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## ENDOPHYTE–HOST ASSOCIATIONS IN FORAGE GRASSES. XI. A PROPOSAL CONCERNING ORIGIN AND EVOLUTION

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### ABSTRACT

*Epichloë typhina* and fungi thought to be closely related to it are widespread as endophytes in the Poaceae. Based on biological features of the fungus–host relationship, associations may be classified as one of three types. In type 1 associations, stromata form on most of the plants harboring endophytes. This type of association has been found in several subfamilies of grasses and sedges. In type 2 associations, stromata are formed on very few of the numerous infected individuals, while in type 3 associations stromata have not been observed on infected plants. The latter two associations are known to occur only in the subfamily Festucoideae. It is evident that coevolution has occurred between *Epichloë* spp. and festucoid grasses resulting in the evolution of mutualisms from primarily pathogenic relationships. It is suggested that this has occurred by selection of grass individuals which possessed the ability to suppress stromata formation by the endophytes. The mechanism for suppression of stromata may involve production of alkaloids which inhibit growth of these fungi.

Key Words: *Acremonium* spp., *Epichloë* spp., endophytes.

Evidence has been accumulating over the course of this century which suggests that a group of fungi, appearing to be related to *Epichloë typhina* (Fr.) Tul., is widespread as endophytes in several genera of the grass subfamily Festucoideae (Vogl, 1898; Sampson, 1933; Neill, 1941; Gould, 1968; Bacon *et al.*, 1977; Morgan-Jones and Gams, 1982; Latch *et al.*, 1984; Shelby and Dalrymple, 1987; White and Morgan-Jones, 1987; White, 1987). A significant body of research shows that these fungi cause a variety of toxic syndromes in grazing mammals and impart resistance to a range of insect pests of grasses (Bacon *et al.*, 1977; Prestidge *et al.*, 1982; Funk *et al.*, 1983; Barker *et al.*, 1985; Clay *et al.*, 1985; Siegel *et al.*, 1987). Toxic syndromes and improved pest resistance are thought to be due to the production of ergot alkaloids by the endophytes, and to increased alkaloid production by grasses in response to the presence of the endophytes (Lyons *et al.*, 1986; Hardy *et al.*, 1985; Bush *et al.*, 1982; Johnson *et al.*, 1985). Mycelium of these endophytes is located intercellularly in leaves and culms, where low concentrations of sugars provide the fungus energy for growth (White and Cole, 1986). Nutritional studies suggest that by converting free glucose into mannitol, which cannot be metabolized by the host grass, the endophytes can gradually accumulate a supply of food with minimum drain on host storage carbohydrates (Kulkarni and Nielsen, 1986).

Although these endophytes are believed to be related to *Epichloë*, most are not known to form perithecia and are not referred to by that epithet. Instead, a classification system based on morphology of the conidial (spermatial) state *in vitro* is employed. In this system, *Acremonium* sect. *Albo-lanosa* Morgan-Jones and Gams was established to accommodate these fungi (Morgan-Jones and Gams, 1982; Latch *et al.*, 1984).

Since the turn of the century, when Vogl (1898) discovered an endophyte in seeds of *Lolium temulentum* L., scientists have speculated about its identity and origin (Freeman, 1906; McLennan, 1920; Neill, 1941; Latch *et al.*, 1984; Siegel *et al.*, 1985). It has only been recently that we have accumulated enough data on these fungi to satisfactorily address questions of origin and evolution (Bacon *et al.*, 1977; Morgan-Jones and Gams, 1982; Funk *et al.*, 1983; Clay *et al.*, 1985; Latch *et al.*, 1984; Barker *et al.*, 1985; White, 1987; White and Bultman, 1987; Siegel *et al.*, 1987). This article is an attempt to explain the distributional patterns and life cycle variations of these fungi from an evolutionary perspective.

