Chapter 1:

General introduction

1. Terminology

Despite many attempts to classify organismic associations (Law, 1985; Lewis, 1985; Starr, 1975), the terminology surrounding host-parasite interactions is not used consistently. To avoid potential confusion, I will first suggest some definitions con-cerning this terminology. I will use the term *symbiosis* in the original sense of de Bary (1879) to describe a physiological integration of two organisms, without giving any qualitative classification of the association. I prefer this definition since it rather underlines the dynamic than the static character of host/parasite interactions. Selective forces on either partner may shift the outcome of their association along a symbiotic continuum, in which mutual interactions are quantified as the potential fitness, or reproductive ability, of the symbiotic partners (Fig. 1; Starr, 1975).

Accordingly, the term *parasite* is used here in a nutritional sense describing a heterotrophic organism whose survival depends on a host from which it obtains either major or minor nutrients, without classifying its effect on host fitness. In contrast to its definition here, *parasite* is often used to describe in particular *antagonistic* symbionts, since many parasites are harmful to host fitness. Here, antagonistic parasites that cause severe host damage are referred to as *pathogens*, which often cause visible disease symptoms. Parasites are called *mutualistic*, if both partners benefit from the association. *Neutral* parasites have no obvious deleterious or beneficial effects on host fitness and *vice verse*. Neutralism corresponds closely with *commensalism*, which describes a situation in which only one partner profits from an association (Cooke, 1977). *Amensalism* describes an intermediate position between antagonism and neutralism, when the association brings costs to only one partner whereas the other is not affected.

The term *virulence* is used in several conceptually distinct ways. Some authors equate it with health or disease severity, others with host or parasite fitness, and others with the ability of a parasite to grow on host tissue. The latter definition is traditionally

	Antagonism	Amensalism	Neutralism	Commensalism	Mutualism
	←		|		→
A	–	–	0	0	+
B	–	0	0	+	+

Figure 1. Positions along the symbiotic continuum defined by the potential fitness of two associated species A and B. (–) Potential fitness decreased; (0) *dito* not affected; (+) *dito* increased.

used in plant systems, where *virulence* has an explicit definition due to its relation with a gene-for-gene interaction between host and parasite (Flor, 1956). After Flor, virulence alleles determine whether a pathogen can or cannot infect a plant. To avoid potential confusion, the term *aggressiveness* was suggested to describe the morbidity and morta-lity of host plants caused by parasite infection. However, according to its definition in animal systems, I will adopt the term *virulence* here to denote symptom severity in host plants. Thus, both *virulence* and *aggressiveness* will be used alternatively to describe the extent that pathogens reduce the fitness of their hosts.

Parasites may be classified according to their dependence on an association with a host: *obligate* symbionts have no or only very limited capacity for a free living existence under natural conditions, whereas *facultative* symbionts are well adapted for existence without hosts while possessing good capability for symbiosis. Host dependence often concerns a parasite's mode of *nutrition*, which may be classified to one of three modes: *saprotrophic* when deriving organic compounds directly from the non-living components of its immediate environment; *necrotrophic* when nutrition is provided by dead host cells, which the parasite has killed itself; *biotrophic* when deriving organic nutrients only from living host cells.

2. Host/parasite associations: coexistence or conflict?

Evolutionary thinking on host-parasite interactions is largely a study of conflicts, which can occur between species, between relatives, and between genes within indivi-duals. Thus,

evolutionary biology is as much the study of maladaptation as it is of adaptation, since participants in conflicts often come off worse than they would have without the conflict. Evolutionary biology is not just selectionist thinking about adaptations, although it is certainly that.

The theory dealing with the evolution in symbiotic environments is much better developed for animal than plant systems (Anderson and May, 1982; May and Anderson, 1983). However, although these models make several assumptions that do not apply for many plant parasites, most of their theoretical considerations can be adapted to plant/parasite associations. Evolution of both hosts and parasites is driven by selection. Any life-history trait of either partner only experiences selection pressure if variation in that trait is correlated with variation in reproductive success, and it only responds to selec-tion if some of that variation is heritable. These processes can lead to adaptation, which may be defined as a state that suggests its evolution because it improved survival or reproduction or both. Although adaptations can be incredibly precise, evolutionary conflicts that are not resolved may inhibit optimal adaptation. In antagonistic associa-tions, hosts often evolve mechanisms to reduce the damage inflicted by the parasite, whereas parasites evolve adaptations to extract resources from their host and to improve their transmission to new hosts. Reduced adaptation in both host and parasite results from costs of both resistance and virulence. However, conflicts between hosts and parasites can be reduced or resolved under certain circumstances, and their associations may shift along the symbiotic continuum towards mutualism.

2.1. The evolution and expression of parasite virulence

Three frameworks have been proposed to understand the evolution and expression of virulence of parasites. Briefly summarised, virulence may be explained as a coinci-dental by-product of unusual, often novel associations, as adaptation of the parasite genotype underlining its beneficial effects to the parasite, or as an ever-changing trait in an ongoing host/parasite arms race. Second, I will introduce models for ecological theories of mutualism. A third paragraph will concern the appropriate level on which symbiotic interactions should be investigated.

(a) Virulence as coincidental by-product. Conventional wisdom holds that successful parasites should evolve to become less virulent over time. This paradigm is based on the idea that parasites that do not harm their hosts have the best long-term chance of survival. Mathematically, a parasite's net reproduction rate can be expressed as $R_0 = \frac{bH}{a+m+b}$, where b is the transmission rate, H the number of hosts, a the rate of parasite-induced host mortality (a measure of virulence), m the rate of parasite mortality within the host and b the rate at which hosts die without parasitic influence. Because the net reproduction rate and the virulence of a parasite are inversely related, low levels of virulence should be selected for with increasing age of an association.

After the coincidental virulence hypothesis, only 'maladapted' parasites are harm-ful to their hosts (Alexander, 1981; Palmieri, 1982). Situations of this kind may arise if parasites accidentally colonise a 'wrong' host or a 'wrong' tissue. Predictions on the level of virulence in novel hosts are difficult to make, but a rough rule of thumb has been suggested: the average virulence in novel hosts decreases with the phylogenetic distance between the normal host and the new host (Ebert, 1994; Morand *et al*., 1996). However, cases of high virulence in novel host/parasite associations exist, for example Ebola virus or Duch elm disease.

(b) Virulence as adaptation of the parasite. The most commonly discussed hypo-theses about the evolution of virulence are based on the idea that virulence evolves because it benefits the parasite (Lipsitch and Moxon, 1997; Read, 1994). Beneficial effects to the parasite may either be direct or indirect. In these models, host evolution is assumed being slow, and the evolution of the disease phenotype being under parasite control.

As direct benefits, certain disease symptoms are thought being associated with increased parasite survival, reproduction or transmission. For example, parasitic castration of hosts may be adaptive since resources usually spent for reproduction become available for parasite growth and reproduction in the vegetative host tissue (Baudoin, 1975; Clay, 1991). Virulence can be indirectly beneficial to parasites although it bears costs by reducing parasite fitness. Indirect benefits may arise since functional constraints between fitness components of a parasite, e.g. survival, repro-duction, and transmission exist (Anderson and May, 1982). Disease expression can then be regarded as an unavoidable side effect correlated with fitness

components of a parasite. Given such trade-offs, evolution would select the parasite strain with the optimal combination of costs (virulence) and benefits (reproduction, transmission and survival) (Read, 1994).

