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FUNGAL GENOTYPE CONTROLS MUTUALISM AND SEX IN *BRACHYPODIUM SYLVATICUM* INFECTED BY *EPICHLOË SYLVATICA**

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The fungal endophyte *Epichloë sylvatica* (Clavicipitaceae, Ascomycota) may obligatorily infect the woodland grass *Brachypodium sylvaticum,* on which it can display two alternative modes of reproduction. During the sexual cycle, external stromata suppress host flowering and production of seed (choke disease), whereas in the asexual cycle the fungus remains asymptomatic and transmits vertically by seeds. Variation in the reproductive system thus determines whether the symbiosis is mutualistic or parasitic. In order to assess the relative effects of each genotype on fungal reproduction, we used naturally infected seed families of *B. sylvaticum* and experimentally infected plants with different combinations of plant and fungal genotypes. The results of one experiment suggested a maternal effect of the host association on the choke rate in the offspring, while the results of a second experiment clearly indicated that the fungal genotype determines stroma formation and thus the mode of reproduction. Since sexual reproduction of the fungus is closely tied with disease expression on the host, the fungal genotype may also be responsible for whether an endophyte association is beneficial or pathogenic. We discuss the results in the light of current theories about the evolution of mutualism and the maintenance of sex.

Keywords: Endophyte – evolution – reproduction mode – symbiosis – transmission mode

INTRODUCTION

According to Law and Lewis [26] selection on the reproductive system of symbionts is different in mutualistic as compared with antagonistic environments. In mutualistic symbioses, such as mycorrhizas or lichens, there should be selection against sex since the constant environment provided by the host generates pressures against continuing evolutionary change. By contrast, antagonistic interactions between the host and a parasite are assumed to be the driving force behind the maintenance of sex [2, 22]. Under a regime of reciprocal selection, parasites may evolve to optimise host exploitation, while hosts evolve to minimise the loss of fitness imposed by the parasite.

Endophytic fungi of genus *Epichloë* (Ascomycota, Clavicipitaceae) can display both mutualistic and antagonistic life cycles and are therefore ideal systems to test

*Dedicated to Professor Lajos Ferenczy on the occasion of his 70th birthday.

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hypotheses on the evolution of mutualism and the maintenance of sex. *Epichloë* species and their asexual *Neotyphodium* anamorphs are widespread endophytes of many cool-season grasses [28, 43]. These fungi live asymptomatically inside the host for most of their life-cycle, except for sexual reproduction, when they form external fruiting structures (stromata) on the flowering tillers. The stromata mechanically suppress the development of host inflorescence and thus prevent flowering and seed-set (choke disease). Conidia (asexual spores) formed on the stromata serve as gametes which have to be transferred by specialised *Botanophila* flies to stromata of opposite mating type for fertilisation [7, 8]. Sexually formed ascospores are thus propagules for the horizontal transmission of the fungus to new host plants. In the alternative asexual cycle, the fungus does not develop disease symptoms, but grows into the ovaries of developing seeds and is vertically transmitted.

A variety of fungal alkaloids produced in infected grasses have distinct anti-herbivore properties and may therefore be beneficial for the host [3, 9]. Other reported benefits resulting from infection include enhanced growth and increased competitive abilities [11, 13, 14, 30], and increased resistance to drought, pests or fungal pathogens [17, 21, 23, 37, 42]. In consequence of the obvious selective advantages for the host, we regard asymptomatic, seed-transmitted endophyte associations as mutualistic, whereas sexually reproducing endophytes which cause the complete or partial loss of seeds are parasitic. Hence, the mode of reproduction and the type of interaction (parasitic versus mutualistic) are closely connected in these fungi.

The type of association formed between an endophyte and host is usually stable, suggesting that major genetic components are involved in disease expression. However, in some hosts stroma formation may be suppressed by rapid elongation of the flowering apex during a critical stage of development, which in turn may be influenced by the environmental conditions [24]. In fact, high levels of nitrogen fertilisation diminish choke rates in infected *Festuca rubra* L. [19, 41]. This indicates that both genotypic and environmental components may influence stroma formation.

