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Challenges of microsatellite isolation in fungi

CYRIL DUTECH¹, JÉRÔME ENJALBERT², ELISABETH FOURNIER³, FRANÇOIS DELMOTTE⁴, BENOÎT BARRÈS⁵, JEAN CARLIER⁶, DIDIER THARREAU⁶, and TATIANA GIRAUD⁷

¹ INRA, Biodiversité, Gènes et Communautés, 33612 Cestas, France

² INRA, Laboratoire de Pathologie Végétale, 78850 Thiverval Grignon, France

³ INRA, Phytopathologie et Méthologie de la Détection Versailles, Route de Saint Cyr, 78026 Versailles, France

⁴ INRA, Santé Végétale, BP 81, 33883 Villenave d'Ornon, France

⁵ INRA, Interactions Plantes-Microorganismes, 54280 Champenoux, France

⁶ Biologie et Génétique des Interactions Plantes-Parasites, CIRAD-INRA-AGRO.M, TA 41/K, 34398 Montpellier, France

⁷ Ecologie, Systématique et Evolution CNRS- Université Paris-Sud, Bâtiment 360, Université Paris-Sud, 91405 Orsay, France

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Corresponding author: Cyril Dutech. INRA, UMR 1202 BIOGECO, Equipe de Pathologie Forestière, Domaine de la Grande Ferrade, BP81, 33883 Villenave d'Ornon Cedex, France
cdutech@bordeaux.inra.fr Tel: +33 5 57 12 26 24. Fax: +33 5 57 12 26 21

Abstract

Although they represent powerful genetic markers in many fields of biology, microsatellites have been isolated in few fungal species. The aim of this study was to assess whether obtaining microsatellite markers with an acceptable level of polymorphism is generally harder from fungi than in other organisms. We therefore surveyed the number, nature and polymorphism level of published microsatellite markers in fungi from the literature and from our own data on seventeen fungal microsatellite-enriched libraries, and in five other phylogroups (angiosperms, insects, fishes, birds and mammals). Fungal microsatellites indeed appeared both harder to isolate and to exhibit lower polymorphism than in other organisms. This appeared to be due, at least in part, to genomic specificities, such as scarcity and shortness of fungal microsatellite loci. A correlation was observed between mean repeat number and mean allele number in the published fungal microsatellite loci. The cross-species transferability of fungal microsatellites also appeared lower than in other phylogroups. However, microsatellites have been useful in some fungal species. Thus, the considerable advantages of these markers make their development worthwhile, and this study provides some guidelines for their isolation.

Introduction

Microsatellite loci, short tandemly repeated motifs of 1-6 bases, also known as simple sequence repeats (SSR), are widely used as genetic markers because of their ubiquity, ease to score, co-dominance, reproducibility, assumed neutrality and high level of polymorphism (Jarne and Lagoda, 1996). They have proved to be invaluable in many fields of biology, from genome mapping to forensics, paternity testing and population genetics (Jarne and Lagoda, 1996; Luikart et al., 2003). Their interest to biologists goes beyond their high polymorphism: when one can assume a model for their evolution, taking into account the number of repeats allows inference of kin relationships among alleles, and they can thus be developed as powerful tools for inferring evolutionary and demographic parameters (Cornuet et al., 1999; Luikart et al., 2003; Michalakis and Excoffier, 1996). The major drawback of microsatellite loci is that they often need to be isolated *de novo* from each species, which can be time-consuming and expensive. Cross-amplification, i.e. amplification of loci from a species other than the one in which they were cloned, is generally possible only among species of the same genera, and even in this case the percentage of cross-amplification is low (Rossetto, 2001). Furthermore, cross-amplification often generates null alleles which can bias genetic analyses (Hardy et al., 2003). In species for which no microsatellite markers for related species are available or cross-amplifiable, recently developed techniques, especially those involving enrichment of genomic DNA in microsatellites (Zane et al., 2002), have rendered the step of microsatellite isolation less laborious and more likely to succeed. However, the task of developing a working primer set from an enriched library can also represent a significant workload (Squirrell et al., 2003).

Microsatellites have been isolated across a wide range of taxonomic groups, but surprisingly little in fungi (Zane et al., 2002; Fig. 1). The low number of population geneticists interested in fungi compared to other organisms certainly explains this rarity, in

1 addition to a preference for anonymous markers such as random amplified polymorphic DNA
2 markers (RAPD), amplified fragment length polymorphisms (AFLP) and inter-simple
3 sequence repeats (ISSR). These markers have only two alleles per locus, but they are easy to
4 develop in large numbers without the fastidious step of building a genomic library, and they
5 generally yield enough polymorphism to differentiate individuals within populations.
6 However, for some fungal species, their lack of species specificity can represent a serious
7 problem. For instance in fungal pathogens, DNA of the focal species can be difficult to isolate
8 from those of the host and of hyperparasites (e.g. mycoparasites; Kiss, 1998), thus requiring
9 an *in vitro* isolation step. Another drawback of AFLP, RAPD and ISSR is dominance, which
10 prevents the detection of heterozygotes in diploid species. Finally, even for haploid species
11 convenient for *in vitro* culture, the problem of anonymity remains, which can introduce
12 serious bias in population genetic studies. Indeed, different alleles from a single locus cannot
13 be easily recognized and markers with occasional non Mendelian behaviour, such as
14 transposable elements, are frequently amplified by these techniques. Amplification problems,
15 such as null alleles are easy to detect in microsatellites, and as minute amounts of template are
16 required, culture can be bypassed.

17 In addition to the low number of population studies on fungi and the preference for
18 anonymous markers, some peculiar biological and genomic traits of fungi may have limited
19 the number of polymorphic microsatellite loci isolated from genomic libraries. First,
20 pathogens which are the most extensively studied species within fungi, have demographic and
21 reproductive traits promoting a low genetic diversity. Crop or human pathogens have for
22 instance often experienced recent bottlenecks, through geographical introduction (Engelbrecht
23 et al., 2004; Milgroom et al., 1992; Rivas et al., 2004) or host shifts (Mackenzie et al., 2001;
24 Paraskevis et al., 2003; Tobler et al., 2003), which can drastically reduce intraspecific genetic
25 diversity. Furthermore, some specific life history traits of fungal pathogens, such as frequent

1 asexual reproduction and recurrent bottlenecks in epidemic cycles, associated with low winter
2 survival and/or selective sweeps following new virulence attributes, are also likely to result in
3 low level of genetic diversity (Goodwin et al., 1994; Guérin and Le Cam, 2004; Hovmøller et
4 al., 2002). Second, fungal genomes may exhibit some peculiarities. Several recent papers
5 have examined the nature and abundance of microsatellites in published partial or complete
6 fungal genomes (Field and Wills, 1998; Karaoglu et al., 2005; Lim et al., 2004).
7 Microsatellites indeed appeared less abundant in these fungal genomes than in other
8 organisms (Morgante et al., 2002; Tóth et al., 2000), had different most abundant motifs
9 (Morgante et al., 2002; Tóth et al., 2000) and long loci were under-represented (Karaoglu et
10 al., 2005; Lim et al., 2004). Lim et al. (2004) reported that ca. 90% of microsatellite loci in 14
11 fungal genomes had low numbers of repeats, i.e. below eight. Long microsatellites, with high
12 numbers of perfect repeats, are more likely than short ones to be polymorphic because of a
13 higher rate of DNA replication slippage or unequal crossing-overs. Several studies have
14 indeed shown that the number of repeats is a good predictor of the level of variability in other
15 organisms (e.g. Brinkmann et al., 1998; Goldstein and Clark, 1995; Thuillet et al., 2002;
16 Vigouroux et al., 2002; Wierdl et al., 1997). If this correlation holds in fungi, most of their
17 microsatellites are expected to exhibit a low polymorphism. Furthermore, most of the
18 microsatellite loci detected in the published fungal genomes (94%) are mononucleotide
19 repeats (Lim et al., 2004) that are seldom used in population genetics because of difficulties in
20 scoring alleles separated by single base pairs. The shortness of microsatellite loci in fungi,
21 their weak representation in the genomes, the low abundance of useful motifs, together with
22 the small size of fungal genomes (between 10 and 40 Mb), may limit the ability to find
23 numerous polymorphic microsatellite, even when a genomic library is available.

24 It is not entirely clear, however, whether the genomic and biological specificities listed
25 above are the major factors limiting the development of microsatellite markers in fungi. First,

1 the conclusions drawn from these genomic studies are limited by the low number of complete
2 fungal genomes available, especially as a huge variability has been found among closely
3 related species in the number and nature of microsatellites (Ellegren, 2004; Karaoglu et al.,
4 2005; Lim et al., 2004). A survey of microsatellite development studies in different fungal
5 species would allow determination whether microsatellites are indeed generally difficult to
6 isolate and are particularly short. Second, another important limitation of genome analyses is
7 the lack of polymorphism assessment, which is the most valuable information for population
8 geneticists. Estimations of demographic or genetic parameters are indeed more powerful with
9 more polymorphic loci (e. g. Paetkau et al., 2004). If, for instance, microsatellite loci with
10 short repeats are reasonably polymorphic in fungi, their predominance in the genomes would
11 not be a problem for the development of useful markers. Comparing the degree of
12 polymorphism of microsatellites in fungi and in other organisms, and assessing whether the
13 correlation between the number of alleles and the number of repeats holds in fungi, are
14 therefore essential for determining whether attempts to develop microsatellites in this
15 kingdom are worthwhile given the investment required.

16 The aims of this paper were therefore to assess the yield of microsatellites from
17 enriched libraries in fungi and to compare the polymorphism of isolated fungal microsatellites
18 to that of other organisms, in order to determine whether obtaining microsatellite markers
19 with an acceptable level of polymorphism is generally harder in fungi than in other
20 organisms. The specific objectives of this paper were thus to (1) assess the yield of our own
21 seventeen microsatellite-enriched libraries, through the different steps, to identify which steps
22 limited the isolation of polymorphic loci; our data are free from publication bias, whereas
23 failures to develop polymorphic markers are rarely published; (2) estimate the general yield of
24 published microsatellite development in fungi; (3) evaluate the possibility of cross-
25 transferability of microsatellites among fungal species, which may represent an alternative to

the fastidious development of a genomic library; (4) assess whether there is a correlation between length and allele number among fungal microsatellites; (5) compare the nature, in particular the size, of fungal microsatellites and their level of polymorphism to those of other groups of organisms. In this study, we considered fungal species *sensus lato*, i.e. including Oomycota, because these organisms share similarities with true fungi in their morphology and life cycles, and many are also responsible for destructive plant diseases (Tyler, 2001).