Besides maximizing the number of offspring, the fitness of most parasites depends on their transmission from host to host (Ebert, 1994; Lipsitch and Moxon, 1997). Transmission can either be *horizontal*, when parasite offspring infects new host gene-rations contagiously, or *vertical*, when propagules directly infect the offspring of an infected host. W. Topley recognized as early as 1919 that natural selection for increased transmissibility might drive parasites towards increased virulence, as direct or indirect consequence of the parasite's host exploitation during propagule production. The connection between rapid replication, transmission and virulence is most obvious in situations of multiply infected hosts, where more competitive parasites have a selective advantage over strains with slower reproduction (Bremmerman, 1983). In such compe-titive situations, the fastest growing parasite will be the one with the most offspring, and natural selection can thereby favor parasite strains of intermediate, or even high, levels of virulence. In other words, parasites are thought to maximize the number of success-fully transmitted offspring by trading off propagule production against host survival, and therefore against their own survival (Bull, 1994; Bull *et al*., 1991; Herre, 1995).

(c) Virulence as result of reciprocal selection. If selection that maximizes the fitness of a parasite reduces the one of its host, the opposite direction of selection is expected to act from the host's point of view. With reciprocal selection of parasites and hosts, virulence is expected to be an ever changing trait, balanced by the antagonistic evolution of hosts and parasites (Ebert and Hamilton, 1996). Such a conflict could produce an escalating genetic 'arms race' between parasites and hosts (Bell, 1982; Holmes, 1983; Jaenike, 1978; Levin, 1975). Thus, an observed level of virulence in a population reflects not only the optimum for the finess of a parasite, but also the evolution of its host to minimize damage (Lipsitch *et al*., 1996).

Ebert and Hamilton (1996) proposed that virulence does not usually escalate in natural populations because genetic diversity among hosts prevents the parasite from evolving host-

genotype specific virulence. Given the high evolutionary rates of parasites due to their generally short generation times (Hafner *et al.*, 1994), sexual reproduction has been suggested as mechanism to improve a host's evolutionary response to parasite evolution. Sexual reproduction produces much more genetic variation in each generation than asexual reproduction does, where the influx of new variation is limited to the mutation rate. Although sexual reproduction has considerable costs, the ongoing creation of hosts with novel gene combinations may play an important role as a defense mechanism, since it provides a moving genetic target that inhibits parasites to evolve their optimal level of virulence (Hamilton, 1980; Jaenike, 1978; Ladle, 1992).

Parasites in a co-evolutionary arms race with their hosts are under strong selection pressure to infect the most common host genotypes. However, since they cannot instantaneously track genetic changes in the host population, such frequency dependent selection can lead to sustained oscillations in host and parasite gene frequencies (Fig. 2). Hence, rare host genotypes have a selective advantage, a situation that Haldane (1949) described as "just because of its rarity, it will be resistant to diseases which attack the majority of its fellows". After Bell (1982), the theory of constant cycling of gene frequencies in host/parasite systems is known as the 'Red Queen hypothesis' (RQH). Following the Red Queen's world, "it takes all the running you can do, to keep in the same place" (Carroll, 1871). Host-genotype specific expression of virulence, a necessary precondition for a RQH to be applicable, was demonstrated for several plant-parasite interactions. Both Flor's (1956) gene-for-gene (GFG) model and the 'matching allele' theory (Frank, 1994; Hamilton *et al.*, 1990) suggest a complementary relation-ship between host and parasite genes that determine disease outcome. However, although mathematical models on the basis of these mechanisms have been developed which show that frequency dependent selection can favor sexual reproduction in host populations (Hamilton, 1980; Hamilton *et al.*, 1990), a conclusive proof from empirical data cannot be reached at present (Clay and Kover, 1996b).

The co-evolution hypothesis allows several predictions for the evolution of viru-lence. First, virulence is expected to increase when parasites spend many generations on an individual host and adapt specifically with the defense of their host's genotype. Consequently, such adaptation to one host genotype may reduce a parasite's ability to exploit another (Ebert and

Hamilton, 1996). Thus, parasites in genetically diverse host populations have to adapt anew whenever infecting new host genotypes. Second, small and/or isolated host populations are probably more vulnerable to severe epidemics and extinction by pathogen infections. For a variety of organisms (Boyce, 1992; Fischer and Matthies, 1998; Menges, 1991), a positive relationship between the size and the average

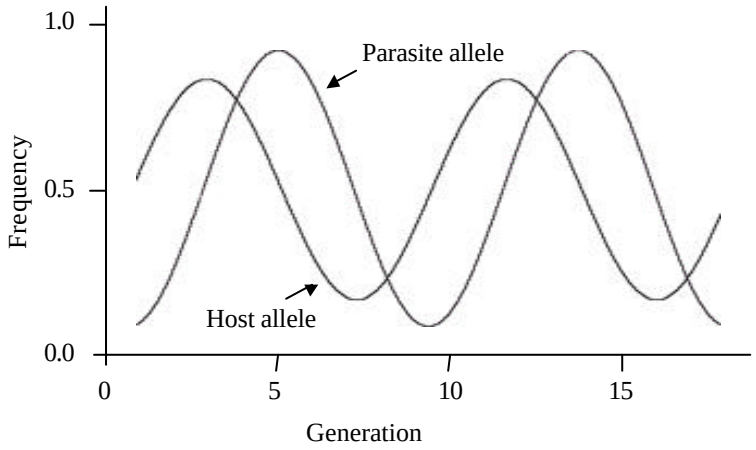

Figure 2. Changes in host and parasite allele frequencies through time-lagged, frequency dependent selection.

of fitness-related characters of populations has been documented (Allee-effect; Allee, 1949). Small host populations have smaller gene pools than large ones, and beneficial effects of genetic recombination through sexual reproduction may consequently be limited. Human activities often result in a loss of areas occupied by natural ecosystems or in a fragmentation of them, and genetic disruption of natural populations can determine the survival of many organisms (Vitousek, 1994). Moreover, hosts and parasites have generally unequal dipersal capacities, and vectors are often involved in parasite life cycles. Thus, host and parasite populations are differently affected by population fragmentation.

2.2. Theories of mutualism

Mutualistic symbioses are ubiquitous, often ecologically dominant, and profoundly influential at all levels of biological organisation (Boucher, 1985; Douglas, 1994; Maynard

Smith and Szathmáry, 1995). It appears that mutualistic associations are more common in tropical communities than elsewhere, probably reflecting their great species diversity and productivity. The selection pressures that drive the evolution in mutualistic associations may differ profoundly from those in antagonistic associations. In environments composed of mutualistic symbionts, frequency dependent selection is expected to favor host genotypes that profit most from these interactions (Law, 1985; Law and Lewis, 1983). Moreover, they are driving their biotic environment to improve more for the frequent than for the rare genotypes. To the degree that a mutualistic symbiont gets adapted to a frequent host, selection against sexual reproduction should increase, since asexual progeny of these genotypes will be more successful than sexual ones due to its genetic homogeneity with the parents. In other words, progeny of asexual mutualists will be more successful than sexual ones, since it is born into a world pre-selected for its mutualistic properities.

