Almost all the wise world
is little else in nature
but parasites or subparasites

Ben Johnson (1606) *Volpone*, act 3, scene 1

Preface

Almost all kinds of organisms live in more or less close association with other organisms, which is generally termed as symbiosis. Depending on the cost and benefit for each participant, these associations span a symbiotic continuum ranging from antagonism to mutualism. Rather than being static, selection pressures on either partner may cause shifts along the symbiotic continuum.

Plant/microbe interactions were perceived for a long time exclusively if situated on the antagonistic end of the symbiotic continuum, i.e. as host/pathogen interactions. Until recently, mutualistic plant/microbe interactions were largely omitted. Most research in this field has focused on rather showy mutualisms such as pollination or fruit dispersal. However, many plants are almost always infected with microorganisms such as rhizobia or micorrhizal fungi. Many of these mutualistic microsymbionts may be considered merely as a special type of plant organ.

Mutualistic symbioses between plants and microorganisms received increased attention as soon as their economic potential was recognized. Research on endophytic fungi that infect grasses, the most familiar and important plant family, commenced when their role in mammalian toxicosis and insect deterrence became largely known. These fungal endophytes of genus *Epichloë* and their anamorphs (*Neotyphodium* species) live intercellulary in the leaves and culms of many grasses. Interest in endophyte research grew as soon as they were

recognised as causative agents of livestock toxicosis. Due to their agronomic importance, most published research focused on few grass cultivars of major pasture and turf grasses. However, studies that were conducted in natural populations revealed abundant genetic diversity of endo-phytic fungi, which is of particular interest since grass/endophyte symbiota are usually characterised by persistent associations of single host and fungal genotypes. Hence, these associations are well suited for studying the effect of genotypic variation on the outcome of the symbiosis, which motivated me to focus on this particular aspect in my thesis.

Thesis outline

Chapter 1 gives a general introduction to host/parasite interactions. It includes definitions on the terminology surrounding this topic and the main concepts on the evolution in symbiotic environments, including the evolution of parasite virulence. More specifically, plant parasites are then classified by their mode of infection, i.e. *local lesion* or *systemic*, and by their effect on host fitness, i.e. *antagonistic*, *neutral* or *mutualistic*. Also included here is a brief introduction on two important plant/fungal mutualisms (lichens and mycorrhizas), whereas *Rhizobium* bacteria will not be considered despite their importance in many plant communities. Finally, I summarise current knowledge on clavicipitaceous fungi that endophytically infect many grasses. This part includes a systematical classification of fungal endophytes, an introduction to their life cycles and a summary of beneficial and detrimental aspects of endophyte infections.

The studies presented in *Chapter 2* and *Chapter 3* are descriptive. They were conducted to investigate the genetic diversity of fungal endophytes in three natural populations of the Swiss Jura Mountains. The locations have been chosen as experimental sites in the framework of a long-term study on biodiversity, a part of the Swiss Priority Programme Environment (SPP-Umwelt). The following chapters (*Chapter 4* to *Chapter 7*) summarise data from different experimental approaches using reciprocal associations of previously defined genotypes of *Bromus erectus*, the dominant grass species in these natural populations, with its natural endophyte *Epichloë bromicola*. For their establishment, I cloned grass genotypes by means of callus culture and artificially inoculated them with previously characterised *E. bromicola* genotypes.

Chapter 2 investigates the infection incidence and genetic diversity of two endo-phyte species, namely *E. bromicola* and *E. festucae* infecting *Bromus erectus* and fine fescue species (*Festuca ovina* and *F. rubra*), respectively. Isolates of each fungal species were collected from three natural populations and investigated with RAPD-PCR (random amplified polymorphic DNA-polymerase chain reaction) for molecular finger-printing. We found abundant genetic diversity in both fungal species, but their genetic population structures

differed widely depending on their modes of reproduction (*sexual* or *asexual*) and transmission (*horizontal* or *vertical*). The results may contribute to an understanding of the consequences of ongoing habitat fragmentation for plant/parasite interactions in general, since they suggest that host and parasite populations are unequally affected due to variation in propagule dispersal.

Chapter 3 compares two techniques for molecular fingerprinting, RAPD-PCR and MS-PCR (microsatellite-PCR), of endophytic fungi. These techniques were applied on a sample of fungal isolates from nine host species collected in a single natural popula-tion. The sample represented all endophyte species (e.g. mating populations) native to Eurasia, two of which were only recently described by Leuchtmann and Schardl (1998). The molecular techniques were evaluated for their potential to identifiy genetic diversi-ty within and genetic differentiation among endophyte mating-populations and host-based subpopulations, and the results were compared with published data from allozy-me analysis. Probably as the most important result, the genetic fingerprints of *E. bro-micola* isolates from different bromegrass species were almost identical, despite their basically different modes of reproduction and transmission.

Chapter 4 describes a system to axenically cultivate endophyte infected grass plants. Unlike in similar investigations with greenhouse derived plants, we were able to reproducibly identify physiological and biochemical alterations in the metabolism of infected hosts and to detect fungal metabolites *in planta* when using this system. Endo-phyte infection affected carbohydrate partitioning and activity of different enzymes, such as chitinase and trehalase. A PCR based quantification of endophyte infection revealed a tenfold mycelial concentration in axenically cultivated plants compared to plants that grew in the greenhouse. Axenic cultures of grass endophyte symbiota are probably useful for future investigations on *in planta* alkaloid and phytohormone production and on the characteristic host specificity of endophytic fungi. Data from experiments using pure fungal cultures and from experimental manipulations using callus tissue and/or cell cultures of different grasses would undoubtedly provide further insight on important aspects of this symbiosis.

Chapter 4 and *Chapter 5* describe the occurrence and distribution of multiple endophyte genotypes infecting individual grass plants. At time of onset of these experiments, “no more

than one *Epichloë* genotype has ever been isolated from an infected plant" (Schardl, 1996). However, mixed genotype inoculations yielded multiply infected host plants. Diagnostic PCR using fungal genotype specific molecular markers revealed that in case of double infections, individual tillers always harbored only one endophyte genotype. Thus, mutual exclusion at the tiller level seemed to characterise the distribution of endophyte genotypes in doubly infected grasses (*Chap-ter 5*). A comparison of the infection success of four fungal genotypes when inoculated alone or in mixture with each of the other isolates revealed complex genotypic effects and interactions on this important trait of the symbiosis (*Chapter 6*).

Chapter 7 describes an ongoing greenhouse experiment, which was designed to investigate plant- and fungal-genotype effects on the fitness of either symbiotic partner in a greenhouse experiment. The experimental setup consisted of five *B. erectus* geno-types that were either uninfected or reciprocally infected with each of four *E. bromicola* genotypes. The plants were cultivated in a single pot experiment in the greenhouse under low and high nutrient supply for one year until harvest. Prior to harvest, endo-phyte infection in each plant was tested and quantified by plating surface sterilized leaf segments on nutrient medium. Other traits of interest were infection persistence and plant and fungal growth. However, the data presented in this chapter are of preliminary character, since the harvested plant material awaits further analyses, such as the quanti-fication of endophyte infection by real-time PCR and of alkaloids (i.e. peramine) accu-mulation *in planta.*

Chapter 8 gives a general discussion of the data presented in this work. Here, my intention was to put my results in the framework of published data on grass/endophyte associations and to point out the significance of experiments using reciprocal plant- and fungal-genotype combinations. Finally, *Chapter 9* summarises the references of the literature cited in this thesis.