCO	Primers Chel F1/R1	Primers O/C	Primers G700/G701				
Test sample (from wasp nest)		1				na	11	na	GR
Test sample (from wasp nest)		1				na	12	na	GR
Test sample (from wasp nest)			1			na	13	na	GR
Test sample (from wasp nest)		1				na	14	na	GR
Test sample (from wasp nest)		1				na	15	na	GR
Test sample (from wasp nest)		1				na	16	na	GR
Test sample (from wasp nest)		1				na	17	na	GR
Test sample (from wasp nest)		1				na	18	na	GR
Test sample (from wasp nest)		1				na	19	na	GR
Test sample (from wasp nest)		1				na	20	na	GR
Test sample (from wasp nest)		1				na	21	na	GR
Test sample (from wasp nest)		1				na	22	na	GR
Test sample (from wasp nest)		1				na	23	na	GR
Test sample (from wasp nest)		1				na	24	na	GR
Test sample (from wasp nest)		1				na	25	na	GR
Test sample (from wasp nest)		1				na	26	na	GR
Test sample (from wasp nest)		1				na	27	na	GR
Test sample (from wasp nest)		1				na	28	na	GR
Test sample (from wasp nest)		1				na	29	na	GR

Legend: Study sites : FR = Fribourg; BE = Bern; GR = Grandcour; Gru = Gruyère

DISCUSSION AND OUTLINE

The global aim of this thesis was to investigate spiders and their natural prey and predator species in agricultural landscapes to gain insight in the structure, dynamics and stability of communities. Three investigations on different topics were performed, which makes it difficult to draw a general synthesis. In the following I will discuss the three different experiments separately and emphasize new issues which can be raised by our results.

1) Bottom-up and top-down control of *Argiope bruennichi* (Araneae: Araneidae) in semi-natural ecosystems

With this study system we were able to demonstrate that the abundance of an intermediate predator, in our case the web-building spider *Argiope bruennichi* can be influenced by both bottom-up (plant and prey) and top-down (predator) effects. Furthermore, the observation of an indirect and direct effect of plant diversity emphasizes the importance of maintaining plant biodiversity in agricultural ecosystems, to preserve high densities of generalist predators for natural pest control.

Synthesis and perspectives

In our experiment, only plant diversity was manipulated. A better approach would be the manipulation of all trophic levels in this system. By doing so, we might have gained a more precise view of the importance of the different factors affecting the abundance of this spider species. In further experiments, we should, for example include the manipulation of prey and predator density. This could be achieved by the observation of the system within cages. First, a clear separation of the effect of prey and predators may help to understand whether the abundance of this spider is more influenced by the availability of prey or by the presence of the predator. Second, hornets also consume Argiope’s prey, and are thus both predators and competitors of this spider. The dynamics of this “intraguild predation” system (Holt & Polis 1997) is worth being studied for its own. Finally, it would be interesting to see whether plants may provide protection from predators. This could be tested by the manipulation of different plant features within cages and by adding a predator.

2) Trends in climate, land use and the dynamics of a ballooning spider assemblage

The main results of this study showed that spider populations appear to be more at risk from the expected intensification of extreme climatic events and changes in land use than from gradually rising temperatures. We were surprised to observe a shift in the phenology of the ballooning population within the relatively short period of eleven years. This indicates that the response of different species to climate change may be faster evolving than expected.

Synthesis and perspectives

From the observation of ballooning spiders collected at one study site only, it is difficult to draw general conclusions. Furthermore, since ballooning spider densities do not one-to-one reflect spider population densities, we are not able to predict the precise relationship between climate or habitat change and spider populations. It would therefore be interesting to concentrate on the continuous collection of spiders 1) at different sites and 2) by the use of different trapping methods. This would however imply a great sampling effort which is generally very costly.

Further interesting questions, which could be addressed at this dataset, are the investigation of interactions between different spider species. Since the response of different species to changing climatic conditions may not be the same, we would expect some species to become more abundant than others. This may provoke an increase or decrease in competitive interactions between species and hence lead to changes in community composition. Methods to investigate competition between species in time-series exist (Ives *et al.* 2003), but not to detect changes in community structure with time. We are currently exploring an approach base on wavelet analyses (Cazelles *et al.* 2008), which yielded promising preliminary results.

