

# Existence of a pattern of reproductive character displacement in *Homobasidiomycota* but not in *Ascomycota*

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## Abstract

Generally, stronger reproductive isolation is expected between sympatric than between allopatric sibling species. Such reproductive character displacement should predominantly affect premating reproductive isolation and can be due to several mechanisms, including population extinction, fusion of insufficiently isolated incipient species and reinforcement of reproductive isolation in response to low hybrid fitness. Experimental data on several taxa have confirmed these theoretical expectations on reproductive character displacement, but they are restricted to animals and a few plants. Using results reported in the literature on crossing experiments in fungi, we compared the degree and the nature of reproductive isolation between allopatric and sympatric species pairs. In accordance with theoretical expectations, we found a pattern of enhanced premating isolation among sympatric sibling species in *Homobasidiomycota*. By contrast, we did not find evidence for reproductive character displacement in *Ascomycota* at similar genetic distances. Both allopatric and sympatric species of *Ascomycota* had similarly low levels of reproductive isolation, being mostly post-zygotic. This suggests that some phylogeny-dependent life-history trait may strongly influence the evolution of reproductive isolation between closely related species. A significant correlation was found between degree of reproductive isolation and genetic divergence among allopatric species of *Homobasidiomycota*, but not among sympatric ones or among *Ascomycota* species.

## Introduction

Different types of reproductive isolation among closely related species may evolve depending on their geographical distribution. Incipient species evolving in different geographic areas (allopatry) have no opportunities to mate with each other; so, reproductive isolation is expected to arise gradually and slowly as a result of independent mutation, genetic drift and indirect effects of natural selection driving local adaptation (Coyne & Orr, 2004, pp. 83–110). By contrast, models of speciation

in sympatry usually involve the rapid selection of reproductive isolation (Coyne & Orr, 2004, pp. 125–178). When incipient species that have diverged in allopatry secondarily come into sympatry, several outcomes are possible: one of the two may go extinct, they may fuse if reproductive isolation is weak enough, or there may be a rapid evolution of premating isolation by selection for avoiding interspecific crosses, if the progeny is unfit because of post-zygotic isolation (Kirkpatrick & Ravigné, 2002). Altogether, stronger reproductive isolation is therefore expected between sympatric than allopatric sibling species. Such reproductive character displacement is expected to mainly affect premating (or prezygotic) reproductive isolation (but see Coyne & Orr, 2004, pp. 365–366) and has extensively been studied in the theoretical literature dealing with reinforcement (Noor, 1999; Servedio & Noor, 2003).

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Enhanced premating reproductive isolation in sympatry compared with allopatry has been detected in many natural cases, for instance among *Drosophila*, damselflies, frogs, fish, crickets, toads, birds, marine organisms and rodents (Coyne & Orr, 2004, pp. 357–360; Lukhtanov *et al.*, 2005; Smadja & Ganem, 2005; and references therein). Experimental data on several taxa have therefore confirmed the theoretical expectations on reproductive character displacement, but are almost exclusively restricted to animals and a few plants (Armbruster *et al.*, 1994; Coyne & Orr, 2004). Further, several studies have failed to detect enhanced isolation in *Drosophila*, toads and grasshoppers (Coyne & Orr, 2004, pp. 361–362). These inconsistencies may reflect the rarity of reinforcement in some taxa or may only be the consequence of looking at sister species with different histories, for instance with different evolutionary ages, or that have been in contact for different periods of time. Coyne & Orr (2004) therefore advocated that we need comparative studies to test for the frequency of reproductive character displacement, controlling for genetic divergence.

Fungi are interesting models for the study of evolution of reproductive isolation (Burnett, 2003). Many can be cultured and crossed under laboratory conditions and mycologists have long reported numerous mating experiments among fungal species. Second, fungi display a huge variety of life cycles, potentially allowing exploration of the influence of parameters such as dispersal ability on the speciation process. Third, numerous species complexes are known in fungi, encompassing multiple recently diverged sibling species. In the present study, we used previously published data on crossing experiments within fungal species complexes to investigate reproductive isolation patterns among close species in the two main groups of true fungi, *Ascomycota* and *Basidiomycota*. Our aims were to assess whether a pattern of reproductive character displacement existed in Fungi and whether reproductive isolation increased with genetic distance, among allopatric and/or sympatric species.