Material and methods

Enriched libraries

The methods used to isolate our microsatellite loci were adapted from two protocols using oligoprobes for the enrichment of genomic libraries. The principle of both methods is the hybridization of restricted genomic DNA on microsatellite oligoprobes, followed by the washing of the non-hybridized genomic fragments. The first protocol, adapted from Edwards et al. (1996), uses membranes on which microsatellite oligoprobes are fixed. The second method is very similar, but uses streptavidin-coated magnetic beads on which biotin-labelled microsatellite oligoprobes are linked. Genomic fragments containing microsatellites hybridize with the oligoprobes, whose biotin links to the streptavidin of the magnetic beads. A magnet therefore allows retention of mostly DNA fragments with microsatellite loci (Kijas et al., 1994). The first method, with a membrane, was used for the species *Cryphonectria parasitica* (Breuillin et al., 2006), *Erysiphe alphitoides* (unpublished data) and *Melampsora larici-populina* (Barrès et al., 2006). The bead method was used in addition for *E. alphitoides* and *M. larici-populina*, and for the 14 other species: *Erysiphe necator* (unpublished data), *Fusarium culmorum* (Giraud et al., 2002b), *Fusarium poae* (unpublished data), *Magnaporthe grisea* (Kaye et al., 2003), *Microbotryum violaceum* (Giraud et al., 2002a), *Microcyclus ulei*

(Le Guen et al., 2004), *Mycosphaerella eumusae* (unpublished data), *Mycosphaerella fijiensis* (unpublished), *Mycosphaerella musicola* (unpublished data), *Penicillium camembertii* (unpublished data), *Penicillium roqueforti* (unpublished data), *Plasmopara viticola* (Delmotte et al., 2006), *Puccinia triticina* (Duan et al., 2003) and *Puccinia striiformis fsp tritici* (Enjalbert et al., 2002). For some species, several libraries had to be produced because of the poor yield of the first one(s).

Twelve out of 17 libraries (70%) were enriched for dinucleotide loci using (AC/TG)_n and (AG/TC)_n oligoprobes, with n= 10 or 15 (Table 1). The five other libraries were enriched using only (AC)₁₀. These two dinucleotide motifs were chosen for enrichment because they had generally been reported as the most frequent in complete fungal genomes (e.g. Lim et al., 2004). Ten libraries were also enriched with trinucleotide motifs (mainly (AAG)₁₀; Table 1).

For each of our enriched libraries, we recorded: (1) the percentage of positive clones, (2) the percentage of redundant sequences, i.e. of identical sequences, (3) the percentage of contaminant clones, i.e. with a significant BLAST value towards a sequence from another species, (4) the percentage of unique sequences, excluding contaminants, with a microsatellite locus (tandemly repeated motifs of 1-6 bases with at least 5 pure repeats, according to the most common definition; Ashley and Dow, 1994 ; Lim et al., 2004), (5) the percentage of unique sequences, excluding contaminants, with a microsatellite locus and suitable flanking regions, (6) the percentage of sequences yielding loci with a clear amplification, (7) the percentage of sequences yielding polymorphic loci at the intra-population level and (8) the percentage of sequences yielding polymorphic loci at the largest measured scale (from inter-population to inter-continental levels, or between populations from different host species). All the above percentages were estimated as ratios over the number of inserts correctly sequenced, except the percentage of positive clones, which was estimated over the total

number of clones with inserts. When several libraries had been built for one species, the average yield was taken for each step. In addition, we recorded for each polymorphic locus: (1) The base composition of the microsatellite motif (2) its perfection (a locus was considered as imperfect if the tandem repeats were interrupted or if several different tandem repeats with more than five repeats each were amplified as a single locus), and (3) the number of tandem repeats (for imperfect loci, number of repeats of the longest perfect microsatellite). The number of repeats was recorded from the sequenced fragment obtained in the library. Finally, we estimated genetic diversity of microsatellite loci as the number of alleles at the largest scale (spatially or inter-hosts). We also recorded the sample size used for assessing polymorphism.

11

Literature search and data extraction

To find studies reporting the development of microsatellite markers in fungi, we searched the bibliographic data bases Web of Knowledge (<http://isi4.newisiknowledge.com/>) and Pubmed (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi>) from January 1985 to June 2006 for all papers with "microsat* and fung* and (isol* or clon* or characteri*)" and "SSR and fung* and (isol* or clon* or characteri*)" in the title, keyword or abstract. We included all data from all papers to which we had access, regardless of the method of microsatellite isolation, except that we kept a single study per species and only the studies having isolated at least two polymorphic dinucleotide loci for comparison between species (see below). For each locus, we recorded the following information when available (1) the length and base composition of the motif, (2) its perfection, (3) the number of repeats of the longest perfect microsatellite (4) the sample size used for assessing polymorphism, (5) the number of alleles. We also recorded the number of loci for which primers could be designed, the percentage of scorable loci and the percentage of polymorphic loci per species, when available.

In addition, cross-species transferability of microsatellite markers in fungi was evaluated from published studies and our own data. We kept 20 studies for which one source species could be clearly identified and data on cross-species transferability was available within a genus, data at lower or higher taxonomic levels being scarce in fungi. In total, 24 source species, 88 target species and 302 primer pairs were tested across these studies. For each target species, a primer pair was considered as transferable when a PCR product of expected size was obtained in at least one individual. We computed the transferability as the mean percentage of loci that were transferable to other species.

To compare the yield and polymorphism of microsatellite development in fungi to those of other organisms, we searched issues of Molecular Ecology Notes from March 2001 to June 2005 for studies reporting isolation of microsatellites in angiosperms, insects, fish (restricted here to Actinopterygii), birds and mammals. These different phylogenetic groups ('phylogroups' hereafter) were chosen to span a wide range of living species and to include at least 50 studies, i.e. a number similar to that of published studies in fungi. We counted the number of primer notes for each phylogroup, and for the 50 most recent, we recorded for each polymorphic dinucleotide locus with a minimum of 5 repeats, the same items as for the fungal bibliographic data above. A few studies had to be discarded because they reported less than two polymorphic dinucleotide markers. The complete dataset included 2,923 microsatellite loci.

Data analyses

Using a Mann-Withney's test performed with Statistica 6.0 (Statsoft 2001), we compared 1) the yield of the different steps of microsatellite isolation, 2) the mean repeat number, and 3) the mean allele number per locus and per species, between our dataset and the published studies on other fungi. For the two latter comparisons, only dinucleotides (the most

isolated motifs in libraries) were retained to remove any possible effect of the length of the motif on allelic diversity.

To analyse the effect of the phylogroup (i.e. fungi, angiosperms, fishes, insects, birds or mammals) on the mean repeat number and on the mean allele number per species, unpublished studies on fungi were discarded and only polymorphic dinucleotide loci were retained, in order to have data similar to the other phylogroups. The phylogroup effect was tested using an analysis of variance, with the GLM procedure of the SAS software (SAS Institute, SAS Publishing, Cary, NC). Variables were Log-transformed for the residuals to reach normality. Pairwise mean comparisons among phylogroups were performed using Student-Newman-Keuls tests (SNK; Means option in GLM, SAS software). For the mean number of alleles per species, we retained only the studies with a minimum of 14 genotyped individuals to reduce the bias of a too small sample size.

The effects of the imperfection, the motif (CA/GT vs GA/CT, the other dinucleotides being too rarely isolated), the number of repeats, the sample size and the species on allele numbers were assessed using a generalized linear model (GENMOD procedure of SAS), assuming a Poisson distribution and a log-link function. Because the “allele” variable was over-dispersed, a scaling parameter was calculated to improve the fit to the Poisson distribution. Full models were first fitted including all factors and all interactions, and then simplified by sequential removal of the least significant highest-order interaction term, retaining significant interactions and all main effects, even when non-significant.

Results

Yield of our 17 fungal microsatellite-enriched libraries

In preliminary experiments, we tried to clone fungal microsatellites without enrichment in two species (*P. striiformis fsp triti* and *P. triticina*). The yield was so low (ca. 0.5% of positive clones) that enrichment appeared unavoidable. In our enriched libraries where the clones were screened for the presence of microsatellites, the mean percentage of positive clones ($\pm$ SE) was 20.2 % ($\pm$ 5.2). Five libraries had more than 30% of positive clones and four had fewer than 6 %.

After the cloning step, several problems were met due to the method of enrichment. First, a non negligible number of redundant clones were recovered in all experiments, probably due to the two PCR steps required for enrichment: the average number of sequences identical to previous sequences had a mean ($\pm$ SE) of 26.2 % ($\pm$ 5.1). Second, in three laboratories, contamination by foreign DNA occurred in six species (*P. roqueforti*, *P. camemberti*, *F. poae*, *P. viticola*, *M. larici-populina* and *C. parasitica*) and could reach up to 69% of the sequences. These contaminant sequences were easily identified: they were repeated several times in the libraries, blasted significantly to sequences in public databases, and/or were sequenced in the previous enrichments performed in the same laboratory. Third, problems in sequencing were met in most of the libraries, in several different laboratories, using either DNA extracted from clones or PCR products purified with various commercial kits. The failure of sequencing reactions seemed to be specific to our adaptors, *Mlu*I (Edwards et al., 1996), that may adopt a particular 3D structure when linked into vectors from the Topo TA Invitrogen kit, impeding sequencing reactions. The problems in sequencing may also be due to the presence of identical adaptors at each end of the insert. Proper sequences could only be obtained using a particular protocol of PCR product purification, using PEG (Rosenthal et al., 1993). Other studies have used adaptors encompassing a restriction site to avoid this problem (Armour et al., 1994; Tenzer et al., 1999).