Epichloë sylvatica Leuchtmann & Schardl infecting *Brachypodium sylvaticum* (Huds.) B. P., a common woodland grass, is an example of an association where both sexual reproduction and seed transmission of the fungus occur to different degrees in natural populations [6, 31]. A previous study, using cloned plants with different symptom types, revealed that disease expression in this association was mainly dependent on the genotypes, while environmental factors had no or only marginal effects [32]. However, the results of that study could not distinguish between fungal and plant genetic factors, although there is circumstantial evidence that the fungal genome alone might control the life cycle [6].

In the present study, we used experimentally infected plants with different combinations of plant and fungal genotypes in order to assess the relative effects of each genotype on fungal reproduction. In the first experiment, plants were grown from naturally infected seed families to evaluate the maternal effects of the host association on the fungal choke rate. The second experiment involved reciprocal combinations of fungal genotypes with different seed families, which should provide a more conclusive answer about the nature of disease control.

MATERIALS AND METHODS

The experimental system

B. sylvaticum is a perennial, caespitose grass of the tribe Triticeae, which is native to temperate Eurasia and North Africa [15, 16]. It is characteristic of natural woodlands and woodland edges, particularly at sites where the tree canopy is open, for example clearings or along forest roads. As a typical clump-forming grass, it has a very limited capacity for lateral vegetative spread. *B. sylvaticum* plants are obligatorily infected by the mostly asymptomatic and seed-transmitted endophyte *E. sylvatica*. This endophyte is a distinct, reproductively isolated biological species, with *B. sylvaticum* as the only known host [29]. Surveys in Switzerland which relied on tissue samples indicated that asymptomatic infection rates of the populations typically reach 100%, but sexual reproduction with stroma formation is rare [6, 31]. In populations where the fungus reproduces sexually, choke-forming plants are usually clustered and consistently appear every year (A. Leuchtmann, pers. observations). In these populations, asymptomatic plants (with only flowering tillers, but infected with seed-transmitted fungi) are still dominant, while some plants exhibit a mixed symptom type (with both flowering and choked tillers) and few plants have all their tillers choked.

Experiment I: Naturally infected seeds

In the first experiment we examined choke formation in the unmanipulated offspring of different plants of *B. sylvaticum* to test whether the type of infection in the maternal plant genotype affects the fungal reproduction mode. Infected *B. sylvaticum* plants (clumps) with and without choke were obtained from natural populations at sites around Zurich and transplanted to pots maintained in the greenhouse (Table 1). In autumn 1996, seeds were collected from 14 of these plants that formed flowering tillers. Since *B. sylvaticum* is mostly selfing [36], particularly under greenhouse conditions, we expect a high degree of genetic similarity among seeds of the same plant (= seed family). Since vertical transmission of *E. sylvatica* is very efficient, all seeds should be infected.

Seed families selected for the experiment were surface sterilised [27], placed on water agar in Petri dishes (up to 10 seeds per dish) and vernalised for 3 weeks at 4 °C. Within 1 week after vernalisation most of the seeds had germinated at room temperature. The seedlings were checked for infection on the agar plate, which could easily be evaluated from emerging hyphae seen under the dissecting scope. After 3 weeks, the infected seedlings were transferred to plant trays filled with potting soil and placed in the greenhouse. One to two months later, young plants were transplanted into a standard potting mix (40% bark peat, 35% regular peat, 20% expanded Lecca clay, 5% clay) in plastic pots (8 cm diameter, 470 ml). A subsample of the plants was checked for infection microscopically by examining excised pieces of leaf

Table 1
Seed families of infected *B. sylvaticum* with symptom type and the number of offspring used in this study. Asymptomatic plants have only flowering tillers where the fungus is seed-transmitted, mixed plants have flowering and choked tillers with stromata, and choked plants have all tillers choked