Four main models of mutualism have been suggested (Boucher *et al*., 1982). Following the *individual selection* model, mutualism is favored because the number of competitors that are benefited is less in a mutualistic association. *Population dynamics* models are based on phase-plane models after the Lodka-Volterra competition theory. If both species attain higher population densities than when alone and mutual benefits decrease as the populations grow larger, they predict stable equilibrium and persistence of mutualistic interactions. Models of *shift* take fluctuations of interactions along the symbiotic continuum into account (see Fig. 1). Finally, the *keystone mutualist* model assumes significant changes in the structure of a community following the removal of a particular symbiont.

2.3. The unit of selection

The appropriate level on which costs and benefits for symbiotic partners have to be investigated may vary among biological phenomens considered. For example, it was shown that individual *Drosophila* flies die if they are 'cured' of naturally existing, bacterial endosymbionts. However, there is no indication that these endosymbiotic bacteria affect the population dynamics in natural host populations (Ehrman, 1983). Other environmental factors, either biotic or abiotic, potentially represent much stronger selective pressures on the population level (Templeton and Johnston, 1982).

However, it is the level of the individual rather than the population, which is important in an evolutionary context, because individuals are the units on which selection acts. Thus, a primary goal of studies concerning host/parasite interactions should be the identification and quantification of costs and benefits to each individual partner in the symbiosis (Herre and West, 1997). Concerning their evolution, interspecific interactions have to be measured by intraspecific, individual-level costs and benefits. Nevertheless, it has to be considered that the significance of fitness differences among individuals lies in the fact that they influence population-level processes. Populations provide the genetic inventory for outcrossing individuals, and their size largely decides the outcome of meiotic recombination of individual genomes. Moreover, the genetic structure of a population may determine the pattern and probability of potential fertilizations.

3. Plant/fungal symbioses

Plants are autotrophic and therefore the primary producers in almost all terrestrial ecosystems. However, a minority of plant species lives without close conjunction with microorganisms, preliminary bacteria or fungi. Symbiotic associations with micro-organisms have been a dominant feature of plant evolution (Clay, 1994). Given the high diversity of plant parasites, I will emphasize here on interactions of plants with *fungi*. Counting just the lichen-forming and mycorrhizal fungi, at least 30% of all fungal species live in association with plants (Carroll, 1992).

Fungi influence all aspects of plant growth and development including germination, vegetative growth, reproductive output, competitive ability, susceptibility to herbivore or pathogen attack, and survival (Agrios, 1988; Burdon, 1987; Burdon, 1993; Jarosz and Davelos, 1995; Pirozynski, 1981). Infection outcome ranges from strongly antagonistic, through commensalistic, to mutualistic. Phytopathogenic fungi can be classsified according to their mode of infection (*local lesion* or *systemic*) and/or according to the outcome of the infection (host *debiliation, castration* or *death*). Such a classification can help to understand

why members of certain groups evolved towards highly aggressive pathogens, while those of other groups did not evolve or maintain high levels of virulence.

3.1. Classification of plant/parasite associations

Plant parasites can first be classified according to their mode of infection, either *local lesion* or *systemic*, and second according to the infection outcome by distin-guishing three categories, *debiliators*, *castrators* and *killers* (Burdon, 1993). Six main classes of plant parasites result (Table 1).

Infection outcome	**Mode of infection**	
	Local lesion	*Systemic*
Host debiliation	• Rusts (*Puccinia/Melampsora*) • Mildews (*Erysiphe*) • Leaf-spot diseases (*Albugo/Peronospora*)	• Viruses
Host castration	• Smuts (*Microbotryum*) • Leaf-spot diseases (*Phomopsis*) • Ergot (*Claviceps*)	• Smuts (*Microbotryum*) • Rusts (*Puccinia*) • Choke (*Epichloë*)
Host death	• Damping off and root rot diseases (*Pythium/Rhizoctonia*)	• Vascular wilts (*Ophiostoma; Cryphonectria*)

Table 1. Classification of plant parasites based on their infection mode and on the outcome of infection on host fitness. For each of six main categories, representative fungal genera that include phytopatho-genic species are given in parentheses. The exception is the class of systemic host debiliators, which includes mainly viruses.

The distinction following infection outcome mirrors to some extent the nutritional characteristics of a parasite. For fungi that kill their hosts, for example the highly aggressive fungi that cause damping off and root rot diseases, evolution towards reduced aggressiveness by constrains is limited due to their necrotrophic infection habit. Even the systemic pathogens in this group, the vascular wilts, do not seem to evolve towards reduced aggressiveness, potentially because less aggressive strains are not selectively favored since hosts are often multiply infected. Direct disruption of the host's reproductive system by castration may be of little damage in environments where reproduction by seed set is neglectible for host fitness

(Eriksson, 1989; Clay, 1991; Clay and Kover, 1996a). However, obligate castrators kill their hosts from an evolutionary point of view, since sterilised plants do not contribute to the gene pools of subsequent generations. *Debiliators* are fungi that cause discrete lesions that individually have little effect on host fitness. Their incidence is usually very variable across space and time by exhibiting annual epidemics, which frequently do not eventuate (Jarosz and Davelos, 1995).

Following the classification of plant parasites according to their infection mode, *local lesion* or *systemic* parasites can be distinguished, each with several characteristic life history traits (Table 2; Clay and Kover, 1996a). Probably the most significant consequence of the systemic infection mode is the fact that systemic parasites often monopolise their hosts, whereas local-lesion infections generally consist of multiple pathogen genotypes on individual hosts. Probably as a result of host monopolisation and the absence of within-host competition among parasite genotypes, systemic parasites have usually much lower replication rates than lesion-forming ones (van der Plank, 1959) and are often capable to control and/or manipulate the growth and reproduction of their hosts. Many systemic parasites reproduce in the flowers of their hosts, and are thereby dependent upon hosts that reach reproductive maturity (Baudoin, 1975). Consequently, they are often long-lived, since they have to exceed the life span of their hosts for successful reproduction and transmission to new hosts. Systemic fungi sometimes induce precocious host flowering or increase flower numbers.

Feature	**Systemic**	**Non-Systemic**
Location of infection	· throughout plant	· highly localized
Number of genotypes infecting host	· one or a few	· many
Occurrence of latent infections	· common	· uncommon
Parasite generation time	· long	· short
Duration of infections	· perennial	· annual
Propagation within host	· by hyphal growth	· by spores
Effect on host survival	· minimal	· detrimental
Location of parasite fruiting	· specific locations	· site of vegetative growth
Effect on host development	· induced changes	· no changes
Frequency of seed transmission	· often common	· rare
Frequency of contagious spread	· low	· high
Frequency of epidemics	· rare	· common

Table 2. Compilation of life history traits characteristic for systemic and non-systemic fungal parasites of plants.

To summarise, a systemic infection habit seems to be a precondition for reduced parasite aggressiveness, but does not necessarily lead to this. Examples of systemic but nevertheless highly aggressive plant parasites are the vascular wilts of trees (Jarosz and Davelos, 1995). In plant pathology, the strong influence of environmental variables on pathogen development and virulence reflects a well known tenet (Agrios, 1988). In addition, the virulence of plant pathogens has to be determined by their effects on five plant traits: survivorship, reproduction, competitive ability, growth and susceptibility to other pathogens or herbivores (Jarosz and Davelos, 1995). Unlike its effect in animals, the damage of parasitic castration can strongly fluctuate with environmental hetero-geneity, depending on the relative importance of sexual (seed set) versus asexual (clonal growth) reproduction (Eriksson, 1989; Schmid, 1990).

