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Genetic differentiation in *Pyrenophora teres* populations measured with AFLP markers

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ABSTRACT

The genetic structure and occurrence of mating types and forms of *Pyrenophora teres*, the causal agent of net blotch on barley, was studied among 278 isolates collected from the northern hemisphere and from Australia. Genetic differentiation was high (F_{CT} 0.238, $P = 0.002$) between *P. teres* f. *teres* (PTT) isolates originating from Northern Europe, North America, Russia and Australia. The *P. teres* population in Australia was clearly divided into two subgroups (F_{CT} 0.793, $P < 0.001$) according to the form identity: PTT and *P. teres* f. *maculata* (PTM), with the PTT samples showing a greater degree of differentiation (F_{ST} 0.573, $P < 0.001$) among Australian states than the PTM samples (F_{CT} 0.219, $P < 0.001$). No differentiation was found among locations within Australian states. Both mating types (MAT1 and MAT2) were equally common (1:1) in several locations in Australia and in Finland. The only exception was Krasnodar, Russia, where only MAT2 was identified. Our results show that the prevalence of sexual reproduction, occurrence of forms of *P. teres*, and genetic differentiation between geographical regions are highly variable. The paper discusses the various effects and outcomes of population selection in Australia and in the northern barley growing regions.

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Introduction

There has recently been an increase in research into understanding the population biology of plant pathogens. Information on the genetic structure of pathogens will help us to improve disease control strategies (Douhan *et al.* 2002; McDonald & Linde 2002). The population structure of several important cereal pathogens is already well known. *Stagonospora nodorum* (Keller *et al.* 1997), *Tapesia yallundae* (Douhan *et al.* 2002), *Mycosphaerella graminicola* (Zhan *et al.* 2003), *Fusarium graminearum* (Zeller *et al.* 2004) and *Rhynchosporium secalis* (Salamati *et al.* 2000) have all shown a high genetic diversity within a small geographical scale without differentiation between locations. The major factors decreasing genetic variation in *M. graminicola* populations are random drift and natural selection, whereas factors increasing genetic variation are mutation, gene flow, and sexual recombination (Zhan & McDonald 2004). McDonald & Linde (2002) have placed several hemibiotrophic cereal pathogens in the same evolutionary risk category together with *M. graminicola*, which do not differ much in their migration (gene/genotype flow) or reproduction system. Understanding the evolutionary potential of

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pathogens would help to estimate the life expectancy of resistance genes and fungicides (McDonald & Linde 2002). Both resistance genes and fungicides may select pathogen populations toward higher tolerance to these control methods. Eventually the effectiveness can be lost. To achieve durable resistance, a comprehensive body of information on pathogen biology is needed.

Net blotch is a foliar disease of barley caused by *Pyrenophora teres* (anam. *Drechslera teres*). It is an important disease in every barley growing region around the world as it decreases barley yield and yield quality. In Finland, it is the most common disease of barley. Two symptom forms of net blotch have been differentiated by Smedegård-Petersen (1971), the spot form (caused by *P. teres* f. *maculata*, PTM) and the net form (caused by *P. teres* f. *teres*, PTT) of net blotch. The two forms are difficult to identify based on spore morphology (Crous *et al.* 1995). In contrast to the other fungal cereal pathogens listed above, genetic differentiation has been observed between *P. teres* populations across wide geographical areas with RAPD and AFLP markers (Peever & Milgroom 1994; Rau *et al.* 2003; Serenius *et al.* 2005). Despite the genetic differentiation, sexual recombination is possible between isolates from distant locations (Peever & Milgroom 1992), and even between the two forms of *P. teres* in the laboratory (Serenius *et al.* 2005; Smedegård-Petersen 1978). However, it is still unknown whether recombination occurs between the two forms in nature (Rau *et al.* 2003). Peever & Milgroom (1992) concluded that *P. teres* populations have not been isolated for a very long time or else there is a regular migration between populations. The two forms of *P. teres* can sometimes be misidentified under field conditions because the symptom type also depends on environmental conditions and the host's genotype (Rau *et al.* 2003; Williams *et al.* 2001). Currently, the two forms can be easily identified with molecular markers (Leisova *et al.* 2005a; Williams *et al.* 2001).