1 In our 17 enriched libraries, the average percentage ($\pm$ SE) of unique sequences,
 2 excluding contaminants, having a microsatellite locus of at least five perfect repeats
 3 (Appendix 1) was only 55.4 % ($\pm$ 4.6) of the correctly sequenced inserts. Among those, the
 4 percentage of useful sequences consistently and sharply decreased along the different steps of
 5 the experiment (Fig. 2). The mean ($\pm$ SE) of the number of loci eventually polymorphic at the
 6 intra-population scale was only of 9.6 % ($\pm$ 2.5). One of the most critical steps was the
 7 suitability of the sequences for primer design (mean $\pm$ SE of 56.9 % $\pm$ 6.2 of unique
 8 sequences with a microsatellite), due to flanking regions with unsuitable base composition or
 9 length, or to microsatellites with too few perfect repeats. The percentage of amplified loci
 10 among those suitable for primer design was generally high (mean $\pm$ SE of 68% $\pm$ 8.2),
 11 although it was very low in some species. In *E. alphitoides*, *E. necator*, *F. poae*, *M. larici-*
 12 *populina*, and *P. viticola*, less than 45 % of the loci retained for primer design could actually
 13 be amplified (Appendix 1). The second most important source of attrition was the level of
 14 polymorphism obtained from amplifiable loci : the percentage of sequences that eventually
 15 yielded polymorphic loci ranged from 0 to 50 % of the initial number of sequenced clones,
 16 with only five species above 20% at the inter-population level (Appendix 1) and a mean ($\pm$
 17 SE) of 17.2 % ($\pm$ 3.5) (Fig. 2). The intra-population level of polymorphism was even lower,
 18 with a mean ($\pm$ SE) of 9.6 % ($\pm$ 2.7) of polymorphic loci (Fig. 2). In four species (the two
 19 *Puccinia* spp. and the two *Penicillium* spp.), no polymorphic loci at all could eventually be
 20 recovered at the intra-population scale (Appendix 1).

21 It was not possible to test here if the method of enrichment (membrane versus beads)
 22 or the length of oligoprobes impacted on the yield of libraries, because the number of studies
 23 was too low and because microsatellite isolation was performed using different methods in
 24 too few species (Table 1). However, regardless of the method, the general trend was a poor
 25 yield of enriched libraries in fungi. Furthermore, in the species for which different methods

1 were used (*M. larici-populina* and *E. alphitoides*), similar results were observed (data not
2 shown), suggesting a lack of protocol effect. There was also no indication that the libraries
3 enriched for both di- and trinucleotides had a better yield than the libraries enriched only for
4 dinucleotides (means of 17% (± 4.9) and 14% (± 4.3) of polymorphic loci isolated,
5 respectively). For four out of the ten species enriched with a trinucleotide oligoprobe, no
6 polymorphic trinucleotide loci could be isolated, and for three of them a single polymorphic
7 locus was recovered (data not shown). The mean percentage of polymorphic loci at the largest
8 scale seemed to be slightly higher in the five genomic libraries enriched using only (AC)₁₀
9 than in those enriched using both (AC/TG)_n and (AG/TC)_n (means of 25 % and 12%
10 respectively; Appendix 1), but there were too few studies to test this difference given the large
11 species effect (see below).

13 ***Comparison between our dataset and the published studies in fungi***

14 We compared the yield of our enriched libraries with that of the published studies in
15 other fungi to detect possible publication bias or specificities in our data. We collected data on
16 microsatellite isolation from 37 fungal species from the literature (Table 1). Among these, 14
17 % used non-enriched libraries and 43% used beads or membranes to enrich libraries. The
18 other methods of microsatellite isolation were based on ISSR (Burgess et al., 2001), FIASCO
19 (Zane et al., 2002), anchored PCR (Zane et al., 2002), and or searches in EST libraries
20 (Appendix 2). Despite the diversity of the methods of microsatellite isolation, the proportion
21 of polymorphic loci relative to the loci tested in fungal species was not significantly different
22 in the literature (mean $\pm$ SE of 49.7 ± 5.0 % among the sequences for which primers were
23 designed, Appendix 2) and in our dataset (53.2 ± 7.4 %; Mann-Withney's test, $Z = -0.43$, $P =$
24 0.67).

As in our libraries, most of the polymorphic loci in published studies on fungi were dinucleotides (69% and 88% for our data). Considering only the dinucleotide loci, the mean number of repeats per locus and per species was similar in our dataset and in the published studies (11.1 ± 0.7 vs 11.9 ± 0.8 ; Mann-Whitney's test, $Z = 0.11$, $P = 0.92$). In the 22 studies in which libraries were enriched for both the dinucleotides $(AC/GT)_n$ and $(AG/CT)_n$, consistently more polymorphic microsatellites were isolated with AC repeats than with AG repeats, regardless of the method of enrichment (5.3 ± 1.3 versus 2.8 ± 0.8 loci per species in the published studies and 8.7 ± 2.5 versus 2.3 ± 0.7 in our data, for $(AC/GT)_n$ and $(AG/CT)_n$ loci respectively).

Cross-species transferability of microsatellite markers between fungal species

Cross-species transferability of microsatellite primer pairs in fungi was estimated based on 24 studies from the literature and our own data. Only 34 % of the 1045 species/primer pair combinations tested within genera were successful in amplifying bands of the expected size. Neither homology, polymorphism nor presence of null alleles in the transferred microsatellite markers were generally assessed.

Comparison of fungal microsatellites with those of other organisms

Only 53% of the published loci were dinucleotides in birds, against ca. 70 % in fungi and fish and more than 80 % in plants, mammals and insects. More than 34% of the published loci were tetranucleotides in birds, against less than 5% in fungi, insects and plants.

Comparison of the 46 published studies on fungal microsatellite development with at least two dinucleotide polymorphic loci and the 50 published Primer Notes on microsatellites isolated from angiosperms, birds, mammals, fish and insects, found no significant difference in mean number of dinucleotide polymorphic loci per species among the phylogroups

(Kruskal-Wallis' test, $H(5,292) = 8.1$, $P = 0.15$). The mean number of polymorphic loci per species was not the lowest in fungi, with a mean $\pm$ SE of 9.3 ± 1.0 ; the other phylogroups ranging from 8.5 ± 0.8 (insects) to 13.6 ± 3.4 (fish).

The number of repeats per dinucleotide locus and per species had a significantly lower mean in fungi (11.8 ± 0.7) than in all the other phylogroups except birds (13.2 ± 0.6). The means of the other phylogroups ranged from 15.3 (insects) to 17.4 (mammals and fish) repeats per locus per species (Fig. 3A). The mean number of alleles per species also had a significantly lower mean in fungi (5.4 ± 0.4) than in all the other phylogroups (Fig. 3B). All these phylogroups had similar means of number of alleles per species, ca. 8 alleles, except fish that had a significantly higher mean (11.6 ± 1.2 ; Fig. 3B).

Factors affecting the diversity of dinucleotide microsatellites

In fungi, the correlation between the mean repeat number and the mean allele number per species was marginally significant ($r=0.28$, $P=0.06$, Fig. 4). The short loci indeed had a very low level of polymorphism, whereas long loci had a larger range of allele numbers, their polymorphism being high for some but still low for others. The mean repeat number and the mean allele number per species were significantly correlated in birds ($r=0.72$, $P<0.001$), insects ($r=0.40$, $P=0.007$) and fish ($r=0.32$, $P=0.03$), but not in mammals ($r=0.07$, $P=0.63$) or in angiosperms ($r=0.19$, $P=0.19$). However, GENMOD analyses on the complete dataset with all loci and taking into account the effects of the other parameters (see below) found the effect of the number of repeats on the number of alleles significant in all the phylogroups, the regression coefficients being consistently lowest in mammals and angiosperms.

A generalized analysis of variance was performed to further investigate which parameters influenced the diversity of individual microsatellite dinucleotide loci in fungi, among motif (considering only AC and AG), imperfection, repeat number, sample size and

species (Table 2). The main source of variation affecting the number of alleles of microsatellites was the differences among species. A significant effect of the number of repeats was also detected, whereas sample size, imperfection and motif were not significant.

Discussion

Consistent problems were met when isolating microsatellite loci from different fungal species. First, the yield of enriched libraries (percentage of positive clones) was low, mostly less than 30%. This percentage is at the lower limit of what has been obtained from other groups of organisms using the same protocols of microsatellite enrichment, which usually leads to 20% - 90 % of positive clones (Zane et al., 2002). Second, the attrition along the different steps from positive clones to polymorphic loci was very high (mean $\pm$ SE of 83.8 % $\pm$ 3.2). This attrition level is similar to that found in plants, which are known to be recalcitrant species for isolating microsatellite markers (Squirrell et al., 2003). For both fungi and plants, the percentage of loci suitable for primer design and the percentage of polymorphic loci seem to be the two most critical steps in microsatellite development. The low percentage of loci suitable for primer design, both in our data and the literature, is partly due to the choice of discarding loci with less than eight perfect repeats. The reason for disregarding them was that very low polymorphism, if any, is generally expected for such short microsatellites. The low polymorphism of short loci observed in fungi, and the positive correlation between number of repeats and number of alleles in fungal species, validated this choice. The final mean percentage of the initial non redundant sequences containing a polymorphic microsatellite at the intra-population level was less than 10%. In several fungal species, no polymorphic loci at

all could be found polymorphic at the within-population level, precluding any estimation of population genetics parameters, such as mode of reproduction or level of gene flow.

In addition to the established redundancy of sequences in enriched libraries (Squirrell et al., 2003), several of our libraries had high rates of contaminant sequences and/or a low success of primer amplification; in particular in *P. viticola*, *M. larici-populina*, *E. alphitoides*, *E. necator*, *P. roqueforti*, *P. camemberti*, and *F. poae*. In the cases of *P. roqueforti*, *P. camemberti* and *F. poae*, contamination was clearly due to the enrichment protocol, involving several PCR steps, because contaminant sequences were from *M. violaceum* which had previously been enriched for microsatellites in the same laboratory. Contaminations by the host DNA, bacteria or saprophytic fungi may explain the lack of amplification with tested primer pairs for the obligate pathogens *P. viticola*, *E. alphitoides*, *E. necator* and *M. larici-populina*. The contamination problems may be exaggerated in obligate pathogens by the small quantity of DNA that can be obtained after cultivation on their host, which favours the amplification of foreign DNA during the enrichment steps.