## Materials and methods

### Data set

We searched the bibliographic data bases Web of Knowledge (<http://isiknowledge.com/>) and Pubmed (<http://www.ncbi.nlm.nih.gov/>) from the last 20 years for all papers with 'hybrid\*' and 'fung\*', 'mating and fung\*', 'cross\*' and 'fung\*', 'biological species and fung\*' and 'reproductive isolation and fung\*' in the title, keyword or abstract. Older papers were found in the citation lists of these articles.

We only considered reproductive isolation within species complexes, i.e. between sibling species nearly indistinguishable morphologically, often grouped under a single species name, but for which evidence of genetic isolation had been reported, such as molecular evidence

of restricted gene flow and/or some level of reproductive isolation under laboratory conditions. Molecular evidence of independent evolution among sibling species were mainly from sequence data showing the existence of divergent monophyletic clades with bootstrap supports higher than 70, but also from isozymes, RFLP, AFLP and DNA/DNA hybridization data showing lack of gene flow (see references in Table 1).

We aimed at comparing patterns of *in vitro* reproductive isolation between allopatric and sympatric species pairs. It can, however, be difficult in some cases to assess whether distributions are allopatric or sympatric. To be conservative regarding the detection of reproductive character displacement, we chose to consider species pairs as sympatric only when: (1) it was clearly indicated in at least one paper; or (2) the geographic distribution of the individuals sampled strongly suggested overlapping distribution areas (e.g. individuals of the two species sampled in the same city, county, country or mountain).

### Fungal life cycles and experimental assessment of pre- versus post-mating isolation

Fungi having very diverse life cycles (Alexopoulos *et al.*, 1996), the description below is obviously an oversimplification of reality, but is given to introduce the terms used in the fungal literature and includes only the main features of fungal life cycles that are relevant for the nature of reproductive isolation.

In *Ascomycota*, sexual reproduction occurs between two strains of opposite haploid mating types (spores or mycelium). Plasmogamy occurs and the dikaryotic stage can be maintained during a few cellular divisions. Karyogamy then takes place, followed by meiosis, leading to asci formation containing haploid ascospores that can disperse. In most pathogenic *Ascomycota*, haploid ascospores germinate on the host and the resulting mycelium grows within or on its host, where sexual reproduction takes place (Alexopoulos *et al.*, 1996).

Regarding *Homobasidiomycota*, a haploid mycelium grows into the ground or a substrate and when two compatible mycelia come into contact, they fuse and produce clamp connections. A dikaryotic mycelium is then formed. The dikaryotic stage can persist for a long time. Fruiting bodies (basidia) are typically produced in basidiocarps (i.e. mushrooms) and yield basidiospores that can passively disperse, germinate and give rise to haploid mycelia.

The life cycle of the parasitic *Microbotryum violaceum*, the single *Basidiomycota* species complex not belonging to *Homobasidiomycota* in our study, can be considered as more similar to *Ascomycota*, with sexual reproduction occurring on the host just after meiosis and with plasmogamy occurring between single cells (Le Gac *et al.*, 2007a).

For *Basidiomycota*, the degree of reproductive isolation was calculated as  $1 - p$ , where  $p$  was the proportion of

Table 1 Species complexes.

