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Genetic diversity and specialisation of *Eudarluca caricis* on some graminaceous *Puccinia* species

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A B S T R A C T

Eudarluca caricis is a common hyperparasite of rusts. A total of 100 cultures were isolated from six *Puccinia* species or forms growing on 10 species of British grasses at two sites approximately 3 km apart. 82 isolates collected in 2005 were partially sequenced at the ITS locus, and amplified fragment length polymorphism profiles generated for 86 isolates from 2005 and 12 from 2007. Partial ITS sequences of most isolates grouped closely, in a clade with previously reported graminaceous *Puccinia* isolates and a number of *Melampsora* isolates. A second clade was very distinct and contained mostly isolates from *Puccinia poarum* on *Poa trivialis*. All isolates had distinct AFLP haplotypes. The *P. poarum* isolates were very distinct from isolates collected from other rusts at the same site. Isolates from *P. brachypodii* f. sp. *arrhenatheri* growing on *Arrhenatherum elatius* in 2005 and 2007 at the same location were distinct ($P < 0.001$). Isolates from each rust or grass in one year and site were more similar than expected from overall variation between isolates ($P < 0.001$). Isolates from *P. coronata* on different grasses clustered together (with isolates from *P. brachypodii* f. sp. *poae-nemoralis*), suggesting partial host rust specialisation in *E. caricis*.

Introduction

Rusts are ubiquitous pathogens of grasses, and are among the most serious problems in agricultural cereal production. Conversely, rusts may play a part in reducing the competitiveness of otherwise invasive plants and increasing biodiversity (Peters and Shaw, 1996). The enemy release hypothesis suggests that organisms which become invasive may do so because they have moved to a new geographic area without the natural enemies – pathogens and pests – which regulate them in their range of origin (Evans, 2008). Natural enemies of rusts are, therefore, of interest from two points of view: they may help regulate agricultural and horticultural pests (Fleming, 1980; Vandermeer et al., 2009; Gordon and Pfender, 2012) and they may reduce the effectiveness of rusts as biocontrol agents.

Natural enemies of rusts include a variety of fungi, for example *Lecanicillium* spp., and animals, for example *Mycodiplosis* sp. flies, of varying degrees of specialisation. The

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ascomycete *Eudarluca caricis* has attracted considerable interest because the asexual form (*Sphaerellopsis filum*) is very common and has a wide host range among the rusts (Kranz and Brandenburger, 1981). It can easily be cultured in artificial media, although it is not found sporulating in nature except in association with rusts. In favourable systems, it can sometimes usefully reduce losses due to certain rusts (Morris et al., 1994; Gordon and Pfender, 2012), although this requires the population of *E. caricis* to be substantial at the start of the season and, therefore, able to increase sufficiently rapidly to slow down rust development before the rust becomes damaging. This is unlikely in most agricultural settings, but can occur if, for example, a population of rust on a weed pre-exists the development of severe epidemics on the crop, providing a reservoir from which *E. caricis* can spread to the rust on the crop. The question of the host range of individual hyperparasite genotypes is, therefore, of practical interest, as well as having theoretical relevance to testing ideas about the functioning of tri-trophic and other co-evolutionary systems (Thompson, 1999).

Host specificity of *E. caricis* was shown by Yuan et al. (1999), who inoculated isolates onto the rust *Melampsora larici-epitea* on willow. Isolates from willow and one from a grass were infectious, but isolates from rusts on *Larix* (Gymnosperm) and *Rubus fruticosa* agg. (Roseacae) were not. Within isolates from *Melampsora* species infecting *Salix*, there were very substantial quantitative differences both in effect on rust isolates and on the spore production of *E. caricis*, and significant quantitative hyperparasite–pathogen interactions (Pei et al., 2010). Similarly, Nischwitz et al. (2005) found quantitative interactions between *E. caricis* isolates and *Melampsora* species (or isolates: multiple isolates were not tested) on poplar. Pei et al. (2010) found quantitative interactions between 12 *E. caricis* isolates and five *Melampsora larici-epitea* isolates. Two isolates from *Puccinia* rusts on grass did not infect *Melampsora*. Previously, Keener (1934) had shown that single isolates from 11 diverse rusts had clear, individually distinct, patterns of host specificity on a test range of 19 angiosperm rusts. However, there is no evidence for specialisation of isolates of *E. caricis* to rusts on an individual host plant species.

Several recent publications have surveyed genetic variation in *E. caricis*, concentrating on isolates from *Melampsora* rusts because of the problems they cause in willow and poplar plantations. Bayon et al. (2006) found little variability among isolates from *Melampsora* rusts in willow and poplar plantations in England with the population dominated by a few clones, but populations changed greatly between years (Bayon et al., 2008). ITS sequences indicate a number of distinct clades within the taxon, and Liesebach and Zaspel (2004) suggested that there were actually two species present. They hypothesised a degree of host separation between these, noting that all their isolates from *Puccinia* lay within one subgroup of the group they denoted “I”.

Despite the importance of rusts as pathogens of cereals and wild grasses, there has been little recent study of *E. caricis* on rusts of grass hosts, and none concerning the genetic structure on different hosts. The aim of the present work was to test three hypotheses: (1) all the isolates from *Puccinia* would lie in the same clade as Liesebach and Zaspel’s isolates from *Puccinia*; (2) the population of *E. caricis* on grass-infecting rusts would be largely clonal; and (3) the population structure of *E. caricis* on grass rusts would be consistent with there being no specialisation of populations on individual species of rust.

Materials and methods

Sampling

Samples were collected from two locations (Table 1). The first was a teaching collection of grasses maintained, since the mid 1980s at the University of Reading as parallel strips 2 m long, about 50 cm wide, and separated by approximately 1 m of bare ground (51.436852 °N,-0.941505 °E). The order of strips was *Bromus erectus* – four unsampled strips – *Holcus mollis* – unsampled – *H. lanatus* – *Dactylis glomerata* – *Anthoxanthum odoratum* – *Cynosurus cristatus* – two unsampled strips – *Festuca pratensis* – *Agrostis gigantea*. Samples from the second site, Shinfield, about 3 km south (51.411437 °N,-0.937411 °E), were from an ungrazed mixed grassland maintained by

Table 1 – Numbers of isolates of *Eudarluca caricis* characterised by AFLP and ITS from each host

Rust species	Host grass	Number of isolates					
		Reading		Shinfield			Total
		2005		2005		2007	
		AFLP	ITS	AFLP	ITS	AFLP	
Puccinia brachypodii f. sp. *poae-nemoralis*	*Arrhenatherum odoratum*	7	7				7
P. brachypodii f. sp. *arrhenatheri*	*Arrenatherum elatius*			13	15	12	29
P. recondita f. sp. *bromina*	*Bromus erectus*	8	8				8
P. coronata	*Agrostis gigantea*	4	2				4
	Festuca pratensis	8	7				8
	Holcus lanatus	8	8	6	7		15
	H. mollis	7	8				8
P. graminis	*Cynosurus cristatus*	9	8				9
	Dactylis glomerata	2	2				2
P. poarum	*Poa trivialis*			16	10		16
	Total ITS		50		32		82
	Total AFLP	53		35		12	100