3.2. Classification of mutualism

Janzen (1985) distinguished four functional main categories of mutualism in terrestrial ecosystems: *seed dispersal, pollination, resource harvest*, and *protection*. *Seed dispersal* and *pollination* are mediated by larger animals and do not apply to fungi. They represent examples of *diffuse* mutualism in which the symbiotic partners are not physically connected. *Resource harvest* and *protection* represent the major mechanisms of plant-fungal associations. In addition, other classes exist among fungal mutualists, including *habitat conditioning*, and *competitive* as well as *physiological enhancement* of host plants (Carroll, 1992).

(a) Harvest mutualism. Harvest mutualism always entails the exchange of goods. It is the rule for heterotrophic fungi, which usually receive fixed carbon as well as nitrogen as a major benefit from their symbiotic partners. In exchange to nutrition by their hosts, fungi typically operate by wrestling and concentrating scarce substances, by liberating insoluble nutrients, or by synthesizing digestive enzymes and/or necessary low molecu-lar weight metabolites. Examples are mycorrhizal fungi and several fungal mutualists of insects.

(b) Protection mutualism. Besides resource harvest, protection from assorted hazards constitutes the second important mode of fungal mutualism. The majority of protective mutualisms have arisen in response to biological stresses, e.g. grazing, predation, parasitism, and toxins in potential food plants. Fungal protection of host plants can be mechanical or chemical. *Mechanically*, dense fungal sheaths of ecto-mycorrhizal fungi can serve as barrier to infection by feeder-root pathogens (Marx, 1970; Perrin and Garbaye, 1983). *Chemically*, a broad range of fungal symbionts protects their hosts by producing secondary metabolites, many of which are toxins and antibiotics. Chemical protection is known in lichens (Lawrey, 1984), mycorrhizas (Marx, 1969), endophytic fungi of trees (Webber, 1981), and clavicipitaceous endo-phytes of grasses.

(c) Mutualism based on physiological enhancement. Fungal mutualisms often lead to enhanced drought tolerance and/or to enhanced vigor and overall performance. Although poorly understood, these effects may relate to alterations of the overall physiological balance of hosts, perhaps through manipulation of plant hormone levels. Effects of this kind have been suggested for VA-mycorrhizal fungi (Allen *et al*., 1980; Allen *et al*., 1982) and for non-mycorrhizal fungi that associate with roots (Brown and Surgeoner, 1993; Sivasithamparam and Parker, 1980), as well as for clavicipitaceous endophytes of grasses.

(d) Combative mutualism. Plants must deal with intra- and/or interspecific compe-tition at some stage of their lives (Harper, 1977). In competitive situations, combatively mutualistic fungi may alleviate competition of host plants by their antagonistic effects on the plant that competes with the host. Examples are weeds in agronomic fields that can harbor latent endophytes such as *Collelotrichum* and *Fusarium*, which are patho-genic to crop plants (Hepperly *et al*., 1985; Raid and Pennypacker, 1987).

(e) Mutualism based on habitat conditioning. Ceratocystis ulmi, causative agent of Dutch elm disease, lives as ectosymbiotic fungus on elm bark beetles, which inoculate trees through feeding. The association is considered mutualistic since wilting caused by the fungus renders the tree more susceptible for further beetle attack and more favorable for growth of the insect larvae (Webber and Brasier, 1984).

It has to be pointed out that a single plant-fungal association may fall in several of the classes distinguished here. It is also important to define the kind of mutualism for each partner individually, since each member in a mutualistic association contributes benefits different from those received.

3.3. Important fungal mutualisms

(a) Mycorrhizas. Mycorrhizal fungi infect the roots of more than 80% of all vascu-lar plant species. They probably represent the most widespread type of plant-fungal interaction. Seven types of mycorrhizas are recognized to date, of which vesicular-arbuscular mycorrhizas (VAM) and the ectomycorrhizas are ecologically most relevant. To date, relatively few (~80) species of VAM fungi with broad host ranges are recogni-sed, which belong to the zygomycetes (family Endogonaceae). VAM fungi have very limited capacity to grow outside host tissue. Hyphae from spores that germinate in the soil infect young root tissue and grow between the cortical cells. Subsequently, they penetrate the cortical cells and form shrublike structures called *arbuscules*, which serve for nutrient exchange with host cells. *Vesicles* are hyphal swellings that are produced between and within cortical cells for lipid storage. In contrast, ectomycorrhizas form a sheath or mantle around the roots of trees and shrubs. Out of this layer, hyphae grow outwardly into the soil and inwardly into the root cortex to form a network between the cells called the *Hartig net*. Usually, root hair development is inhibited and infections cause structural changes of the roots. An ectomycorrhizal fungus may associate with different trees, and conversely, one tree can associate with different fungi.

The outcome of mycorrhizal colonization of plant roots is manyfold. Most important is the improvement of the root function for the release and uptake of nutrients from the soil, in particular of phosphorous (Smith and Read, 1997). Moreover, enhanced water uptake is a widely reported effect, which increases drought tolerance of host plants (Allen, 1991; Smith and Smith, 1996; Smith and Read, 1997). Besides exchange of nutrients, mycorrhizal infections concern other aspects of plant/fungal mutualisms as well. There is evidence that mycorrhizal infections provide both mechanical (Marx, 1970; Perrin and Garbaye, 1983) and chemical (Marx, 1969; Trofast and Wickberg, 1977) protection against root pathogens. Given

the broad spectrum of advantages con-ferred to hosts infected with mutualistic fungi, why are not all plants in a community infected? The reason attests to the costs associated with the maintenance and growth of the fungi. For mycorrhizal fungi, examples where VAM infection reduced plant growth most likely reflect these costs (Bethlenfalvay *et al*., 1982; Buwalda and Goh, 1982). In both VAM and ectomycorrhizas, the development of an association with roots is inhibited, and established associations can revert to their nonsymbiotic condition, if abundant nutrients are present in the soil (Smith, 1980). Based on evidence that host plants are capable to actively regulate mycorrhizal infections to levels that optimize their fitness (Smith, 1987), mycorrhizal fungi have been considered being 'controlled pathogens' (Melin, 1962).

(b) Lichens. In lichen symbioses, the fungus (*mycobiont*) usually comprises most of the lichen thallus, while the alga or cyanobacteria (*phytobiont*) provide photosyntha-tes. The lichen thallus is the result of a morphological transformation of the fungal symbiont and to a lesser degree of the photobiont. Lichen symbioses have been very successful through their capacity to survive extreme cold, heat and drought stress (Hale, 1983). Main types of thalli are crustose, foliose and fruticose. The proportion of each symbiont in a thallus is highly variable. Interestingly, as few as ~200 species of photo-bionts attribute to 15'000 lichen species, whereas many species of fungi are involved, most of which belong to the Ascomycota. Most lichens reproduce asexually and sexually. Asexual propagules typically incorporate both algal and fungal cells, which are released in discrete units of varying size. Sexually reproducing, ascomycetous lichen fungi typically produce numerous ascocarps and release ascospores that have to reestablish an association with a photosynthetically active partner (Ahmadjan, 1982).

Physiologically, the photobiont excretes most of its photosynthetic products and, in the case of cyanobacteria, much of the nitrogen they fix, whereas respiration is mostly that of the mycobiont. Mycobionts are obliged to secure adequate illumination, to compete for space, and to facilitate gas exchange of photobionts. Similar to the situation in mycorrhizal fungi, the relationship between fungus and alga in a lichen symbiosis is thought to be that of controlled parasitism. The fungus parasitizes the alga, but under natural conditions, parasitism is slow and infected algal cells may live for years. Like in mycorrhizal associations, abundance of external

nutrients inhibits the symbiotic transformation of the fungus and causes a breakdown of established associations.