Published studies on microsatellite development from fungi experienced the same difficulties as our work, regardless of the method used. The yield of libraries was consistently low, both in terms of quantity and quality of loci isolated, with a few exceptions. It is likely that many attempts to develop microsatellite in other fungal species have never been published because the number of polymorphic loci isolated is too low, as was the case for our libraries: only nine of our 17 microsatellite developments have been published, five of the eight unpublished libraries having yielded each fewer than three loci polymorphic at the intra-population level. The proportion of polymorphic loci relative to the number of loci tested and the mean number of alleles per locus in fungi may therefore be yet lower than suggested by this survey.

1 Specific difficulties in developing fungal microsatellites were further revealed when
2 comparing microsatellites to those isolated in other organisms. The mean number of loci
3 isolated from fungi was similar to that of birds or insects, but they were significantly shorter
4 in fungi than in other phylogroups, except birds. This difference was even stronger when
5 considering all published microsatellites, i.e. with fewer than five repeats, which were not
6 included in our study according to our microsatellite definition. These very short
7 microsatellites represented almost 15 % of all the published loci in fungi, against less than 5%
8 in the other phylogroups. Despite a preference for loci with the highest numbers of repeats,
9 generally assumed to be the most polymorphic, the scarcity of long microsatellites in the
10 fungal libraries has certainly constrained the choice towards very short microsatellites. The
11 methods used to isolate microsatellites in fungal species may have affected the statistics of the
12 cloned loci, in particular their length. However, most of the methods used in fungi were also
13 used in the other phylogroups (Zane et al., 2002); enrichments with beads or membranes
14 being prominent in all the groups. Despite the noise that the different methods of isolation
15 may have introduced, the present literature survey based on more than 2200 dinucleotide loci
16 and 250 species showed that microsatellite loci isolated from fungal species had both
17 significantly fewer repeats and fewer alleles than those from the other phylogroups examined.

18 In addition, our study showed that there is little to expect from cross-species
19 transferability of microsatellites in fungi. Within genera, only 34% of the loci tested could be
20 transferred, which appears much lower than in animals or plants. In plants for instance, 76.4
21 % of 1800 species/primer combinations tested within genera were successful (Rossetto,
22 2001). This discrepancy may be due to higher levels of sequence divergence between fungal
23 species within genera in comparison to animals and plants. In fact, the percentage of protein
24 identity between three fungal species belonging to the genus *Aspergillus* has been found to be
25 comparable to that between mammals and fish (i.e. 66 to 70%; Galagan et al., 2005). This

lower transferability in fungi compared to other organisms suggests that the need to build a specific library for each species to be studied is even more crucial in fungi.

A first explanation for the low number and the lack of diversity of microsatellite loci in fungi is linked to the genomic specificities of these organisms. A general low density of microsatellites has been found in the complete fungal genomes available (Morgante et al., 2002; Tóth et al., 2000) as well as a rarity of long motifs (Lim et al., 2004; Karaoglu et al., 2005). Both the low density and the shortness of microsatellites should lower their propensity to hybridize on the probes used for enrichment. Furthermore, the positive correlation between microsatellite polymorphism and number of repeats in perfect tandem was shown to hold in fungi. Therefore, the general shortness of microsatellites in fungal genomes should not only impact on the number of isolated loci, but also, and more importantly, on their level of polymorphism. So far, we do not have any strongly supported explanation for the low density and the low level of repeat number observed in fungal microsatellites. It should, however, only depend on genomic processes limiting the number and the size of microsatellites but not on other fungal specificities such as life history traits. Measures of mutation rates, greatly facilitated in fungi by their asexual reproduction and rapid generation times, would be valuable for assessing the existence of an efficient mismatching repair system in fungi that limits expansion of microsatellites, as suggested by Karaoglu et al. (2005).

Restricted sampling design may also be invoked to explain the low polymorphism of fungal microsatellites. However, we recorded the results of fungal diversity from the largest spatial scale tested in each study, we retained only the studies with more than 14 strains, and the sampling scheme was often chosen to maximise diversity by testing the loci on populations that were as different as possible, i. e. from different countries or continents, or from different host species. There was no indication that the sample scale in fungi was less

representative of species diversity than that in the other phylogroups. The analysis of variance furthermore confirmed that the effect of sampling size on diversity was not significant in our survey.

In addition to genomic characteristics, i.e. shortness of loci, the lower polymorphism observed in fungal microsatellites compared to those in other phylogroups may be due to some specificities of their life-history traits or history. For instance, the species in which long microsatellites (i.e. higher than 12 perfect repeats in average) have been found but that exhibited a mean diversity lower than five alleles per locus were generally pathogens on few cultivated plants, or were recently introduced (e.g. *Ascochyta rabei*, *Cryphonectria parasitica*, *Magnaporthe grisea*, *Microcyclus ulei*, *Mycosphaerella fijiensis*, *M. musicola*, *Paecilomyces fumosoroseus*, *Phytophthora infestans*). These results are consistent with reduced diversity in these species being due to bottlenecks following introductions and/or recent host shifts, as may be the case in many studied fungi. However, additional analyses are required because the diversity in the life-history traits and in the population history of the species precluded detailed analyses testing for their effects on microsatellite polymorphism.

Although the overall yield of microsatellite development was generally low in fungi, microsatellites have been isolated and have proven to be polymorphic within populations in a few species. In fact, the main factor explaining the level of polymorphism among fungal species was the species identity. We could not identify clear factors explaining the high polymorphism in certain species. The most polymorphic of our 17 species (*C. parasitica*, *F. culmorum*, *M. violaceum* and *M. mycosphaerella*) were not necessarily those having the highest numbers of sequenced clones, tested primer, of individuals sampled, or largest spatial scales sampled, nor did they have any obvious peculiar history or reproductive mode. In *M. violaceum*, the diversity was certainly over-estimated because of the existence of cryptic

1 sibling species (Giraud et al. 2002a), but it does not seem to be the case in the other above
2 species. We could not find any obvious factors explaining the level of diversity per species in
3 the literature survey of fungal microsatellites either. Because there is no apparent rule, a good
4 strategy when deciding whether a microsatellite library is worth constructing may therefore be
5 to first evaluate the polymorphism of the species using other markers, such as AFLP, ISSR or
6 RAPD. Such methods do not require the investment that microsatellites do for development
7 and may serve as a preliminary test for the level of polymorphism in the focal species. For
8 instance, our own AFLP analyses conducted in parallel to the microsatellite ones on five
9 fungal species (Lopez-Villavicencio et al. 2005; Enjalbert et al. 2005; unpublished data)
10 indicated that when two individuals presented on average less than one band difference per
11 AFLP profile (using 2 selective bases on each primer), polymorphism was difficult to find
12 with microsatellites. We advocate that if AFLP on a given sample yields less than 2 %
13 difference between individuals per profile (i.e., one band difference on a profile with fifty
14 bands), microsatellites probably will not be polymorphic, and building enriched microsatellite
15 libraries may just be a waste of time and money. More efficient alternatives should then be
16 sought. For instance, polymorphism can be looked for in fragments generated by RAPD or
17 ISSR and specific primers can be developed such as sequence characterised amplified regions
18 (SCAR). Relative to a microsatellite library, this method is a rapid screen of putative
19 polymorphic loci. In addition, the rapid development of genomics may offer a new route for
20 efficiently developing powerful markers in fungi. In particular, expressed sequence tags or
21 extensive genomic libraries provide interesting material for finding long microsatellites (Kaye
22 et al. 2003; Breuillin et al. 2006) or other markers, such as single nucleotide polymorphism
23 (SNPs).

24

1 If microsatellite development is to be performed, the present study provides some
2 guidelines. First, this survey points to the necessity of using enriched protocols for isolation of
3 microsatellite markers. Our rare attempts to directly clone microsatellites from total DNA
4 failed and 86% of the published microsatellite fungal isolations were performed using
5 enriched protocols or searches in databases. It was not possible to test the effect of the
6 methods of enrichment on the yield of libraries and on the polymorphism of the loci isolated,
7 although the rare studies using several methods on a single species gave no indication of any
8 differences. However, we found enrichment with beads more convenient than with
9 membranes. Furthermore, it is worth noting that the ISSR method, more specifically used in
10 fungi, seemed to preferentially isolate short microsatellites, but comparisons of several
11 methods of isolation on the same species are required to provide compelling evidence for such
12 a bias. Second, regarding the choice of motif for enrichment, AG appears the most frequent in
13 the published fungal genomes (Lim et al., 2004). In contrast, we found that in the studies in
14 which libraries were enriched for both the dinucleotides AC and AG, consistently more
15 polymorphic microsatellites were isolated with AC repeats than with AG repeats. However,
16 the analyses of fungal genomes also underlined a high variability among species regarding the
17 most frequent motifs, with several genomes with a low density of AG motif and higher levels
18 of AT (Lim et al., 2004). The AT motif should, however, be avoided as self-complementarity
19 makes this motif difficult to amplify, and the number of repeats is generally low (Karaoglu et
20 al., 2005). As it is difficult to decide *a priori* which motifs are the most abundant in a given
21 species, the best strategy may be to enrich for both the AC and AG motifs in order to increase
22 the probability to recover numerous and long loci. In addition, although our libraries did not
23 yield many useful trinucleotide markers, enriching libraries for trinucleotides may be worth
24 trying. Such motifs indeed represented 24% of all the polymorphic published loci in fungi.
25 Third, our analyses confirmed that, after species identity, the best predictor of polymorphism

was the length of the longest perfect microsatellite. For developing polymorphic microsatellites, loci with the highest number of perfect repetitions should therefore be the best candidates. However, we found that it may be hard in many fungal species to isolate several microsatellite loci with more than eight repeats. Many loci with five to seven repetitions were polymorphic within populations in some fungal species, and those may thus be worth testing.

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References

- Armour, J., Neumann, R., Gobert, S. Jeffreys, A., 1994. Isolation of human simple repeat loci by hybridization selection. Hum. Mol. Genet. 3, 599-605.
- Ashley, M.V., Dow, B.D., 1994. The use of microstellite analysis in population biology: Background, methods and potential applications. In: Schierwater, B., Streit, B., Wagner, G., DeSalle, R. (Eds.), Molecular Ecology and Evolution, Approaches and Applications. Birkäuser Verlag, Basel, pp. 185-201.