Failure to identify the forms of *Pyrenophora teres* has complicated the analyses of *P. teres* populations leaving unanswered questions concerning the amount and distribution of genetic variation and random mating within the populations. Our objective was to test, whether the population structure of *P. teres* within a small geographical area (e.g. a field) resembles that on a wider geographical area (e.g. a country, state or continent). The second objective was to test the occurrence of the forms and mating types of *P. teres* in different geographical areas. The final objective was to estimate the factors affecting the evolutionary potential of *P. teres*, based on the observed occurrence of mating types, estimated random mating, and genetic differentiation between locations.

Materials and methods

Isolates

Two collections of *Pyrenophora teres* isolates, one from MTT Agrifood Research Finland (MTT) and the second from South Australian Research and Development Institute (SARDI), were used in this study. Both collections included isolates from discrete geographic populations, e.g. a field, as well as random single isolates collected over larger geographical areas. The MTT collection included 167 single conidial isolates originating mostly from Finland but also from other European countries, North America and Australia. Details concerning the origin of the isolates are given in Tables 1 and 2. Finnish *P. teres* isolates covering the whole barley growing region in Finland (FIN89, FIN90, FIN91, FIN92, FIN94, and FIN95) were collected from official variety trials (Kangas *et al.* 2005) from the barley variety Arve. Some of those isolates collected before year 1995 were previously analysed with RAPD markers (Peltonen *et al.* 1996) (Table 1). Most of the isolates were random isolates of different origins but there were two population samples (FIN01 and RUS) (Table 2). A sample of 52 isolates (FIN01) was collected hierarchically from Ylistaro, Finland in 2001 as described earlier (Serenius *et al.* 2005). Infected Leaves were collected from Krasnodar, Russia in 2002 and were received from O. Afanasenko, All Russian Research Institute for Plant Protection (VIZR), St Petersburg, and a *P. teres* population (RUS) of 44 isolates was established at MTT.

The SARDI collection (139 isolates) consisted only of Australian single conidial isolates. Details concerning the origin and number of the isolates are listed in Table 3. Samples were collected by taking ten infected leaves per field at random from a transect of 10 m, and from several fields over states of Western Australia (WA) and South Australia (SA). Field samples from WA were from a transect of 350 km and field samples from SA from a single region called the Lower Eyre Peninsula, and were from a transect of 50 km. These samples were collected in order to study the population structure and variation within a state in Australia. A few samples were also collected from New South Wales (NSW), Queensland (QSL) and Victoria (VIC; Table 3). Those samples were included to study the total variation and migration within the continent of Australia.

Fungal culture and DNA isolation

All single conidial isolates were established from infected dry leaf material according to McDonald (1967). At MTT, isolates were maintained in a freezer at −70 °C until needed. Single conidial isolates were first grown on 2.5 % V8 agar under near UV light at +18 °C, and subsequently on yeast extract as described earlier (Serenius *et al.* 2005). The mycelium was harvested, washed, lyophilised, and ground for DNA isolation, which was done by using DNeasy plant mini kit (QIAGEN, Hilden, Germany) as described in Serenius *et al.* (2005). The DNA extracted was diluted to a concentration 25 ng μl^{-1} before AFLP analysis at MTT.

In SARDI, single conidial isolates were grown in potato dextrose broth for 14 d before the fungal material was filtered from the solution and freeze-dried as described in Williams *et al.* (2001). Approximately 4 g fresh tissue was freeze-dried and DNA was extracted with 3 ml extraction buffer according to the method of Raeder & Broda (1985) as described in Williams *et al.* (2001).

Genotypic characterisation with specific PCR markers and AFLP

The mating types (MAT1 or MAT2) of *Pyrenophora teres* isolates were identified with specific PCR markers as described earlier

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