3) Spider molecular barcode to track trophic links in food webs

In this study we use the trophic interaction between a wasp species and its spider prey to show the potential of molecular tools to track trophic links in food webs. Despite criticism of the use of cytochrome oxydase I for the identification of species, our results highlight two decisive advantages of this method: 1) the identification of species lacking a clear morphological differentiation, like immature spiders and 2) the better understanding of otherwise invisible trophic links in food webs by the identification of species from prey remains.

Synthesis and perspectives

The established methods to track trophic links between the wasp *Trypoxylon figulus* and its spider prey will, in a next step, be used to fully characterize the prey community of this wasp in our experimental system. Wasp females may collect different prey species in habitats differing in plant structure and diversity. Furthermore, plant diversity and structure may affect the parasitoid community of the wasp larvae. The method established to identify spider species can be applied as well for the identification of parasitoid species, which will allow gaining insight in the network of interactions involving *T. figulus* larvae. Establishing the barcodes for all (or the most common) species in our system would certainly be a breakthrough to determine and quantify trophic interactions at the scale of the whole food web. Currently, the lack of such high-quality and quantitative data is a major hurdle for the development of food web models, a primary task in the face of current global change.

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Curriculum vitae

PERSONAL

Name	Bruggisser Odile
Date of birth	30. 11. 1980
Present address	3014 Bern Greyerzstrasse 81
E-mail	odile.bruggisser@unifr.ch
Nationality	Swiss
Civil status	single
Language skills	German (native language) French (very good knowledge in spelling and writing) English (very good knowledge in spelling and writing) Spanish (good knowledge in spelling and writing)

EDUCATION

2006 – present	**PhD thesis**: Understanding the structure of interactions and the dynamics of spider populations in agricultural ecosystems. Unit of Ecology and Evolution, University of Fribourg
2005 – 2006	**Diploma-thesis**: Effect of vineyard management on biodiversity at three trophic levels (plants, grasshoppers, spiders). Community Ecology, University of Bern
2004 – 2005	Exchange semester: Universidad Autonónoma Madrid, Spain
2001 – 2004	Undergraduate Studies: University of Bern, Switzerland.
1994 – 2001	High school: Deutsches Gymnasium Biel

WORKSHOPS AND COURSES

2009 May	Project Management in Research
2008 Feb-May	Amphibienkurs: Feldherpetologischer Kurs (Karch)
2008 April	Field Animal Experimentation - Practical Module 1
2007 Nov-Dec	Scientific Writing Clinic
2007 Sep-Oct	An Introduction to the Practice of Statistics Using R
2005 Jul	Summer academy on Alp Flix, course of spider taxonomy and systematic

ACTIVITIES IN THE FIELD OF BIOLOGY

2009	"Journée de la Biodiversitée" en Parc naturel regional Gruyère Pays-d'Enhaut, Switzerland: animation of young pupils on the ecology of spiders
2008	"Geo Tag der Artenvielfalt" in the Parc Ela, Bergün, Switzerland. Expert in spider taxonomy
2007	"Geo Tag der Artenvielfalt" in the Sensegraben, Switzerland. Expert in spider taxonomy

PUBLICATIONS AND PRESENTATIONS

Publications:

Bruggisser OT, Schmidt-Entling MH, Bacher S (2010): Effects of vineyard management on biodiversity at three trophic levels. *Biological conservation*, **143,** 1521-1528.

Bruggisser O, Schmidt MH, Bacher S (2005): Effect of vineyard management on biodiversity at three trophic levels. *Verhandlungen der Gesellschaft für Ökologie*, Band **35,** 173.

Oral presentations:

Bruggisser OT, Blandenier G, Bersier LF (2009) The role of plants in an Argiope bruennichi – prey system. 25th European Congress of Arachnology (ECA), Alexandroupolis, **Greece.** *(Third price for "best student's talk")*

Bruggisser OT, Blandenier G, Bersier LF (2008) Spider community dynamics and climate change. BES Annual Meeting 2008, Imperial College, London, **England.**

Bruggisser OT, Blandenier G, Bersier LF (2007) Climate change increases the variability of spider dispersal dynamics ESA/SER Joint Meeting San Jose, **USA.**

Bruggisser OT, Schmidt MH, Bacher S (2006) Effect of vineyard management on spider biodiversity in northern Switzerland. 23th European Congress of Arachnology (ECA), Sitges, **Spain.**

Posters:

Bruggisser OT, Blandenier G, Bersier LF (2009) Detecting shifts in competitive interactions in time series. Biology 09, Bern, **Switzerland.**