3.4. The impact of parasites in natural plant populations

Given the high diversity of plant parasites, general predictions for host and parasite fitness on the population and community level are difficult to make. The classification suggested above already simplifies the fact that the population dynamics and genetics of each parasite is individually unique. Hence, parasite populations are likely to be variable with respect to their virulence and their response to selective pressures exerted by their hosts. Studies on the genetic diversity of either symbiotic partner in natural populations are needed to test the hypothesis that genetically diverse rather than genetically uniform host populations are more likely to selectively favor a range of virulent pathogen genotypes. On the other hand, genetically poor populations such as agronomic cultures are more prone for severe epidemics if compatible, highly aggressive parasites once invade a population. Factors that determine the extent to which such epidemics occur include (i) the size and genetic structure of host populations (which often represent habitat remnants), (ii) the dispersal powers of the parasite within and between patches, and (iii) the average number of parasite generations that individual host populations last.

Fungal mutualisms involve direct pairwise interactions between individual partners as well as interactions between populations of the partners. For example, mycorrhizal fungi can interconnect the roots of a whole population of conspecific plants. Moreover, fungal mutualists in both mycorrhizas and lichen symbioses have limited host specifity or almost completely lack it, which allow the occurrence of third-party or more complex interactions. Communities can vary in the species composition of mycorrhizal fungi (Johnson *et al.*, 1992), which induce different growth responses in infected host species. It has recently been demonstrated that that both species composition and richness of mycorrhizal fungi can affect plant species composition, variability and productivity on the community level (van der Heijden *et al.*, 1998). Computer models suggest that community-level effects might explain the stability of mutualistic associations, rather than inherent constraints on their growth (Dodds, 1988).

4. Clavicipitaceous endophytes of grasses

Clavicipitaceous fungi (Hypocreales, Ascomycota) are parasites of grasses, insects, and other fungi (Jones and Clay, 1987). Within the Clavicipitaceae, species of the genera *Atkinsonella*, *Balansia*, *Balansiopsis*, *Epichloë* and *Myriogenospora* (tribe Balansieae) infect many grasses and sedges. Clavicipitaceous endophytes colonise the intercellular spaces of their hosts, and the systemic infections often persist over the lifetime of their hosts. The infections rarely have negative effects on vegetative host tissues (Christensen *et al*., 1997). However, as a typical feature of clavicipitaceous plant parasites, they express disease symptoms in the reproductive organs of their hosts (Sampson, 1933; Kohlmeyer, 1974; Leuchtmann, 1992). Also characteristic is the production of toxic alkaloids with anti-insecticidial and anti-mammalian activity. Indeed, the interest in fungal endophytes grew rapidly as soon as they were recognized as causative agents for toxicosis suffered by livestock grazing infected grass (Bacon, 1977).

In this work the term *"endophyte"* refers to fungi of genus *Epichloë* and their *Neotyphodium* anamorphs. To date, nine species (mating populations) were described within this genus (Table 3), based on experimental matings, cultural characteristics and ascospore morphology (Leuchtmann and Schardl, 1998; Schardl and Leuchtmann, 1999). Other proposed species are mostly associated with tropical grasses, and have therefore been incorporated into genus *Parepichloë* (White, 1998).

Two major life-history transitions within the Clavicipitaceae were of crucial im-portance for the evolution of mutualistic *Neotyphodium* endophytes. These transitions affected on one hand the mode of infection, and on the other in close relatonship the mode of reproduction and transmission.

4.1. Localized *versus* systemic infection

Claviceps species cause ergot disease by infecting single ovules of grass inflores-cences (Fig. 3). Ovule infection results in the formation of a sclerotium instead of a seed. Sclerotia overwinter on the ground and produce infective ascospores the following

Mating Population	Fungal species	Host species	Continent	Trans-mission[a]
MP-I	*E. typhina*[b]	*Anthoxanthum odoratum*	Europe	–
MP-I	*E. typhina*	*Dactylis glomerata*	Europe	–
MP-I	*E. clarkii*[b]	*Holcus lanatus*	Europe	–
MP-I	*E. typhina*	*Phleum pratense*	Europe/Asia	–
MP-I	*E. typhina*	*Poa spp.*	Europe	–
MP-I	*E. typhina*	*Lolium perenne*	Europe	–
Anamorph	*N. lolii*[c]	*L. perenne*	Europe	|
Hybrid anamorph	LpTG-2	*L. perenne*	Europe	|
MP-II	*E. festucae*	*Festuca spp.*	Europe	– |
MP-III	*E. elymi*	*Elymus spp.*	N. America	– |
MP-IV	*E. amarillans*	*Agrostis hiemalis*	N. America	– |
MP-IV	*E. amarillans*	*Sphenopholis obtusata*	N. America	– |
MP-V	*E. baconii*	*Agrostis spp.*	Europe	–
MP-V	*E. baconii*	*Calamagrostis villosa*	Europe	–
MP-VI	*E. bromicola*	*Bromus spp.*	Europe	– |
MP-VII	*E. sylvatica*	*Brachypodium sylvaticum*	Europe/Asia	– |
MP-VIII	*E. glyceriae*	*Glyceria striata*	N. America	–
MP-IX	*E. brachyelytri*	*Brachyelytrum erectum*	N. America	– |
Anamorph	*N. uncinatum*	*Festuca pratensis*	Europe	|
Hybrid anamorph	*N. coenophialum*[d]	*Festuca arundinacea*	Europe	|
Hybrid anamorph	FaTG-2	*F. arundinacea*	Europe	|
Hybrid anamorph	FaTG-3	*F. arundinacea*	Europe	|

[a] – = horizontal, | = vertical
[b] Morphospecies *E. typhina* and *E. clarkii* are grouped as MP-I since they are interfertile in mating tests
[c] LpTG-1
[d] FaTG-1

Table 3. Described species (mating populations) within genus *Epichloë* sensu stricto including their *Neotypho-dium* anamorphs. Abbreviations: MP: mating population; Lp: *Lolium perenne*; Fa: *Festuca arundinacea*; TG: taxonomic grouping.

spring (Hoffmann and Schmutterer, 1983). In contrast, infections with endophytic species are systemic in above ground host tissue. Endophyte infected plants always originate from seeds. They are infected either through ascospore mediated spread or by invasion of seeds with the fungus that already colonized the mother plant. The success of contagious spread of both ergot and endophytic fungi is strongly influenced by tempo-ral coordination of ascospore release and the susceptible stage of ovaries. In ergot, this coordination is favored in cold and wet springs,

and environmental fluctuations cause highly variable disease incidence (Eleuterius, 1974). Epidemic outbreaks of ergot occurred in the middle age, which led to severe toxicosis in humans and animals (Schumann, 1991). Contagious spread of sexual endophytes is not epidemic. The persistence of their infections makes their incidence less prone to environmental fluctuation in natural populations. Together with the systemic infection mode, the loss of a life stage outside host tissue acted to selectively favor endophyte strains that caused minimal damage to the vegetative performance of hosts.