Seed family	Symptom type of mother plant	Experiment I	Experiment II	
			1996	1997
AG1	asymptomatic	25		80
AG4	asymptomatic	20	24	30
AG5	asymptomatic			40
LR13	asymptomatic			80
LR14	asymptomatic	39	28	70
S3	asymptomatic	36		40
S5	asymptomatic	39	54	70
VR6	asymptomatic	22	27	70
D2	mixed	33		40
LR1	mixed			40
S1	mixed	29	8	72
S2	mixed	37	28	70
S4	mixed	29	44	70
VR1	mixed	30	31	70
VR4	mixed	29	27	12
A1	choked	7		
DW4	choked	21		
DW18	choked			81

sheath epidermis [25]. The plants hibernated in an unheated greenhouse, and in spring 1997 the pots were placed in the soil of an experimental garden at the Botanical Garden Zurich, and kept there throughout the whole experiment. Plants were irrigated as necessary, kept shaded during the summer months, and fertilised in spring 1998 with solid long-term fertiliser (Hauert Rasen Tardit®). At the end of the second season in 1998, the numbers of flowering and choked tillers of each plant were counted.

Experiment II: Artificial combinations

For the second experiment, different genotypes of *B. sylvaticum* were experimentally inoculated with distinct *E. sylvatica* strains to create novel combinations. Seeds from 15 mother plants (Table 1) were collected in 1996 and 1997 and treated as described in experiment I, except for a heat treatment step after surface sterilisation that should kill the residing endophyte. Seed families were subjected to heat treatment for 3 weeks at 37 °C and high humidity [34]. This method can successfully kill the endophyte without substantially reducing the viability of seeds of *B. sylvaticum* (Kurz and Leuchtmann, unpublished). Moreover, endophytes which remain viable after heat treatment grow out from germinating seeds and can easily be detected

Table 2
Identity of *E. sylvatica* fungal strains with symptom type of the *B. sylvaticum* plant, symptom type of the tiller from which the strain was isolated, and the number of experimentally infected seedlings used in experiment II. Fungi from asymptomatic tillers were seed-transmitted and fungi from choked tillers formed stromata on the original host

Fungal strain	Symptom type of plant	Symptom type of tiller	1996	1997
DW7	asymptomatic	asymptomatic		40
LR14	asymptomatic	asymptomatic		50
S3	asymptomatic	asymptomatic	28	20
VR2	asymptomatic	asymptomatic	39	12
DW20T6	mixed	asymptomatic		106
DW2T2	mixed	asymptomatic		101
VR1T2	mixed	asymptomatic		100
VR7T2	mixed	asymptomatic		50
DW20T3	mixed	choked		106
DW2T1	mixed	choked		100
DW3T1	mixed	choked		30
DW6T5	mixed	choked		20
S1	mixed	choked	98	
VR1T6	mixed	choked		100
A1	choked	choked	28	
DW11	choked	choked		30
DW13	choked	choked		30
DW4	choked	choked	78	
DW5	choked	choked		40

under the microscope [31]. Endophyte-free seedlings in the one-to-three-leaf stage were then inoculated with one of 16 selected fungal strains (Table 2). Following a well-established procedure [25], we made a longitudinal cut with a fine hypodermic needle through the pseudostem of the seedling just above the apical meristem, and inserted a small piece of mycelium from agar culture into this cut. For inoculation of the up to 10 seedlings per Petri dish only one fungal strain was used to prevent unintentional infections. We used at least 10 seedlings per combination of seed family and fungal strain. After inoculation, the seedlings were kept in the Petri dishes at room temperature for another two weeks so that the fungi could establish infections under humid growing conditions. Surviving seedlings, and later young plants, were checked for infection, transplanted, potted and maintained in the experimental garden in the same way as those in experiment I. For an initial test of the infection procedure in autumn 1996, we infected 271 seedlings and hibernated them in the greenhouse as described in experiment I. For a second series, in spring 1997 we infected another 935 seedlings.