*Systematic position and life cycle.*—*Epichloë* is classified in the tribe Balansiae of the family Clavicipitaceae of the Ascomycotina with the morphologically and biologically similar genera *Atkinsonella* Diehl, *Balansia* Speg., and *Myriogenospora* Atk. (Diehl, 1950; Rykard *et al.*, 1984). In the genus *Epichloë*, the most widely

recognized taxon is *E. typhina*. Mycelium of this fungus is endophytic, perennating intercellularly within tissues of leaves and culms (Sampson, 1933). The endophyte egresses from its host during elongation of the culm, at which time the inflorescence and usually some of the surrounding leaves are enveloped with mycelium forming a stroma (Kirby, 1961). Spermatia are produced on the surface of the stroma. These must be transferred to a stroma of the opposite mating type, by wind or most commonly by insects, before perithecia will develop (White and Bultman, 1987). After fertilization, perithecia form in the stroma, which thickens, turning from white to pale orange in color. Within perithecia numerous asci, each containing eight filiform multiseptate ascospores, are produced. Ascospores disarticulate within the ascus to form single-celled "part-spores." These are forcibly ejected from the ascus, in rapid succession, into the air (Ingold, 1971). Early research suggested that ascospores infect tillering buds of grasses (Butler and Jones, 1949), but this has proven difficult to confirm (Western and Cavet, 1959).

#### TYPES OF ASSOCIATIONS

Based on biological features of the grass-fungus association, *Epichloë typhina* and endophytes thought to be related to it may be classified as one of three types, as follows.

*Type 1.*—Stromata are formed on most, if not all, infected individuals so that sexual reproduction by infected host plants is completely suppressed. The fungus completes its sexual cycle at the expense of the host plant. This type of association is broadly distributed in several subfamilies of the Poaceae and in some sedges. Populations of plants with this type of infection tend to remain small, spreading predominantly as the grass clone spreads vegetatively. Although ascospores are produced, they do not appear to infect adjacent uninfected plants. *Agrostis tenuis* Sibth. and *Dactylis glomerata* L. are known to harbor this type of association (Bradshaw, 1959; Western and Cavet, 1959). This association is the classic "choke disease" of grasses, and its negative impact on host individuals suggests that it is correctly classified as a pathogenic association.

*Type 2.*—Stromata are produced on only a few (1–10%) of the infected individuals in a popu-

lation. Often within an infected grass clone on which stromata form, only a few of the numerous culms produced bear stromata. This type of association minimizes the negative impact on infected grasses, and has been found exclusively in the subfamily Festucoideae of the Poaceae. The grass species *Agrostis hiemalis* (Walt.) B.S.P., *Bromus anomalus* Rupr., and *Elymus canadensis* L. harbor this association. Infected populations tend to contain the endophyte in 50–75% of the individuals. Often infection is present at this level throughout the range of the host grass. Evidence suggests that the endophyte in this type of association spreads by clonal growth of the host grass, and probably also by ascospore infection and seed transmission (White, 1987; White and Bultman, 1987).

*Type 3.*—Stromata have not been found on infected plants and apparently are not produced (Halisky *et al.*, 1985; Siegel *et al.*, 1987; White, 1987). This association has been found only in festucoid grasses. Grasses with this association include *Festuca arundinacea* Schreb., *F. versuta* Beal., *Lolium perenne* L., *L. temulentum* L., *Stipa eminens* Cav., and *S. robusta* (Vasey) Scribn. In many of these grasses the endophyte is present in over 90% of the individuals throughout the range of the species. This type of association cannot be classified as pathogenic since the host plant is not detrimentally affected; rather this is a purely mutualistic association.

#### DISCUSSION

It seems reasonable to suggest that mutualistic associations (type 3) have evolved from pathogenic associations (type 1). Associations classified as type 2 appear to represent an intermediate stage in that evolution. The trend toward mutualism is as much a function of host evolution and adaptation as it is that of the endophyte. In populations of *E. canadensis* containing *E. typhina*, a type 2 association, stromata tend to form on the same few grass clones season after season. Comparative studies of isolates from plants with and without stromata reveal no obvious differences (unpubl. data). This seems to suggest that certain grass individuals in the population possess the ability to suppress formation of stromata, while others lack that ability. This regulation of endophyte development is probably due to the production of alkaloids by these grasses. Recent experiments have shown that halostach-

ine, an alkaloid produced by *F. arundinacea*, reduces the growth rate of endophyte cultures *in vitro*; the degree of reduction depends on concentration of the alkaloid (unpubl. data). This and similar alkaloids produced by many festucoid grasses (Yates and Tookey, 1965) probably enables them to control development of their endophyte associates. The fact that endophyte-containing lines of fescue and ryegrass, when compared to corresponding endophyte-free lines, produce higher levels of alkaloids, such as loline and peroline, gives additional support to the hypothesis that plant-produced alkaloids function in regulation of endophyte development (Jones, 1981; Bush *et al.*, 1982).