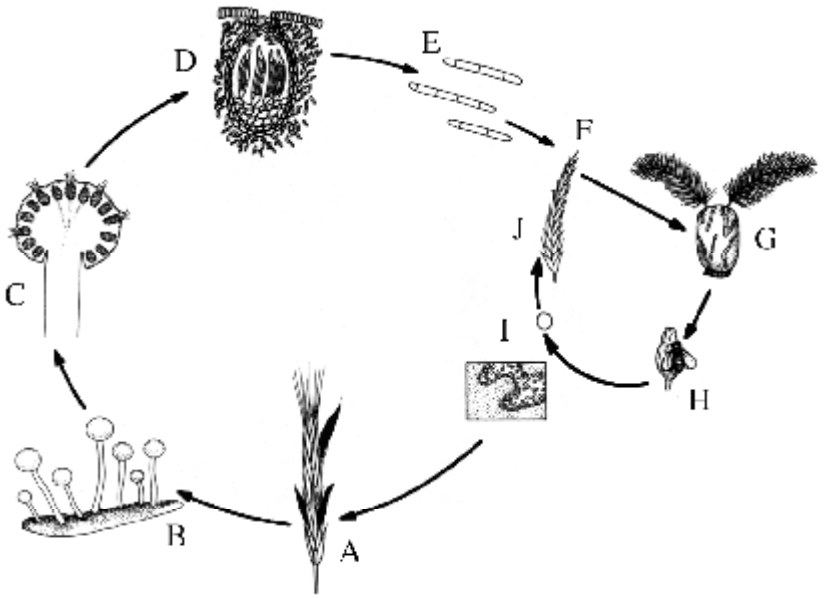

Figure 3. Life cycle of ergot (after Schumann, 1991). *Claviceps* species form sclerotia on inflorescenses of a variety of host grasses (A), which overwinter on the ground and germinate the following spring (B-D). Ascospores (E) are ejected from mature perithecia (D), which can infect ovules through stigma of flowering hosts (G). Flies are attracted through the production of 'honeydew', a fungal compound containg conidia (H-I), which may cause secondary infections by translocation through the vector (J). During seed ripening, infected ovules develop to sclerotia instead of seed.

The systemic infection mode of clavicipitaceous endophytes probably enabled them to monopolise an individual host plant (Jarosz and Davelos, 1995). In fact, most infec-ted grasses harbor only a single endophyte genotype (Bultman and White, 1988; Groppe *et al*., 1995; Leuchtmann and Clay, 1989). However, considerable abundance of multip-ly infecting *E. sylvatica* genotypes on individual *Brachypodium sylvaticum* hosts in natural populations

was recently reported (Mejier and Leuchtmann, 1999). Multiple infections with non-systemic ergot fungi may occur, but ovule infections spatially separate the fungal genotypes involved and prevent colonization of the same host tissue, and thereby within host competition among fungal genotypes.

4.2. Horizontal *versus* vertical transmission

The second important transition in the evolution of mutualism in grass/endophyte symbioses affected the mode of fungal reproduction and transmission and took place within the genus *Epichloë* (Fig. 4). Sexually reproducing *Epichloë* species form an ectophytic fruiting structure, a so-called stroma (pl. stromata), which develops around

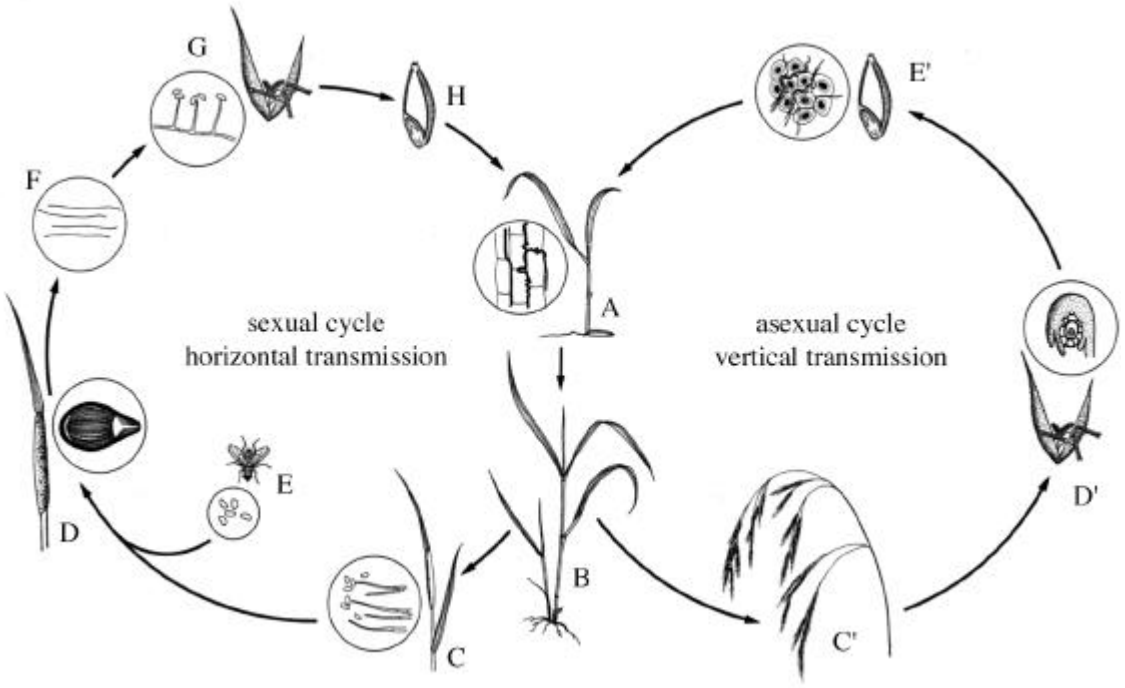

Figure 4. Sexual and asexual life-cycle of clavicipitaceous endophytes (after Leuchtmann and Schardl, 1998). Fungal endophytes systemically colonize above ground host tissue with convoluted hyphae growing in the intercellular spaces (A). In the *sexual* cycle, hyphae grow into the elongating culm (B) and proliferate mycelium for stroma formation (C), which surrounds the immature inflorescence and finally arrests inflorescence maturation. Fertilization occurs by transfer of spermatia (conidia) of opposite mating type by a fly vector (E). If the parents are conspecific (same mating population, see Table 3), perithecia containing asci develop (D) and filamentous ascospores are ejected (F). Germinating ascospores on host florets cause new infections through developing seeds (G). In the *asexual* cycle, hyphae invade the floral

meristems (C'-D') and the ovules of the florets (E'), such that the fungus is transmitted in the next generation of seeds.

the emerging inflorescence and thereby suppresses host flowering and seed set (choke disease). The mating system is heterothallic. Thus, conidia of opposite mating type are required for successful fertilization of a stroma (White and Bultmann, 1987). Flies of genus *Botanophila* (Bultman, 1995; Bultmann *et al*., 1995), which parasitise on stromal tissue and translocate conidia in order to fertilize stromata. Sexual ascospores are actively ejected from mature stromata, which by chance land on immature inflorescen-ces of susceptible hosts. Viable ascospores germinate and invade ovules on young inflorescenses through stigmata. Iterative conidiation steps after ascospore germination probably elongate the infective time span and multiply the amont of fungal inoculum (Bacon and Hinton, 1991). Nevertheless, experimental investigations suggested low efficiency of horizontal transmission via ascospores (Chung and Schardl, 1997). This is in agreement with the fact that epidemic spread of sexual endophytes have never been observed (Leuchtmann and Clay, 1997). Horizontal transmission may also cause infection of vegetative grass tissue, which was recently described for *E. sylvatica* infecting *B. sylvaticum* (Brem and Leuchtmann, 1999).