As the number of seeds available from mother plants with high choke rates was limited, it was not feasible to inoculate seedlings of all plants with all 16 fungal strains. In addition, the death of seedlings and young plants and also unsuccessful infections further reduced the number of plants which could be used for the experi-

ment. In autumn 1998, at the end of the growing season, choke rates were determined in all plants that formed reproducing tillers. For unknown reasons, many of the plants grew only vegetatively and their choke rates could not be assessed.

Data analysis

Analyses are based on plant choke rates, defined as the number of choked tillers divided by the total number of reproducing tillers (choked and flowering). The choke rates were logit transformed to normalise residuals. The logits of the choke rate were then analysed by using ANOVA. Because of the unbalanced data set and the empty cells in experiment II, we applied the sequential ANOVA method described by Snedecor and Cochran [40] and Searle [38] to test for significance of the interaction term. First, we computed the error sum of the squares of the full model with the two main effects including the interaction term, and then we computed the error sum of squares for a reduced model without the interaction term. The sum the of squares of the interaction term is the difference between the two sums of squares. The reason for choosing this method was the incomplete and unbalanced nature of the data, combined with the random character of the main effects (seed family and fungal genotype), which caused statistical problems under the full model.

RESULTS

Experiment I: Naturally infected seeds

We observed highly significant differences in choke rates among plants originating from seeds of different, naturally infected plants of *B. sylvaticum* (Tables 3 and 4). The results ordered by increasing average choke rates of different seed families indicated a clear dichotomy. Twelve seed families exhibited choke rates of 8% or less, with most of them close to zero, whereas 2 families (A1 and Dw4) had rates of over 97%. No intermediate choke rates were observed among the examined seed families.

There was a high degree of correlation between the disease symptoms observed in the mother plant and those expressed in the offspring. Seed families A1 and Dw4 originated from mother plants which were completely choked, except for a few escaping tillers which provided a limited number of seeds (Table 1). The other seed families were from plants with intermediate levels of choking or asymptomatic plants. Obviously, fungal strains which normally choke their host completely are capable of seed transmission, although they rarely get the chance to do so.

Experiment II: Artificial combinations

We created novel associations of plant and fungal genotypes by artificial inoculations, using seed families of 15 different mother plants of *B. sylvaticum* (Table 1) and 16 endophyte strains (Table 2). The reproduction mode of the fungus was then stud-

ied in reproducing plants of these associations. Representatives of 73 combinations from the 240 possible combinations survived and formed reproducing tillers at the end of the experiment (Table 5). An unexpectedly high number of plants of the second inoculation series died (35.6%, as compared with 2.2% in the first series) or did not form reproducing tillers (57% of the surviving plants, compared to 2.4% in the first series) and therefore could not be scored. This caused an extra 18 empty cells in the table and very small sample sizes for most of the reported choke rates. Similarly to the results of experiment I, most choke rates were either close to 1 or close to zero with few intermediate values (Table 5).

Statistical analysis of the available data using the full model showed no significant interaction between seed families and fungal genotypes (Table 6). Because of the incomplete and unbalanced nature of the data the interaction term is not suitable to test the significance of the main effects (fungal genotype and seed family). Therefore, we tested the main effects against the residual of a reduced model with-

Table 3

Results of experiment I. Choke rates in plants from naturally infected seed families of *B. sylvaticum.* Values are given as means of the logit transformed choke rates with standard deviations, and as back-transformed mean choke rates.