Species complex	Life style and hosts for parasites	Sympatry			Allopatry			ITS accession numbers	Reference
		Number of species pairs	Mean IR $\pm$ SE	Genetic distance $\pm$ SE	Number of species pairs	Mean IR $\pm$ SE	Genetic distance $\pm$ SE		
(A) <i>Ascomycozia</i> <i>Ascochyta</i> sp.	Parasite (legumes)	4	0.56 $\pm$ 0.26	0.014 $\pm$ 0.006			DQ383950, 52-54	Kaiser <i>et al.</i> (1997), Hernandez-Bello <i>et al.</i> (2006), Peever <i>et al.</i> (2007)	
<i>Ascobotus</i> sp.	Saprophyte	1	1.00		2	0.88 $\pm$ 0.13	No	Meinhardt <i>et al.</i> (1984)	
<i>Botrytis cinerea</i>	Parasite (fruits, legumes, flowers)	1	0.75	0.00			E. Fournier pers. comm.	Fournier <i>et al.</i> (2005), E. Fournier and P. Leroux pers. comm.	
<i>Ceratocystis</i> sp.	Parasite (trees)	15	0.32 $\pm$ 0.08	0.023 $\pm$ 0.003	4	0.25 $\pm$ 0.18	U75618, 20, 26, AY214001, AY233907, 21, 25	Harrington & McNew (1998), Harrington <i>et al.</i> (1996)	
<i>Cochliobolus</i> sp.	Parasite (grasses and cereals)	9	0.81 $\pm$ 0.04	0.015 $\pm$ 0.005			AF071332, AF158105, 9, 10, AF163074, AB179836	Berbee <i>et al.</i> (1999), Nelson (1960)	
<i>Cryphonectria</i> sp.	Parasite (chestnut trees)	1	1.00	0.087			AY141873, AY143076	Hoegger <i>et al.</i> (2002)	
<i>Epichloë</i> sp.	Parasite (grasses)	13	0.50 $\pm$ 0.00	0.027 $\pm$ 0.004	11	0.50 $\pm$ 0.00	L07131, 36, 38, U57665, X62987, AB105953	Moon <i>et al.</i> (2004), Schardl <i>et al.</i> (1997), Schardl & Leuchtman (1999)	
<i>Erysiphe graminis</i>	Parasite (grasses)	6	0.50 $\pm$ 0.11	0.022 $\pm$ 0.006			AJ313137-40	Hlura (1978), Wyand & Brown (2003)	
<i>Giberella fujikuroi</i>	Parasite (cereals)	13	1.00 $\pm$ 0.00	0.032 $\pm$ 0.007	2	1.00 $\pm$ 0.00	U34555-60, U34568	Leslie (1991, 1995), O'Donnell <i>et al.</i> (1998)	
<i>Histoplasma capsulatum</i>	Parasite (humans, animals)	2	0.63 $\pm$ 0.13	0.028 $\pm$ 0.006	1	0.50	AF322377, 78, 86, 87	Kasuga <i>et al.</i> (1999), Taylor <i>et al.</i> (1999)	
<i>Neurospora</i> sp.	Saprophyte	9	0.50 $\pm$ 0.04	0.002	1	0.50	AY681192-93	Dettman <i>et al.</i> (2003)	
<i>Ophiostoma picea</i>	Parasite (trees)	3	1.00 $\pm$ 0.00	0.035 $\pm$ 0.008	2	1.00 $\pm$ 0.00	AY194505, AY573250, AY618239	Brasier & Kirk (1993), Uzunovic <i>et al.</i> (2000)	
<i>Ophiostoma ulmi</i> , <i>O. novo-ulmi</i> and <i>O. himal-ulmi</i>	Parasite (trees)	1	0.50	0.007	2	0.50 $\pm$ 0.00	AF196232, 34, 36	Brasier (2001), Brasier & Mehrotra (1995)	
<i>Phomopsis</i> sp.	Saprophyte	1	1.00				No	Brayford (1990)	
<i>Saccharomyces</i> sp.	Saprophyte	13	0.31 $\pm$ 0.06	0.017 $\pm$ 0.002	2	0.25 $\pm$ 0.00	AY130306-09, AY046148, EF457568	Naumov (1996), Liti <i>et al.</i> (2006), Murphy <i>et al.</i> (2006)	
<i>Venturia inaequalis</i>	Parasite (Apple trees, Pyracantha)	1	0.50	0.018			B. Le Cam, pers. comm.	Le Cam <i>et al.</i> (2002)	

Table 1 (Continued)