Neotyphodium species seem to lack any sexual stage and vetically invade the seeds of infected hosts. Since they do not cause any disease symptoms, these associations are considered entirely mutualistic. Asexual *Neotyphodium* endophytes lack any possibility to contagiously infect new hosts. Thus, they necessarily have to provide benefits to their hosts, since infected hosts would alternatively be outcompeted by uninfected conspeci-fics in natural populations. How benefits are mediated will be the subject of the next section. Asexual endophytes probably evolved polyphyletically from sexual species (White, 1988; Schardl *et al.*, 1994). Several findings suggest the importance of para-sexual mechanisms for their evolution, such as interspecific hybridizations of fungal genomes (Schardl *et al*., 1994; Tsai *et al*., 1994). A well documented example is *N. coenophialum* infecting tall fescue, which possesses three copies of the b-tubulin gene, each ultimatively derived from a different sexual *Epichloë* species. Close contact of fungal hyphae is required for anastomosis and karyogamy,

and hybridizations therefore suppose close contact of different endopyhte genotypes within a single host.

Probably the closest adapted grass/endophyte associations represent those where both the host and the fungus has the capability to reproduce sexually through a mixed life history strategy of the endophyte. In these pleiotropic associations, horizontal and vertical transmission can occur on an individual host plant, whereas seed transmission dominates in the majority of the infected plants. A continued balance between host and fungal sexual cycles, which probably best assures net mutual benefit in the long term, has been found only in the subfamily Pooideae (Catalán *et al*., 1997), and here within grass genera *Brachyelytrum*, *Brachypodium*, *Elymus* and *Festuca* (Schardl, 1996).

What are the consequences of the different transmission modes? Most intriguing is the inverse relation of host and fungal reproduction through meiotic recombination. It has been speculated that the suppression of a host's sexual reproduction by parasites may act as mechanism to prevent the generation of resistant host progeny (Clay, 1991; Clay and Kover, 1996a). This idea is supported by the fact that in *Danthonia* hosts infected with *Atkinsonella hypoxylon*, only outcrossing, chasmogamous flowers are aborted upon infection, whereas selfed, cleistogamous flowers develop to viable seeds, which the fungus invades for vertical transmission (Clay, 1984; Clay and Jones, 1984). However, empirical genetic data that suggest frequency dependent selection do not exist, and the adaptive significance of host castration through fungal endophytes remains speculative.

Following the RQH, selection that favors hosts resistance requires that parasites significantly reduce the fitness of their hosts. However, sexual *Epichloë* endophytes share many beneficial properties of asexual *Neotyphodium* species, for example the production of protective alkaloids (Bacon, 1986; Cheplick, 1988; Clay, 1989). Why should infected hosts that enjoy protective effects evolve resistance to endophytes, irrespective whether these reproduce sexually or asexually? Fungal reproduction and transmission are closely related, and selection pressures on each trait are difficult to separate. On one hand, abandonment of sexual reproduction may be selectively favored since genetically recombined parasite offspring may be less adapted to an increasingly favorable environment within host tissue (Law, 1985; Law

and Lewis, 1983). On the other hand, the transition may also be driven by selection for increased efficiency of endophyte transmission. The generally higher incidences of asexual endophytes compared to those of sexual ones in natural populations support such argumentation.

Descriptive studies concerning the genetic diversity in natural endophyte popula-tions address the effects of different modes of fungal reproduction. Those concerning endophyte incidence and genetic population differentiation more likely address the effects of different transmission modes. When interpreting genetic data from endophyte populations, it has to be considered that the way how host and fungal life cycles are linked is either complete for asexual or opposite for sexual endophytes. Parallel analyses of both the genetic diversity and the differentiation among in host and endophyte populations could also provide insight on the adaptive significance of sexual reproduc-tion and host castration. If such studies are conducted only for the fugal partner, they provide data on the effects of different transmission modes on gene flow among isolated endophyte populations.

The genetic structures of endophyte populations are likely to reflect not only life history variation among fungal species, but selection on host fitness as well (Bucheli and Leuchtmann, 1996). In particular, this may be true for asexual endophytes where complete linkage of fungal and host reproduction allows an estimation of seed dispersal rates by analysing the distribution patterns of the associated endophyte genotypes. Indeed, the genomes of asexual endophytes are inherited like mitochondrial and chloro-plast genes in the maternal host lineage (Schardl and Phillips, 1997).

4.3. Costs and benefits of endophyte infections

Current knowledge suggests that the cost of bearing endophyte infection is low. The abundance of mycelium in the intercellular spaces in above ground host tissue is generally low (White, 1987b). Variation among different tissues may exist, and it was suggested that concentrations are highest in meristematic tissues and in leaf sheaths (Hinton, 1985; Siegel, 1987; Fineran, 1983). Such variation probably results from the availability of extracellular sucrose and other sugars (Farrar, 1987). However, one study reported lower endophyte

abundance in leaf sheaths compared to leaf blades (Groppe and Boller, 1997). Indirect evidence for low nutritional costs comes from experiments where effects on host growth were compared under different nutrient supply (Cheplick *et al*., 1989). Growth reduction upon endophyte infection was identified only if nutrient availability was low, whereas under intermediate and high nutrient supply, endophyte infection improved plant growth. This suggests that costs from supplying nutrients to the fungus are significant if hosts suffer severe nutrient limitation. Direct assessments of the costs of endophyte infection *in planta* are rare. Few reports describe the specific expression of enzymes upon endophyte infection, in one case involving a protease (Lindstrom and Belanger, 1994; Lindstrom *et al*., 1993) and in the other an invertase (Lam *et al*., 1995). Activities of both enzymes were strongest induced when endophytes grew on nutrient poor media or in host tissue, suggesting their involvement in nutrient uptake. Availability of nutrients may correlate with the growth rate of an endophyte. *In vitro* growth studies showed inter- and intraspecific variation and its dependence on the kind of carbon that was supplied (Christensen and Latch, 1991; Christensen *et al*., 1991; White, 1987a; White *et al*., 1991). Asexual *Neotyphodium* isolates generally grow slower than sexual *Epichloë* species that form stromata. In order to successfully sterilize a host, the fungus has to grow rapidly enough to trap the expanding inflorescence pri-mordium (White *et al*., 1993; White and Morrow, 1991).

Endophytes seem to be almost invisible to their hosts, since no defense reactions were described in natural grass/endophyte associations. However, seedling inoculations with endophytes from distinct host species revealed evidence for mechanisms that determine resistance due to host specificity, which however are not clearly understood (Christensen, 1995; Koga *et al*., 1993a). Inoculations of *D. glomerata* seedlings with the progeny from crossings of its native endophyte with a non-compatible one yielded limited infection success suggesting that several genes determine grass/endophyte compatibility (Chung *et al*., 1997).

Beneficial effects of endophyte infections cam be categorised as *intrinsic* or *extrin-sic* (Clay, 1993). Intrinsic mechanisms include direct alterations of the biochemistry, physiology and/or morphology of infected hosts, most of which are poorly understood. Evidence exists for infection induced changes of the phytohormone balance of host plants (Porter *et al*.,

1985). Evidence for genotypic variability here arises from *in vitro* studies, which showed that endophytes produce the major active auxin, *b*-indole acedic acid (IAA), in a strain dependent manner. Published data report higher growth rates and biomass production (Clay, 1987; Marks and Clay, 1990), higher photosynthetic rates (Belesky *et al*., 1987) and improved drought resistance (Arachevaleta and Radcliffe, 1989; Elmi and West, 1995, Hill, 1990) of infected hosts. Again, nutrient availability might determine a host's physiological response to endophyte infection. For example, enhanced host performance was observed at high nutrient levels, whereas endophyte infection was neutral or even negative at low nutrient levels (Cheplick *et al*., 1989; Marks and Clay, 1990).