- 1 Barrès, B., Dutech, C., Andrieux, A., Caron, H., Pinon, J., Frey, P. 2006. Isolation and
2 characterization of 15 microsatellite loci in the poplar rust fungus, *Melampsora larici-*
3 *populina*, and cross-amplification in related species. Mol. Ecol. Notes 6, 60-64.
- 4 Breuillin, F., Dutech, C., Robin, C., 2006. Genetic diversity of the chesnut blight fungus
5 *Cryphonectria parasitica* in four French populations assessed by microsatellite
6 markers. Mycol. Res. 110, 288-296.
- 7 Brinkmann, B., Klintschar, M., Neuhuber, F., Huhne, J., Rolf, B., 1998. Mutation rate in
8 human microsatellites: influence of the structure and length of the tandem repeat. Am.
9 J. Hum. Genet. 62, 1408-1405.
- 10 Burgess, T., Wingfield, M., Wingfield, B., 2001. Simple sequence repeat markers distinguish
11 among morphotypes of *Sphaeropsis sapinea*. App. Environ. Microbiol. 67, 354-362.
- 12 Cornuet, J.-M., Piry, S., Luikart, G., Estoup, A., Solignac, M., 1999. New methods employing
13 multilocus genotypes to select or exclude populations as origins of individuals.
14 Genetics 153, 1989-2000.
- 15 Delmotte, F., Chen, W.-J., Richard-Cervera, S., Greif, C., Papura, D., Giresse, X., Mondor-
16 Genson, G., Corio-Costet, M.-F., 2006. Microsatellite loci from the grape downy
17 mildew (*Plasmopara viticola*). Mol. Ecol. Notes. 6, 379-381.
- 18 Duan, X., Enjalbert, J., Vautrin, D., Solignac, M., Giraud, T., 2003. Isolation of twelve
19 microsatellite loci, using an enrichment protocol, in the phytopathogenic fungus
20 *Puccinia triticina*. Mol. Ecol. Notes 3, 65-67.
- 21 Edwards, K.J., Barker, J.H.A., Daly, A., Jones, C., Karp, A., 1996. Microsatellite libraries
22 enriched for several microsatellite sequences in plants. Biotechniques 20, 758-760.
- 23 Ellegren, H., 2004. Microsatellites: Simple sequences with complex evolution. Nature Rev.
24 Genet. 5, 435-445.

- 1 Engelbrecht, C., Harrington, T., Steimel, J. Capretti, P., 2004. Genetic variation in eastern
2 North American and putatively introduced populations of *Ceratocystis fimbriata* f.
3 *platani*. Mol. Ecol. 13, 2995-3005.
- 4 Enjalbert, J., Duan, X., Vautrin, D., Giraud, T., Vallavieille Pope, C. de, Solignac, M., 2002.
5 Isolation of twelve microsatellite loci, using an enrichment protocol, in the
6 phytopathogenic fungus *Puccinia striiformis* f.sp. *tritici*. Mol. Ecol. Notes 2, 563-565.
- 7 Enjalbert, J., Duan X., Leconte M., Hovmøller M., Vallavieille-Pope., C. de, 2005. Genetic
8 evidence of local adaptation of wheat yellow rust (*Puccinia striiformis* f. sp *tritici*)
9 within France. Mol. Ecol. 14, 2065-2073.
- 10 Field, D., Wills, C., 1998. Abundant microsatellite polymorphism in *Saccharomyces*
11 *cerevisiae*, and the different distributions of microsatellites in eight prokaryotes and *S.*
12 *cerevisiae*, result from strong mutation pressures and a variety of selective forces.
13 Proc. Natl. Acad. Sci. 95, 1647-1652.
- 14 Fisher, P., Gardner, R. Richardson, T., 1996. Single locus microsatellites isolated using 5'
15 anchored PCR. Nuc. Acids Res. 24, 4369-4371.
- 16 Galagan, J.E., Calvo, S.E., Cuomo, C., Ma, L.J., Wortman, J.R., Batzoglou, S., Lee, S.I.,
17 Basturkmen, M., et al., 2005. Sequencing of *Aspergillus nidulans* and comparative
18 analysis with *A. fumigatus* and *A. oryzae*. Nature 438, 1105-1115.
- 19 Giraud, T., Fournier, E., Vautrin, D., Solignac, M., Shykoff, J.A., 2002a. Isolation of 44
20 polymorphic microsatellite loci in three host races of the phytopathogenic fungus
21 *Microbotryum violaceum*. Mol. Ecol. Notes 2, 142-146.
- 22 Giraud, T., Fournier, E., Vautrin, D., Solignac, M., Vercken, E., Bakan, B., 2002b. Isolation
23 of eight polymorphic microsatellite loci, using an enrichment protocol, in the
24 phytopathogenic fungus *Fusarium culmorum*. Mol. Ecol. Notes 2, 121-123.

- 1 Goldstein, D., Clark, A., 1995. Microsatellite variation in North American populations of
2 *Drosophila melanogaster*. Nuc. Acids Res. 23, 3882-3886.
- 3 Goodwin, S., Cohen, B., Fry, W., 1994. Panglobal distribution of a single clonal lineage of
4 the Irish potato famine fungus. Proc. Natl. Acad. Sci. USA 91, 11591-11595.
- 5 Guérin, F., Le Cam, B., 2004. Breakdown of the scab resistance gene Vf in apple leads to a
6 founder effect in populations of the fungal pathogen *Venturia inaequalis*.
7 Phytopathology 94, 364-369.
- 8 Hardy, O.J., Charbonnel, N., Fréville, H., Heuertz, M., 2003. Microsatellite allele sizes: a
9 simple test to assess their significance on genetic differentiation. Genetics 163, 1467-
10 1482.
- 11 Hovmøller, M.S., Justesen, A.F., Brown, J.K.M., 2002. Clonality and long-distance migration
12 of *Puccinia striiformis* f. sp. *tritici* in north-west Europe. Plant Pathol. 51, 24-32.
- 13 Jarne, P., Lagoda, P.J.L., 1996. Microsatellites, from molecules to populations and back.
14 Trends Ecol. Evol. 11, 424-429.
- 15 Karaoglu, H., Lee, C.M.Y., Meyer, W., 2005. Survey of simple sequence repeats in
16 completed fungal genomes. Mol. Biol. Evol. 22, 639-649.
- 17 Kaye, C., Milazzo, J., Rozenfeld, S., Lebrun, M., Tharreau, D., 2003. The development of
18 simple sequence repeat markers for *Magnaporthe grisea* and their integration into an
19 established genetic linkage map. Fung. Genet. Biol. 40, 207-214.
- 20 Kijas, J.M.H., Fowler, J.C.S., Garbett, C.A., Thomas, M.R., 1994. Enrichment of
21 microsatellites from the Citrus genome using biotinylated oligonucleotide sequences
22 bound to streptavidin-coated magnetic particles. Biotechniques 16, 656- 660.
- 23 Kiss, L., 1998. Natural occurrence of *Ampelomyces* intracellular mycoparasites in mycelia of
24 powdery mildew fungi. New Phytol. 140, 709-714.

- 1 Le Guen, V., Rodier-Goud, M. Troispoux, V. Xiong, T.C., Brottier, P., Billot, C., Seguin, M.,
2 2004. Characterization of polymorphic microsatellite markers for *Microcyclus ulei*,
3 causal agent of South American leaf blight of rubber trees. Mol. Ecol. Notes 4, 122-
4 124.
- 5 Lim, S., Notley-McRobb, L., Lim, M., Carter, D.A., 2004. A comparison of the nature and
6 abundance of microsatellites in 14 fungal genomes. Fung. Genet. Biol. 41, 1025-1036.
- 7 Lopez-Villavicencio, M., Enjalbert, J., Hood, M.E., Shykoff, J.A., Raquin, C., Giraud, T.,
8 2005. The anther smut disease on *Gypsophila repens*: a case of parasite sub-optimal
9 performance following a recent host shift? J. Evol. Biol. 18, 1293-1303.
- 10 Luikart, G., England, P.R., Tallmon, D., Jordan, S., Taberlet, P., 2003. The power and
11 promise of population genomics: from genotyping to genome typing. Nature Rev.
12 Genet. 4, 981-994.
- 13 Mackenzie, J.S., Chua, K.B., Daniels, P.W., Eaton, B.T., Field, H.E., Hall, R.A., Halpin, K.,
14 Johansen, C.A., Kirkland, P.D., Lam, S.K., McMinn, P., Nisbet, D.J., Paru, R., Pyke,
15 A.T., Ritchie, S.A., Siba, P., Smith, D.W., Smith, G.A., van den Hurk, A.F., Wang,
16 L.F., Williams, D.T., 2001. Emerging viral diseases of Southeast Asia and the western
17 Pacific. Emerg. Infect. Dis. 7, 497-504.
- 18 Michalakis, Y., Excoffier, L., 1996. A generic estimation of population subdivision using
19 distances between alleles with special interest to microsatellite loci. Genetics 142,
20 1061-1064.
- 21 Milgroom, M.G., Liparis, S., Wang, K.R., 1992. Comparison of genetic diversity in the
22 chestnut blight fungus *Cryphonectria (Endothia) parasitica*, from China and the
23 United States. Mycol. Res. 96, 1114-1120.
- 24 Morgante, M., Hanafey, M., Powell, W., 2002. Microsatellites are preferentially associated
25 with non repetitive DNA in plant genomes. Nature Genet. 30, 194-200.

- 1 Paetkau, D., Slade, R., Burden, M., Estoup, A., 2004. Genetic assignment methods for the
2 direct, real-time estimation of migration rate: a simulation-based exploration of
3 accuracy and power. *Mol. Ecol. Notes* 13, 55-65.
- 4 Paraskevis, D., Lemey, P., Salemi, M., Suchard, M., Van de Peer, Y., Vandamme, A. M.,
5 2003. Analysis of the evolutionary relationships of HIV-1 and SIVcpz sequences
6 using Bayesian inference: Implications for the origin of HIV-1. *Mol. Biol. Evol.* 20,
7 1986-1996.
- 8 Rivas, G., Zapater, M., Abadie, C., Carlier, J., 2004. Founder effects and stochastic dispersal
9 at the continental scale of the fungal pathogen of bananas *Mycosphaerella fijiensis*.
10 *Mol. Ecol.* 13, 471-482.
- 11 Rosenthal, A., Coutelle, O., Craxton, M., 1993. Large-scale production of DNA sequencing
12 templates by microtitre format PCR. *Nuc. Acids Res.* 21, 173-174.
- 13 Rossetto, M., 2001. Sourcing of SSR markers from related plant species. In: Henry, R.J. (Ed.),
14 *Plant Genotyping : the DNA fingerprinting of plants*. CABI Publishers, New York, pp.
15 211-224.
- 16 Shinde, D., Lai, Y.L., Sun, F.Z., Arnheim, N., 2003. Taq DNA polymerase slippage mutation
17 rates measured by PCR and quasi-likelihood analysis: (CA/GT)(n) and (A/T)(n)
18 microsatellites. *Nuc. Acids Res.* 31, 974-980.
- 19 Squirrell, J., Hollingsworth, P.M., Woodhead, M., Russell, J., Lowe, A.J., Gibby, M., Powell,
20 W., 2003. How much effort is required to isolate nuclear microsatellites from plants?
21 *Mol. Ecol.* 12, 1339-1348.
- 22 Stephan, W., Langley, C.H., 1998. DNA polymorphism in *Lycopersicon* and crossing-over
23 per physical length. *Genetics* 150, 1585-1593.