Seed family	No. of plants	Logit transformed choke rate	SD	Back-transformed choke rate
LR14	37	–9.67	2.02	0.0001
S3	35	–9.63	2.06	0.0001
D2	33	–9.03	3.05	0.0001
AG4	19	–8.66	3.49	0.0002
AG1	21	–8.15	5.26	0.0003
S2	37	–8.05	3.75	0.0003
VR1	32	–7.74	4.69	0.0004
VR4	28	–7.72	3.90	0.0004
S4	26	–5.98	4.66	0.0025
S5	39	–3.71	4.49	0.0239
VR6	20	–2.98	4.83	0.0485
S1	29	–2.43	5.36	0.0811
A1	6	3.86	8.07	0.9793
DW4	12	5.18	8.00	0.9944

Table 4

ANOVA of choke rates of experiment I. The identity of the infected seed family had a highly significant effect on the choke rate in the offspring.

Source	Sum of squares	df	Mean square	F	P
Seed family	4570.17	13	351.55	19.33	<0.0001
Residual	6545.66	360	18.18		

Table

Results of experiment II. Back-transformed mean choke rates in *B. sylvaticum* plants originating from (number of reproducing plants which were scored

Seed family	Fungal genotype							
	DW7	S3	VR2	DW20T6	DW2T2	VR1T2	VR7T2	DW20T3
AG1				0 (4)	0 (1)	0.001 (7)		
AG4							0 (2)	
AG5							0 (8)	
LR13				0 (5)	0 (7)	0 (10)		
LR14	0 (1)			0.500 (2)	0 (3)	0 (4)		
S3							0 (3)	
S5				0 (3)	0 (5)	0 (1)		0 (1)
VR6				0.001 (3)	0.005 (2)	0 (3)		
LR1	0 (6)						0 (6)	
S2		0.005 (22)		0 (1)		0 (7)		
S4			0 (4)	0 (1)	0.500 (2)	0 (4)		
VR1			0.091 (30)		0 (1)	0 (4)		
VR4				0 (5)				0 (6)
DW18					0 (4)	0 (4)		0 (1)
S1			0.003 (8)			0 (5)		

5
artificially infected combinations of different seed families and fungal genotypes
at the end of the experiment are given in parenthesis)

Seed family	Fungal genotype							
	DW2T1	DW3T1	VR1T6	A1	DW13	DW4	DW5	S1
AG1	0.023 (5)		0 (5)					
AG4		0.500 (8)				0.987 (20)		
AG5		0.016 (10)					1 (1)	
LR13	0.001 (9)		0 (6)					
LR14	0.033 (3)		0 (3)	0.270 (27)				
S3		0.926 (4)						
S5	0.006 (2)		0 (4)					0.132 (50)
VR6	0.006 (4)		0 (3)			0.932 (26)		
LR1							1 (1)	
S2	1 (2)		0 (2)				1 (1)	
S4	0.033 (3)		0 (5)					0.526 (41)
VR1	0.053 (7)		0 (6)					
VR4						0.983 (25)		
DW18	0.480 (5)		0 (2)		1 (1)			
S1	0.926 (9)		0 (5)					

Table 6
ANOVA of experiment II. The effect of the fungal genotype on the choke rate was highly significant only under a reduced model, where the effects of fungal genotype and seed family were tested against the residual mean square without the interaction term (for details see text)

Source	Sum of squares	df	Mean square	F	P
Fungal genotype (adjusted for seed family)	7583.02	15	505.53	14.49	<0.0001
Seed family (adjusted for fungal genotype)	550.23	14	39.30	1.13	0.3313
Residual (reduced model)	16569.94	475	34.88		
Seed family × fungal genotype	1864.88	42	44.40	1.31	0.1007
Residual (full model)	14705.06	433	33.96		

out the interaction. The effect of the fungal genotype was highly significant. This was further underscored by a mean square of 505.53 which was more than 10 times larger than the second largest mean square of the interaction (44.4). There, results of our experiments therefore clearly suggest that the fungal genotype, and not the seed family, determines the choke rate.