Extrinsic effects resulting from endophyte infection are those that affect the inter-actions of infected hosts with other species in their environment. They are largely of protective nature, which applies to attack by herbivores, pathogens and competitors (Bacon, 1986; Siegel, 1987). The best understood and probably the most important mechanism is mediated by the production of toxic alkaloids that accumulate within host tissue. Four alkaloid classes have so far associated with endophyte infections: *ergot alkaloids* (lysergic acid and ergovaline), *indolediterpenes* (e.g. paxilline and lolitrem B), *pyrrolopyrazines* (peramine) and the *saturated aminopyrrolizidines*, which are collectively called 'lolines'.

All alkaloid classes have activity against insects (Porter, 1994), whereas activity against vertebrates was reported for all but peramine (Raisbeck, 1991; Dew, 1990). Thus, endophyte infections have both beneficial and detrimental agronomical aspects, depending on the alkaloid classes produced *in planta.* Anti-mammalian activities are associated with livestock maladies known as 'ryegrass staggers', most commonly ob-served in sheep grazing on infected perennial ryegrass, or as 'fescue toxicosis', which collectively describes a variety of disease symptoms in cattle that graze *N. coeno-phialum* infected tall fescue. Due to the protective character of alkaloid production, the outcome of endophyte infections is largely determined by fluctuations of herbivore pressure in natural plant communities.

4.4. Symbiotic traits affected by genotypic variation of hosts and endophytes

The main purpose in this work was to investigate effects of both the host and the fungal genotype on the outcome of grass/endophyte associations. I used reciprocal associations of chosen host and fungal genotypes in most of my experiments. For their establishment, genotypes of *Bromus erectus* Huds. were cloned by means of callus cultures. As soon as regenerants were obtained from tissue cultures, they were inocu-lated with previously chosen *E. bromicola* genotypes. Once established, a variety of experiments could be conducted using these symbiota. The relevance of experimental investigations on the genotype level is manyfold.

(a) Using allozyme analysis, several studies showed that abundant genetic diversity within natural endophyte populations exists, even within those of asexually reproducing endophyte species (Bucheli and Leuchtmann, 1996; Leuchtmann, 1994; Leuchtmann and Clay, 1989b; Leuchtmann and Clay, 1990; see also Chapter 2). Is not clearly under-stood how this variation is generated and maintained in asexual endophytes. Using PCR based tools, we could confirm the abundance of genetic diversity and found different degrees of genetic differentiation among natural endophyte populations, which probably reflected the different transmission and dispersal modes sexual and asexual endophytes, and their close relation to host reproduction (see Chapter 2).

(b) Variation in probably one of the most important aspects of endophytic fungi exists, namely the classes and amounts of fungal alkaloids produced *in planta.* Thus, the protective effects of endophyte infections are likely to vary due to genotypic variation of host and fungal species. Substantial variation among *Neotyphodium* isolates for anti-biosis against different test fungi was described based on *in vitro* studies. Considerable variation in alkaloid profiles was also identified in native populations of tall fescue and perennial ryegrass (Agee and Hill, 1994; Christensen *et al*., 1993) and in tall fescue seeds (Welty *et al*., 1994), while this variation seemed to correlate with the host rather than the fungal genotype. Peramine is the only alkaloid that has been identified in infected *B. erectus* grass on which most of the experiments presented here were conducted (Leuchtmann *et al*., 2000). Production of peramine seems to be under control of a single locus (Schardl and Phillips, 1997), what makes it an interesting candidate for studies concerning effects of genotype interactions of host and fungus. The question whether alkaloid concentration and fungal biomass correlates *in planta* remains unclear. Such a correlation was identified for the concentration of both peramine and

lolitrem B in perennial ryegrass (Ball *et al*., 1995). In contrast, another study showed that peramine concentrations did not correlate with those of ergovaline in tall fescue (Agee and Hill, 1994), which suggested that no general correlation exists between fungal biomass and alkaloid concentrations. Since mutualism in this symbiosis is to considerable extent of protective nature, strong selection pressures are to be expected if variation in alkaloid production is heritable.

(c) The characteristic host specificity of endophytic fungi suggests that host grasses have evolved mechanisms to recognize an invading fungus. However, such mechanisms have not been identified so far, but evidence for their existence comes from cross inoculations among host species, which may lead to successful colonization of the 'non-native' fungi, although they are in most cases not persistent (Christensen, 1995; Koga *et al*., 1993b). Host specificity has been demonstrated to be under genetic control and thus being heritable (Chung *et al*., 1997). Genotypic variation in compatibility may crucially affect the genetic composition of endophyte populations. Such variation may affect the persistence of natural endophyte infections as well, which could explain the generally low incidences of sexual endophytes. Those are difficult to explain in established com-munities where host turnover is low, since ongoing contagious spread via ascospores should cumulate over time and lead to an increase of the prevalence of horizontally transmitted endophytes.

(d) Another trait which is potentially affected by genotypic variability is the expression of disease symptoms, e.g. the extent to which inflorescenses are aborted by stroma formation. With respect to this trait, studies involving *E. bromicola* are of particular interest for two reasons. First, many infected *B. erectus* plants in natural populations do not express disease symptoms, or stromata are formed only on a pro-portion of the reproductive tillers (Groppe *et al*., 1999). It remains unclear whether the host or the fungus controls stroma formation, or whether this trait rather depends on abiotic factors. Second, *E. bromicola* infects not only *B. erectus* but also other brome-grass hosts (*B. benekenii* and *B. ramosus*), on which stroma formation has never been observed (Leuchtmann and Schardl, 1998). Interestingly, *E. bromicola* isolates from *B. erectus* could successfully establish infections on those other bromegrass hosts, but no persistent infections were observed when *B. erectus* plantlets were inoculated with isolates from *B. benekenii* or *B. ramosus*.

Several interesting questions arise: does genotypic variation exist for such limited cross-compatibility? Do *E. bromicola* isolates from *B. erectus* express stroma on the novel bromegrass hosts, or do they invade their seeds, which has never been observed in *B. erectus*? Experiments of this kind would help to understand which symbiotic partner controls stroma expression, and to which degree variability in this trait exists.

(e) Experiments with clavicipitaceous endophytes have several methodological advantages over those with other plant/fungal associations, which make them interesting in particular for investigations on the genotype level. First, these fungi can be cultivated on synthetic nutrient media. Second, enophyte/grass associations usually consist of single genotype entities that last over the live span of the host. Artificial inoculation techniques are well established, and mixed genotype inoculation experiments allow investigations on the competitive performance of fungal genotypes (see Chapter 6). Several techniques are available for infection diagnosis, e.g. leaf plating and sensitive, PCR-based tools (Groppe *et al*., 1995). Size variable loci in the fungal genomes can be used as genetic markers in order to identify endophyte genotypes alone or in mixture. Moreover, a PCR-based technique was recently established to quantify fungal biomass within plant tissue (Groppe and Boller, 1997).