- 1 Tenzer, I., degli Ivanissevich, S., Morgante, M. Gessler, C., 1999. Identification of
2 microsatellite markers and their application to population genetics of *Venturia*
3 *inaequalis*. *Phytopathology* 89, 748-753.
- 4 Thuillet, A.C., Bru, D., David, J., Roumet, P., Santomi, S., Sourdille, P., Bataillon, T., 2002.
5 Direct estimation of mutation rate for 10 microsatellite loci in durum wheat, *Triticum*
6 *turgidum* (L.) Thell. ssp durum desf. *Mol. Biol. Evol.* 19, 122-125.
- 7 Tobler, K., Ackermann, M., Griot, C., 2003. SARS-agent and lessons to be learned from
8 pathogenic coronaviruses of animals. *Schweiz Arch. Tierh* 145, 316-322.
- 9 Tóth, G., Gáspári, Z., Jurka, J., 2000. Microsatellites in Different Eukaryotic Genomes:
10 Survey and Analysis. *Genome Res.* 10, 967 - 981.
- 11 Tyler, B.M., 2001. Genetics and genomics of the oomycete–host interface. *Trends Genet.* 17,
12 611-614.
- 13 Vigouroux, Y., Jaqueth, J.S., Matsuoka, Y., Smith, O.S., Beavis, W.F., Smith, J.S.C.,
14 Doebley, J., 2002. Rate and pattern of mutation at microsatellite loci in maize. *Mol.*
15 *Biol. Evol.* 19, 1251-1260.
- 16 Wierdl, M., Dominska, M., Petes, T.D., 1997. Microsatellite Instability in Yeast: Dependence
17 on the Length of the Microsatellite. *Genetics* 146, 769-779.
- 18 Zane, L., Bargelloni, L., Patarnello, T., 2002. Strategies for microsatellite isolation: a review.
19 *Mol. Ecol.* 11, 1-16.
- 20

Figure legends

Figure 1. Number of primer notes published in Molecular Ecology Notes between March 2001 and June 2005 (black bars) on fungi, angiosperms, fish, birds, mammals and insects. For fungi, microsatellite isolation reports found in other journals are represented in grey.

Figure 2. Change in the average percentage of useful sequences along the different steps of microsatellite isolation for 17 fungal microsatellite-enriched libraries. Percentages are given for each step relative to the initial number of inserts correctly sequenced. Maximum and minimum values are given by the vertical lines and dashed lines indicate limits of the first and the third quartiles. See Appendix 1 for details on the libraries.

Figure 3. Boxplots of the number of repeats (A), and of the number of alleles (B) for fungi, angiosperms, fishes, birds, mammals and insects. Statistics are represented on the means per species for all primer notes published in Molecular Ecology Notes between March 2001 and June 2006 (and published in all journals for fungi). Boxes indicate quartiles, dark squares means and vertical traits minimal and maximal values. Different letters indicate significantly different groups of means in a SNK pairwise comparison test ($P < 0.05$).

1 **Figure 4.** Mean number of alleles per species plotted against mean number of repeats (of the
2 longest pure tandem repeat) for all available data on fungal dinucleotide microsatellites. The
3 regression line is drawn ($r=0.28$, $P=0.06$).
4

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Table 1. Fungal species for which the authors built microsatellite-enriched libraries, with their characteristics.

Species	Order	Method of enrichment	Motif for enriched libraries	Number of screened individuals
<i>Cryphonectria parasitica</i>	Ascomycota	membrane	(AC) ₁₅ (AG) ₁₅ (AAG) ₁₀	113
<i>Erysiphe alphitoides</i> (2 libraries)	Ascomycota	Membrane beads	(AC) ₁₅ (AG) ₁₅ (AAG) ₁₀ (AC) ₁₅ (AG) ₁₅	8
<i>Erysiphe necator</i> (2 libraries)	Ascomycota	beads	(TC) ₁₀ (TG) ₁₀	15
<i>Fusarium culmorum</i>	Ascomycota	beads	(AC) ₁₀ (AAG) ₁₀	20
<i>Fusarium poe</i>	Ascomycota	beads	(AC) ₁₀	20
<i>Magnaporthe grisea</i>	Ascomycota	beads	(TC) ₁₅ (AC) ₁₅	6
<i>Melampsora larici-populina</i> (5 libraries)	Basidiomycota	membrane beads beads	(AC) ₁₅ (AG) ₁₅ (AAG) ₁₀ (AC) ₁₅ (AG) ₁₅ (TC) ₁₀ (TG) ₁₀	30
<i>Microbotryum violaceum</i> (3 libraries)	Basidiomycota	beads	(AC) ₁₀ (AAG) ₁₀	30
<i>Mycosphaerella musicola</i>	Ascomycota	beads	(TC) ₁₅ (AC) ₁₅	15
<i>Mycosphaerella fijiensis</i>	Ascomycota	beads	(TC) ₁₅ (AC) ₁₅	15
<i>Mycosphaerella eumusae</i>	Ascomycota	beads	(TC) ₁₅ (AC) ₁₅	15
<i>Mycrocyclus ulei</i>	Ascomycota	beads	(TC) ₁₅ (AC) ₁₅	16
<i>Penicillium roqueforti</i> (4 libraries)	Ascomycota	beads	(AC) ₁₀ (AAG) ₁₀ (CAC) ₁₀ (GGA) ₁₀	5
<i>Penicillium camembertii</i>	Ascomycota	beads	(AC) ₁₀ (AAG) ₁₀	5
<i>Plasmopara viticola</i>	Oomycota	beads	(TC) ₁₀ (TG) ₁₀ (GAA) ₁₀ (TAA) ₁₀	100
<i>Puccinia striiformis fsp tritici</i>	Basidiomycota	beads	(AC) ₁₀ (AG) ₁₀ (AAC) ₁₀ (AAG) ₁₀	96
<i>Puccinia triticina</i>	Basidiomycota	beads	(AC) ₁₀ (AG) ₁₀ (AAC) ₁₀ (AAG) ₁₀	15

Table 2. Results of the GENMOD analysis testing for an effect of repeat number, imperfection and motif of the loci, species and sample size on the allele number of microsatellites ($R^2=0.49$).

Source	D. F.	Chi Square	P
Number of repeats	1	25.34	<0.0001
Imperfection	1	1.59	0.2076
motif	1	0.48	0.4888
Species	40	223.39	<0.0001
Sample size	1	0.49	0.4828

Number of published papers

Dutech et al. Figure 1

Dutech et al. Figure 2

Number of repeats

Figure 3A Dutech et al.

Number of alleles

Figure 3B Dutech et al.

Dutech et al. Figure 4

Appendix 1. Proportion of useful loci along the different steps of microsatellite development in 17 fungal species. The percentages are relative to the initial number of sequenced clones.

Species	Number of sequenced clones	Percentage of unique sequences with a microsatellite	Percentage of sequences suitable for primer design	Percentage of sequences yielding correct amplification	Percentage of sequences yielding polymorphic loci at the largest scale	Percentage of sequences yielding intra-population polymorphic loci
<i>Cryphonectria parasitica</i>	58	44.8	29.3	27.6	17.2	17.2
<i>Fusarium culmorum</i>	33	57.6	27.3	27.3	24.2	23.7
<i>Fusarium poae</i>	137	63.5	14.6	1.5	1.5	1.5
<i>Erysiphe alphitoides</i>	110	19.1	9.1	0.0	0.0	0.0
<i>Erysiphe necator</i>	96	79.2	18.8	8.3	3.1	2.1
<i>Magnaporthe grisea</i>	96	46.9	20.8	10.4	10.4	10.4
<i>Melampsora larici-populina</i>	208	17.8	17.8	7.2	6.7	6.3
<i>Microbotryum violaceum</i>	143	42.0	30.8	30.8	30.8	13.0
<i>Microcyclus ulei</i>	52	46.2	26.9	25.0	21.2	21.2
<i>Mycosphaerella eumusae</i>	58	63.8	32.8	19.0	15.5	15.5
<i>Mycosphaerella fijiensis</i>	111	46.8	43.2	29.7	29.7	29.7
<i>Mycosphaerella musicola</i>	64	53.1	34.4	18.8	18.8	18.8
<i>Penicillium camembertii</i>	24	66.7	54.2	50.0	50.0	0.0
<i>Penicillium roqueforti</i>	23	43.5	43.5	39.1	17.4	0.0
<i>Plasmopara viticola</i>	186	37.1	19.9	4.8	3.8	3.8
<i>Puccinia striiformis fsp tritici</i>	79	75.9	35.4	30.4	15.2	0.0
<i>Puccinia triticina</i>	130	82.3	27.7	23.8	9.2	0.0
Mean		52.1 ± 4.7	28.6 ± 3.9	20.8 ± 3.5	16.2 ± 3.2	9.6 ± 2.5

Appendix 2. Isolation and characterization of microsatellite loci in fungal species