DISCUSSION

The results of experiment I using naturally infected seed families of *B. sylvaticum* suggested a maternal effect of the host association on the choke rate in the offspring, while reciprocal combinations of fungal and plant genotypes with different symptom types in experiment II clearly indicated that the fungal genotype determines stroma formation. Hence, the maternal effect observed in experiment I was induced by the seed-transmitted endophyte strain. Moreover, the fungal genotype may also be responsible for whether an endophyte association is beneficial or pathogenic, since the stromata formed during sexual reproduction of the fungus cause the disease.

As compared with the genotypic effects of the fungus, environmental factors appear to have no or only marginal effects on the choke rate of infected *B. sylvaticum* plants. In a previous study, using cloned plants with different degrees of choking, the effects of shading, elevated CO_2 concentration and fertilisation were examined and proved to be negligible [32]. Only fertilisation slightly stimulated the disease expression in some clones, which is contrary to expectations and may have had other reasons than those of phenotypic variation. Horizontal transmissions mediated by ascospores can be frequent and may have confounded the original infection status in some plants of that experiment [32]. In other endophyte associations exhibiting variable degrees of choking, environmental factors can be far more important. High lev-

els of nitrogen fertiliser reduced choke rates in *Festuca rubra* infected with *E. festucae,* probably in consequence of the growth stimulation of the flowering stems [19, 41].

Unlike the *E. festucae/F. rubra* association, *E. sylvatica* consists of individual genotypes which are either seed-transmitted or choke-forming and which form reproductively isolated asexual or sexual subpopulations [6]. These subpopulations are not only confined to individual plants of a larger host population, but may be separated at the tiller level of multiple infected plants [31]. Ecologically, the two subpopulations occupy the same niche, but differ in their mode of reproduction and their means of transmission. Selection may act on either of these traits, while genotypes are competing for new host plants or within already infected host plants.

A remarkable characteristic of the asexual subpopulation is its wide distribution with very high levels of infection typically reaching 100%. All *B. sylvaticum* host populations so far examined in Switzerland were infected mainly by asymptomatic endophytes [6, 31], as were samples from Italy, The Netherlands, England and Sweden (Leuchtmann, unpubl. data). Among isolates from all these populations, a single isozyme genotype was predominantly found suggesting a clonal origin of the asexual endophytes in this area. Associations of *B. sylvaticum* with this particular genotype must have had (and still have) a strong selective advantage over uninfected plants, which may have allowed successful spread after the first invasion. However, it is not entirely clear what the benefits for the host plant are in this mutualistic association, which resulted in the observed high levels of infection with this asymptomatic endophyte strain. *Epichloë* spp. and their anamorphs are well known to produce a variety of physiologically active alkaloids with distinct anti-herbivore properties [35, 39]. Moreover, a recent study has shown that larvae of the insect herbivore *Spodoptera frugiperda* performed significantly better on a diet of uninfected leaves of *B. sylvaticum* as compared with the infected control, even though the chemicals responsible for this effect are not known [5]. The increased resistance of infected plants to herbivory could be an important factor, although such biotic selection forces are usually density-dependent, and spatially and temporally structured. Additional benefits which may arise from endophyte infection and which are well documented for other host grasses are growth stimulation and improved seedling establishment which should increase competitive abilities of infected plants [11, 13, 30]. However, preliminary results from competition experiments involving *B. sylvaticum* do not support this hypothesis (D. Brem, unpubl.).

The sexual subpopulation of *E. sylvatica*, by contrast, is genetically more diverse and characterised by rare and patchy distribution [6]. In the few host populations in Switzerland where sexually reproducing strains are regularly observed, stroma formation is restricted to a subset of infected plants and grass clumps are often only partly choked. Moreover, some sexual strains appear to be capable of seed transmission as well, although very rarely, which distinguishes them from other stroma-forming *Epichloë* species [6, this study].