In type 2 associations, individuals on which stromata form probably produce low levels of these inhibitory compounds, allowing development of the endophyte on these few individuals. Outbreeding of the host grass appears to be an important feature in the maintenance of type 2 associations, since only in grass populations in which sufficient genetic variability is maintained will individuals producing both low and high levels of inhibitory compounds be evident. A common feature of grasses bearing type 3 associations is a narrow range of genetic diversity due to 1) selective inbreeding, as is the case for *F. arundinacea* and *L. perenne*, where years of cultivation have resulted in varieties which are low in genetic diversity and high in alkaloid content (Braverman, 1986), and 2) naturally inbreeding species, such as *F. versuta*, *S. eminens*, and *S. robusta*, which are often composed of small marginal populations where genetic diversity is minimal (Silander, 1985).

The question remains as to why type 2 and 3 associations should be present in only festucoid grasses. The answer probably lies in the coevolution of *Epichloë* with the Festucoideae. Both *Epichloë* and festucoid grasses tend to occur in cooler temperate climates and are predominantly absent from areas with warmer climates, where other grass subfamilies predominate. It seems probable that an early ancestor of the festucoid grasses established an association with a fungus species ancestral to modern *Epichloë* endophytes. The primary impetus for the grass to participate in that association was the benefit of fungus-produced alkaloids. As endophytes and hosts co-evolved and selective pressure, imposed by the presence of the endophyte, favored increased alkaloid production by grasses, additional ben-

efits were realized in the increased concentrations of defense-related compounds.

In mutualistic associations, where one organism is contained within another organism, selection against sexual reproduction of the endobiont frequently occurs. Years of asexual reproduction often results in the loss of the capacity for sexual reproduction (Law, 1985). This phenomenon may be evident where some endophytes are concerned. Endophytes isolated from *F. versuta* and *S. robusta*, type 3 associations, grow very slowly and do not produce spermatia when cultured *in vitro* (White and Cole, 1986; White, 1987). Since spermatia play an integral role in the sexual cycle, this suggests a loss of capacity for sexual reproduction. Endophytes of type 3 associations rely exclusively on vegetative propagation and seed transmission, which involves only mycelial growth from the parent plant to the embryo in the seed. Without continued use of spermatia, mutations may accumulate affecting the capacity of the endophyte to produce them. It thus seems reasonable that once a type 3 association is established, the endophyte should gradually lose the ability to form spermatia and probably stromata as well. Endophytes which appear to be in the process of losing the ability to form spermatia, as evidenced by slow growth and abnormal development of conidiogenous cells in culture, include *Acremonium lolii* Latch, Christensen and Samuels and *A. chisosum* White and Morgan-Jones, endophytes of *L. perenne* and *S. eminens*, respectively (Latch *et al.*, 1984; White and Morgan-Jones, 1987). It is interesting to note that *A. coenophialum* Morgan-Jones and Gams, an endophyte of *F. arundinacea*, shows no tendency toward loss of ability to form spermatia *in vitro*, even though stromata have not been reported on this grass. This may be explained by the fact that *F. arundinacea* has only come to be extensively cultivated in this century (Gould, 1968). It seems likely that in populations of *F. arundinacea*, where natural genetic variability has been conserved, stromata may be forming on some individuals in a type 2 association. Such populations may be present in Europe where varieties of *F. arundinacea*, presently in widespread cultivation, are thought to be native (Siegel *et al.*, 1987).

Further research is necessary to test the hypothesis proposed in this article, but if proven accurate, this proposal is potentially significant from both applied and basic perspectives. For

example, there has been recent interest in obtaining cultivars of endophyte-containing grasses for turf purposes (Funk *et al.*, 1983; Latch and Christensen, 1985; Siegel *et al.*, 1987). This may be accomplished by locating populations of grasses with type 2 associations, and selectively breeding endophyte-containing plants on which stromata do not form. Additionally, as a control strategy, grass breeders have produced endophyte-free cultivars of tall fescue under the assumption that the endophyte cannot reinfect grasses. This may be only a temporary solution. Since these grasses are genetically unchanged and therefore still compatible with the endophyte, reinfection from other host species on which stromata occur seems likely (White, 1987). From a basic perspective, these fungi and their hosts represent biological systems in which coevolution has resulted in relationships which are beneficial to both groups of organisms. Continued study of these associations may increase our understanding of how evolution has progressed in both festucoid grasses and the Balansiae.

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