OUR DATA

Species	Method of library enrichment	Nb of tested primers	Percentage of scorable loci	Percentage of polymorphic loci	Nb of loci used for analyses ⁽¹⁾	Nb of repeats ⁽²⁾	Nb of alleles ⁽²⁾	Nb of strains tested	References
<i>Cryphonectria parasitica</i>	Membrane and EST ⁽³⁾	17	94	59	8	13.3	4.9	113	Breuillin <i>et al.</i> (2006)
<i>Erysiphe necator</i>	Beads	18	44	11	DA	DA	DA	DA	Unpublished
<i>Fusarium culmorum</i>	Beads	9	100	89	7	13.0	6.1	20	Giraud <i>et al.</i> (2002a)
<i>Fusarium poae</i>	Beads	20	10	10	DA	DA	DA	DA	Unpublished
<i>Magnaporthe grisea</i>	Beads and EST ⁽³⁾	19	50	30	24	16.7	2.9	6	Kaye <i>et al.</i> (2003) and unpublished
<i>Melampsora larici-populina</i>	Beads and membrane	37	41	35	12	9.7	4.9	30	Barrès <i>et al.</i> (2005)
<i>Microbotryum violaceum</i>	Beads	44	100	100	44	11.4	5.4	30	Giraud <i>et al.</i> (2002b)
<i>Microcyclus ulei</i>	Beads	14	93	93	11	15.0	2.9	16	Le Guen <i>et al.</i> (2004) and unpublished
<i>Mycosphaerella eumusae</i>	Beads	19	58	47	9	8.6	2.9	15	Unpublished
<i>Mycosphaerella fijiensis</i>	Beads	48	69	69	33	10.2	4.6	15	Unpublished
<i>Mycosphaerella musicola</i>	Beads	19	63	63	12	10.2	4.1	15	Unpublished
<i>Penicillium camembertii</i>	Beads	13	100	92	11	12.2	2.6	5	Unpublished
<i>Penicillium roqueforti</i>	Beads	19	84	37	4	11.8	3.5	5	Unpublished
<i>Plasmopara viticola</i>	Beads	15	40	40	6	8.2	4.5	100	Delmotte <i>et al.</i> (2006)
<i>Puccinia striiformis fsp tritici</i>	Beads	28	86	43	11	7.3	2.5	96	Enjalbert <i>et al.</i> (2002)
<i>Puccinia triticina</i>	Beads	36	86	33	12	8.1	3.2	15	Duan <i>et al.</i> (2003)
Mean		23.4 ± 3.0	69.9 ± 7.0	53.2 ± 7.4	14.6 ± 2.9	11.1 ± 0.7	4.1 ± 0.3⁽⁴⁾	44.4 ± 12.1	

LITERATURE DATA

Species	Method of library enrichment	Nb of tested primers	Percentage of scorable loci	Percentage of polymorphic loci	Nb of published loci ⁽¹⁾	Nb of repeats ⁽²⁾	Nb of alleles ⁽²⁾	Nb of strains tested	References
<i>Alternaria brassicicola</i>	Beads	21	81	57	12	8.7	2.9	46	Avenot <i>et al.</i> (2005)
<i>Armillaria ostoyae</i>	Membrane	12	100	100	12	9.9	7.2	23	Langrell <i>et al.</i> (2001)
<i>Ascochyta rabiei</i>	Non enriched	37	70	54	20	12.6	4.2	22	Geistlinger <i>et al.</i> (2000)
<i>Aspergillus flavus</i>	GenBank ⁽⁵⁾	NA	NA	NA	6	13.4	7.4	20	Tran-Dinh & Carter (2000)
<i>Aspergillus parasiticus</i>	GenBank	NA	NA	NA	6	13.4	5.2	15	Tran-Dinh & Carter (2000)
<i>Aspergillus fumigatus</i>	Non enriched	8	100	50	4	19.3	14.0	100	Bart-Delabesse <i>et al.</i> (1998)
<i>Beauveria brongniartii</i>	Beads	11	91	91	10	11.8	3.8	96	Enkerli <i>et al.</i> (2001)
<i>Beauveria bassiana</i>	Beads	NR	NR	NR	8	20.0	6.3	24	Rehner & Buckley (2003)
<i>Botryosphaeria parva</i>	ISSR ⁽⁶⁾	20	NR	35	5	9.0	3.0	82	Slippers <i>et al.</i> (2004)
<i>Botryosphaeria rhodina</i>	ISSR	19	68	42	5	5.0	4.0	9	Burgess <i>et al.</i> (2003)
<i>Coccidioides immitis</i>	Non enriched	9	100	100	9	13.6	9.9	25	Fisher <i>et al.</i> (1999)
<i>Cryphonectria eucalypti</i>	FIASCO ⁽⁷⁾	NR	NR	NR	8	20.3	7.0	20	Nakabonge <i>et al.</i> (2005)
<i>Cylindrocladium parasiticum</i>	ISSR	25	NR	48	8	7.5	3.5	17	Wright <i>et al.</i> (2006)
<i>Dothistroma pini</i>	Anchored and EST	9	78	33	4	6.7	2.7	12	Ganley & Bradshaw (2001)
<i>Fusarium oxysporum</i>	ISSR	NR	NR	NR	9	12.5	11	64	Bogale <i>et al.</i> (2005)
<i>Heterobasidion annosum</i>	Beads	NR	NR	NR	5	11.3	6.5	19	Johannesson & Stenlid (2004)
<i>Laccaria bicolor</i>	Beads	150	26	5	7	8.7	7.0	12	Jany <i>et al.</i> (2006)
<i>Leptosphaeria maculans</i>	Non enriched and EST	36	94	19	6	9.5	2.3	14	Hayden <i>et al.</i> (2004)
<i>Lobaria pulmonaria</i>	Beads	21	57	52	11	14.5	8.3	30	Walser <i>et al.</i> (2003)
<i>Melampsora lini</i>	FIASCO	41	83	27	10	7.5	4	102	Barett & Brubaker (2006)
<i>Metarhizium anisopliae</i>	Beads	14	100	100	8	10.6	5.4	34	Enkerli <i>et al.</i> (2005)
<i>Mycosphaerella fijiensis</i>	Beads	19	58	58	10	20.2	5.0	29	Neu <i>et al.</i> (1999)
<i>Mycosphaerella musicola</i>	Beads	48	NR	54	24	22.1	3.4	24	Molina <i>et al.</i> (2001)
<i>Mycosphaerella populorum</i>	EST	10	NR	50	3	11	5	77	Feau <i>et al.</i> (2006)
<i>Paecilomyces fumosoroseus</i>	Beads	9	100	89	8	14.4	3.6	26	Dalleau-Clouet <i>et al.</i> (2005)

<i>Phaeosphaeria nodorum</i>	EST	73	NR	12	11	6.0	6.0	121	Stukenbrock et al. (2005)
<i>Phytophthora cinnamomi</i>	Membrane	10	40	40	4	20.5	5.3	5	Dobrowolski et al. (2002)
<i>Phytophthora ramorum</i>	Beads	24	58	29	7	10	3.5	26	Prospero et al. (2004)
<i>Phytophthora infestans</i>	Non enriched and EST	108	NR	11	13	15.3	4.7	90	Lees et al. 2006
<i>Pisolithus microcarpus</i>	RAPD-PCR	25	NR	24	4	5.5	4.0	30	Hitchcock et al. 2006
<i>Pisolithus spp.</i>	ISSR	8	100	75	3	5.0	4.5	39	Kanchanaprayudh et al. (2002)
<i>Pleurotus eryngii</i>	Beads	6	83	83	5	10.0	5.0	22	Della Rosa et al. (2004)
<i>Russula brevipes</i>	Enriched	26	23	23	5	6.3	5.7	27	Bergemann et al. (2005)
<i>Sclerotinia sclerotiorum</i>	Membrane	70	NR	36	17	9.9	3.7	44	Sirjusingh & Kohn (2001)
<i>Sphaeropsis sapinea</i>	ISSR	22	82	68	3	8.5	8.0	40	Burgess et al. (2001)
<i>Tuber magnatum</i>	Beads	23	91	35	8	18.5	7.5	370	Rubini et al. (2004)
<i>Venturia inaequalis</i>	Beads	52	40	40	17	10.7	10.1	44	Guérin et al. (2004)

Mean **31.2 ± 5.8** **75.0 ± 5.3** **49.7 ± 5.0** **8.6 ± 0.8** **11.9 ± 0.8** **5.8 ± 0.4⁽⁴⁾** **52.2 ± 10.8**

NA: Not applicable

NR: Not reported

⁽¹⁾ Number of polymorphic loci with a minimum of five repeats

⁽²⁾ Only dinucleotide loci considered

⁽³⁾ Loci isolated from an expressed sequence tags (EST) library

⁽⁴⁾ Loci isolated from genomic data available at <http://www.ncbi.nlm.nih.gov/>

⁽³⁾ Loci isolated from an enriched library using an intergenic simple sequence repeats (ISSR) protocol (Burgess et al., 2001)

⁽⁴⁾ Loci isolated from an enriched library using an anchored protocol (Fisher et al., 1996)

⁽⁵⁾

⁽⁶⁾ Loci isolated from an enriched library using a FIASCO protocol (Zane et al., 2002)

⁽⁷⁾ Mean estimated using only the studies with at least 14 haplotypes.