The question arises as to the evolutionary scenario under which such a highly balanced symbiosis has evolved and what the conditions are for maintaining two sepa-

rate endophyte subpopulations. The family Clavicipitaceae, in which *Epichloë* belongs, is largely comprisesd of parasites of grasses, insects and other fungi [1]. It is therefore reasonable to assume that the ancestral forms of *Epichloë* were also parasites and that current seed-transmitted endophytes have evolved through a series of coevolutionary changes in the reproductive systems of host and fungi [12]. *B. sylvaticum* may originally have been infected only by choke-forming strains of *Epichloë* which sterilise their hosts and which typically form low levels of infections, as seen for example in grasses infected by *E. typhina* [28]. Mutations in the host populations that restore fertility were then favoured by increasing the reproductive capacity of the plants and by significantly reducing the cost of infection. This may have been particularly important for caespitose grasses such as *B. sylvaticum* which have no or very limited means of vegetative spread. The restoration of host fertility is followed by the loss of sexual reproduction on the part of the fungi, which became asymptomatic, seed-transmitted endophytes. Alternatively, mutations in the fungal population may have given rise to strains which were no longer able to choke the host plants completely and then became seed-transmitted.

With the emergence of the seed-transmitted fungi, several preconditions for the evolution of mutualism are met, as postulated by Frank [18] and Genkai Kato & Yamamura [20]**.** Both partners potentially benefit from waste products of the other partner (the fungus from nutrients leaking into the intracellular space, and the host from protective fungal alkaloids) whereby Frank's threshold is passed [18], and the inhabitant is in control of the mode of transmission [20]. Seed-transmitted associations are indeed mutualistic as indicated by empirical evidence gained from this and many other grass hosts. In addition, according to Law and Lewis [26], mutualistic environments will generate selective pressures against sex of a symbiotic inhabitant, which should further promote the spread of asexual endophytes. The result of this may be seen in the many populations of *B. sylvaticum* which are infected solely by asymptomatic endophytes. A potential drawback for the seed-transmitted endophytes over evolutionary time is the accumulation of deleterious mutations due to the lack of sex, which is known as Muller's ratchet [33]**.** It is interesting to note that *B. sylvaticum* is the only selfing species of *Brachypodium* which leads to a much higher seed production as compared with the outcrossing species of the genus [36]. Moreover, it is the only *Brachypodium* species which is known to be infected by seed-transmitted endophytes. Whether self compatibility in *B. sylvaticum* was caused by the endophyte infection, or conversely facilitated the enormous success of this association, cannot be decided at the moment.

Why then is the sexual subpopulation still persistent in *B. sylvaticum*? The answer to this question may be founded on characteristics which distinguish many successful pathogens. *E. sylvaticum* has an efficient means for contagious spread in the form of wind-dispersed ascospores. These ascospores may mediate new infections through the invasion of host florets and developing seeds [10], or vegetative tissues of stems and leaves [4]. In an experimental setting, up to one-third of endophyte-free plants transplanted to natural sites within stroma-forming plants of *B. sylvaticum* became infected after 2 years, indicating that horizontal transmission of *E. sylvatica* was very

frequent [4]. In natural stands where typically all plants are already infected mostly by asymptomatic strains, horizontal transmissions may be lower because new incoming strains would have to compete with the resident endophyte in the host. In fact, recent findings suggest that infection by seed-transmitted endophytes can make host plants less susceptible to superinfection by choke-forming strains [32]. However, multiple infections of single plants by genetically different endophyte strains do occur in natural populations of *B. sylvaticum* [31]. These strains often differ in their symptom type and always occupy different parts of a plant, which may have been a result of competitive displacement after superinfection. Finally, choke-forming-strains undergo sexual recombination regularly, which enables them to evolve rapidly enough to overcome any resistance of the host genotypes and to win the coevolutionary "arms race" between the asymptomatically infected host plant and the choke-forming parasite. What we find today might be a sexual subpopulation which remained in a transitional stage towards mutualism with the capacity to form choke, but with the potential for seed transmission.

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