References

- Avenot, H., Dongo, A., Bataille-Simoneau, N., Iacomini-Vasilescu, B., Hamon, B., Peltier, D., Simoneau, P., 2005. Isolation of 12 polymorphic microsatellite loci in the phytopathogenic fungus *Alternaria brassicicola*. Mol. Ecol. Notes 5, 948-950.
- Barrès, B., Dutech, C., Andrieux, A., Caron, H., Pinon, J., Frey, P., 2006. Isolation and characterization of 15 microsatellite loci in the poplar rust fungus, *Melampsora larici-populina*, and cross-amplification in related species. Mol. Ecol. Notes 6, 60-64.
- Barrett, L.G., Brubaker, C.L., 2006. Isolation and characterization of microsatellite loci from the rust pathogen, *Melampsora lini*. Mol. Ecol. Notes 6, 930-932.
- Bart-Delabesse, E., Humbert, J.F., Delabesse, E., Bretagne, S., 1998. Microsatellite markers for typing *Aspergillus fumigatus* isolates. J. Clin. Microbiol. 36, 2413-2418.
- Bergemann, S.E., Miller, S.L., Garbelotto, M., 2005. Microsatellite loci from *Russula brevipes*, a common ectomycorrhizal associate of several tree species in North America. Mol. Ecol. Notes 5, 472-474.
- Bogale, M., Wingfield, B.D., Wingfield, M.J., Steenkamp, E.T., 2005. Simple sequence repeat markers for species in the *Fusarium oxysporum* complex. Mol. Ecol. Notes 5, 622-624.
- Breuillin, F., Dutech, C., Robin, C., 2006. Genetic diversity of the chestnut blight fungus *Cryphonectria parasitica* within four French populations assessed by microsatellite loci. Mycol. Res. 110, 288-296.
- Burgess, T., Wingfield, M.J., Wingfield, B.D., 2003. Development and characterization of microsatellite loci for the tropical tree pathogen *Botryosphaeria rhodina*. Mol. Ecol. Notes 3, 91-94.
- Burgess, T., Wingfield, M.J., Wingfield, B.W., 2001. Simple sequence repeat markers distinguish among morphotypes of *Sphaeropsis sapinea*. Appl. Environ. Microbiol. 67, 354-362.
- Dalleau-Clouet, C., Gauthier, N., Risterucci, A.M., Bon, M.C., Fargues, J., 2005. Isolation and characterization of microsatellite loci from the entomopathogenic hyphomycete, *Paecilomyces fumosoroseus*. Mol. Ecol. Notes 5, 496-498.
- Della Rosa, V., Cappuccio, I., Fanelli, C., Urbanelli, S., 2004. Isolation and characterization of microsatellite markers in two basidiomycete species: *Pleurotus eryngii* and *P. ferulae*. Mol. Ecol. Notes 4, 271-273.
- Delmotte, F., Chen, W., Richard-Cervera, S., Greif, C., Papura, D., Giresse, X., Mondor-Genson, G., Corio-Coste, M., 2006. Microsatellite DNA markers for *Plasmopara viticola*, the causal agent of downy mildew of grapes. Mol. Ecol. Notes 6, 379-381.
- Dobrowolski, M.P., Tommerup, I.C., Blakeman, H.D., O'Brien, P.A., 2002. Non-mendelian inheritance revealed in a genetic analysis of sexual progeny of *Phytophthora cinnamomi* with microsatellite markers. Fungal Genet. Biol. 35, 197-212.
- Duan, X., Enjalbert, J., Vautrin, D., Solignac, M., Giraud, S., 2003. Isolation of 12 microsatellite loci, using an enrichment protocol, in the phytopathogenic fungus *Puccinia triticina*. Mol. Ecol. Notes 3, 65-67.
- Enjalbert, J., Duan, X., Giraud, T., Vautrin, D., de Vallavielle-Pope, C., Solignac, M., 2002. Isolation of twelve microsatellite loci, using an enrichment protocol, in the phytopathogenic fungus *Puccinia striiformis f.sp. tritici*. Mol. Ecol. Notes 2, 563-565.
- Enkerli, J., Kolliker, R., Keller, S., Widmer, F., 2005. Isolation and characterization of microsatellite markers from the entomopathogenic fungus *Metarhizium anisopliae*. Mol. Ecol. Notes 5, 384-386.

- Enkerli, J., Widmer, F., Gessler, C., Keller, S., 2001. Strain-specific microsatellite markers in the entomopathogenic fungus *Beauveria brongniartii*. *Mycol. Res.* 105, 1079-1087.
- Feau, N., Jacobi, V., Hamelin, R.C., Bernier, L., 2006. Screening of ESTs from *Septoria musiva* (teleomorph *Mycosphaerella populorum*) for detection of SSR and PCR-RFLP markers. *Mol. Ecol. Notes* 6, 356-358.
- Fisher, M.C., White, T.J., Taylor, J.W., 1999. Primers for genotyping single nucleotide polymorphisms and microsatellites in the pathogenic fungus *Coccidioides immitis*. *Mol. Ecol.* 8, 1082-1084.
- Ganley, R.J., Bradshaw, R.E., 2001. Rapid identification of polymorphic microsatellite loci in a forest pathogen, *Dothistroma pini*, using anchored PCR. *Mycol. Res.* 105, 1075-1078.
- Geistlinger, J., Weising, K., Winter, P., Kahl, G., 2000. Locus-specific microsatellite markers for the fungal chickpea pathogen *Didymella rabiei* (anamorph) *Ascochyta rabiei*. *Mol. Ecol.* 9, 1939-1941.
- Giraud, T., Fournier, E., Vautrin, D., Solignac, M., Vercken, E., Bakan, B., Brygoo, Y., 2002a. Isolation of eight polymorphic microsatellite loci, using an enrichment protocol, in the phytopathogenic fungus *Fusarium culmorum*. *Mol. Ecol. Notes* 2, 121-123.
- Giraud, T., Fournier, E., Vautrin, D., Solignac, M., Shykoff, J.A., 2002b. Isolation of 44 polymorphic microsatellite loci in three host races of the phytopathogenic fungus *Microbotryum violaceum*. *Mol. Ecol. Notes* 2, 142-146.
- Guerin, F., Franck, P., Loiseau, A., Devaux, M., Le Cam, B., 2004. Isolation of 21 new polymorphic microsatellite loci in the phytopathogenic fungus *Venturia inaequalis*. *Mol. Ecol. Notes* 4, 268-270.
- Hayden, H.L., Wilson, L.M., Cozijnsen, A.J., Howlett, B.J., 2004. Characterization and cross-species amplification of microsatellite loci in the plant pathogenic fungus *Leptosphaeria maculans*. *Mol. Ecol. Notes* 4, 480-481.
- Hitchcock, C.J., Chambers, S.M., Cairney, J.W.G., 2006. Development of polymorphic simple sequence repeat markers for *Pisolithus microcarpus*. *Mol. Ecol. Notes* 6, 443-445.
- Jany, J.L., Bousquet, J., Gagne, A., Khasa, D.P., 2006. Simple sequence repeat (SSR) markers in the ectomycorrhizal fungus *Laccaria bicolor* for environmental monitoring of introduced strains and molecular ecology applications. *Mycol. Res.* 110, 51-59.
- Johannesson, H., Stenlid, J., 2004. Nuclear reassortment between vegetative mycelia in natural populations of the basidiomycete *Heterobasidion annosum*. *Fungal Genet. Biol.* 41, 563-570.
- Kanchanaprayudh, J., Lian, C., Zhou, Z., Hogetsu, T., Sihanonth, P., 2002. Polymorphic microsatellite markers of a *Pisolithus sp* from a Eucalyptus plantation. *Mol. Ecol. Notes* 2, 263-264.
- Kaye, C., Milazzo, J., Rozenfeld, S., Lebrun, M.-H., Tharreau, D., 2003. The development of simple sequence repeat markers for *Magnaporthe grisea* and their integration into an established genetic linkage map. *Fungal Genet. Biol.* 40, 207-214.
- Langrell, S.R.H., Lung-Escarmant, B., Decroocq, S., 2001. Isolation and characterization of polymorphic simple sequence repeat loci in *Armillaria ostoyae*. *Mol. Ecol. Notes* 1, 305-307.
- Le Guen, V., Rodier-Goud, M., Troispoux, V., Xiong, T.C., Brottier, P., Billot, C., Seguin, M., 2004. Characterization of polymorphic microsatellite markers for *Microcyclus ulei*, causal agent of South American leaf blight of rubber trees. *Mol. Ecol. Notes* 4, 122-124.
- Lees, A.K., Wattier, R., Shaw, D.S., Sullivan, L., Williams, N.A., Cooke, D.E.L., 2006. Novel microsatellite markers for the analysis of *Phytophthora infestans* populations. *Plant Pathology* 55, 311-319.

- Molina, C., Kaemmer, D., Aponte, S., Weising, K., Kahl, G., 2001. Microsatellite markers for the fungal banana pathogen *Mycosphaerella musicola*. Mol. Ecol. Notes 1, 137-139.
- Nakabonge, G., Cortinas, M.N., Roux, J., Gryzenhout, M., Wingfield, B.D., Wingfield, M.J., 2005. Development of polymorphic microsatellite markers for the fungal tree pathogen *Cryphonectria eucalypti*. Mol. Ecol. Notes 5, 558-561.
- Neu, C., Kaemmer, D., Kahl, G., Fischer, D., Weising, K., 1999. Polymorphic microsatellite markers for the banana pathogen *Mycosphaerella fijiensis*. Mol. Ecol. 8, 523-525.
- Prospero, S., Black, J.A., Winton, L.M., 2004. Isolation and characterization of microsatellite markers in *Phytophthora ramorum*, the causal agent of sudden oak death. Mol. Ecol. Notes 4, 672-674.
- Rehner, S.A., Buckley, E.P., 2003. Isolation and characterization of microsatellite loci from the entomopathogenic fungus *Beauveria bassiana* (Ascomycota : Hypocreales). Mol. Ecol. Notes 3, 409-411.
- Rubini, A., Topini, F., Riccioni, C., Paolucci, F., Arcioni, S., 2004. Isolation and characterization of polymorphic microsatellite loci in white truffle (*Tuber magnatum*). Mol. Ecol. Notes 4, 116-118.
- Sirjusingh, C., Kohn, L.M., 2001. Characterization of microsatellites in the fungal plant pathogen, *Sclerotinia sclerotiorum*. Mol. Ecol. Notes 1, 267-269.
- Slippers, B., Burgess, T., Wingfield, B.D., Crous, P.W., Coutinho, T.A., Wingfield, M.J., 2004. Development of simple sequence repeat markers for *Botryosphaeria* spp. with Fusicoccum anamorphs. Mol. Ecol. Notes 4, 675-677.
- Stukenbrock, E.H., Banke, S., Zala, M., McDonald, B.A., Oliver, R.P., 2005. Isolation and characterization of EST-derived microsatellite loci from the fungal wheat pathogen *Phaeosphaeria nodorum*. Mol. Ecol. Notes 5, 931-933.
- Tran-Dinh, N., Carter, D., 2000. Characterization of microsatellite loci in the aflatoxigenic fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Mol. Ecol. 9, 2170-2172.
- Walser, J.C., Sperisen, C., Soliva, M., Scheidegger, C., 2003. Fungus-specific microsatellite primers of lichens: application for the assessment of genetic variation on different spatial scales in *Lobaria pulmonaria*. Fungal Genet. Biol. 40, 72-82.
- Wright, L.P., Wingfield, B.D., Crous, P.W., Brenneman, T., Wingfield, M.J., 2006. Isolation and characterization of microsatellite loci in *Cylindrocladium parasiticum*. Mol. Ecol. Notes 6, 110-112.