Species complex	Life style and hosts for parasites	Sympatry		Allopatry		Genetic distance $\pm$ SE	ITS accession numbers	Reference
		Number of species pairs	Mean IR $\pm$ SE	Number of species pairs	Mean IR $\pm$ SE			
(B) <i>Basidiomycota</i>								
<i>Amylostereum</i> sp.	Saprophyte/parasite (trees)	3	1.00 $\pm$ 0.00	0.033 $\pm$ 0.003	2	0.53 $\pm$ 0.08	DQ383950, 52-54	Boidin & Lanquetin (1984)
<i>Armillaria</i> sp.	Saprophyte/parasite (trees)	38	1.00 $\pm$ 0.00	0.028 $\pm$ 0.006	40	0.96 $\pm$ 0.02	No	Anderson <i>et al.</i> (1989)
<i>Collybia dryophila</i>	Saprophyte	12	1.00 $\pm$ 0.00		33	0.76 $\pm$ 0.08	E. Fournier pers. comm.	Vilgalys & Johnson (1987)
<i>Flammulina</i> sp.	Saprophyte/parasite (trees)	9	0.91 $\pm$ 0.08	0.031 $\pm$ 0.003	6	0.91 $\pm$ 0.09	U75618, 20, 26, AY214001, AY233907, 21, 25	Hughes <i>et al.</i> (1999), Petersen <i>et al.</i> (1999)
<i>Fomes pinicola</i>	Saprophyte/parasite (trees)	1	1.00		2	0.75 $\pm$ 0.00	AF071332, AF158105, 9, 10, AF163074, AB179836	Mounce & Macrae (1938)
<i>Hebeloma</i> sp.	Ectomycorrhizal	53	1.00 $\pm$ 0.00	0.010 $\pm$ 0.002			AY141873, AY143076	Aanen & Kuyper (1999), Aanen <i>et al.</i> (2000)
<i>Heterobasidium annosum</i>	Parasite (trees)	3	0.92 $\pm$ 0.04	0.017 $\pm$ 0.001	6	0.66 $\pm$ 0.13	L07131, 36, 38, U57665, X62987, AB105953	Harrington <i>et al.</i> (1989), Johannesson & Stenlid (2003), Stenlid & Karlsson (1991)
<i>Hyphoderma setigerum</i>	Saprophyte	8	1.00 $\pm$ 0.00	0.136 $\pm$ 0.009	6	1.00 $\pm$ 0.00	AJ313137-40	Nilsson <i>et al.</i> (2003)
<i>Laccaria laccata</i>	Ectomycorrhizal	4	1.00 $\pm$ 0.00		6	1.00 $\pm$ 0.00	U34555-60, U34568	Mueller (1991)
<i>Lentinula</i> sp.	Saprophyte	3	1.00 $\pm$ 0.00	0.116	6	0.50 $\pm$ 0.22	AF522377, 78, 86, 87	Mata <i>et al.</i> (2001)
<i>Microbotryum violaceum</i>	Parasite (Caryophyllaceae)	26	0 $\pm$ 0.00	NA			AY681192-93	Le Gac <i>et al.</i> (2007a, b)
<i>Marasmius androsaceus</i>	Saprophyte	1	1.00 $\pm$ 0.00		2	0.50 $\pm$ 0.25	AY194505, AY573250, AY618239	Gordon & Petersen (1997)
<i>Peniophora</i> sp.	Saprophyte	1	1.00 $\pm$ 0.00		2	0.45 $\pm$ 0.23	AF198232, 34, 36	Chamuris (1991)
<i>Pleurotus</i> sp.	Saprophyte	23	1.00 $\pm$ 0.00	0.124	12	0.42 $\pm$ 0.15	No	Vilgalys & Sun (1994)
<i>Polyporus</i> sp.	Saprophyte/parasite (trees)	10	1.00 $\pm$ 0.00	0.124 $\pm$ 0.013	9	0.94 $\pm$ 0.04	AY130306-09, AY046148, EF457568	Hoffmann (1978), Macrae (1967), McKay (1962), Kausarud <i>et al.</i> (2006)
<i>Serpula himantioides</i>	Saprophyte	1	1.00	0.015	1	1.00	B. Le Cam, pers. comm.	Kausarud <i>et al.</i> (2006)
<i>Sistotrema</i> sp.	Ectomycorrhizal	7	1.00 $\pm$ 0.00		4	1.00 $\pm$ 0.00	No	Ullrich (1973)

For each species complex used in the study, lifestyle and hosts for parasites, number of species pairs used in this study, mean degree of reproductive isolation detected *in vitro* and mean genetic distance among sympatric and allopatric species pairs, respectively, and references (A, *Ascomycota*, B, *Basidiomycota*).

crosses showing evidence of plasmogamy initiation, i.e. clamp connections in *Homobasidiomycota* and conjugations in *M. violaceum*. This represents a quantitative measure of the degree of pre-mating reproductive isolation, very similar to the measure of prezygotic isolation used by Coyne & Orr (1989). As basidiocarps (i.e. fruits) are very difficult to obtain *in vitro*, data were not available for calculating levels of post-mating reproductive isolation in *Homobasidiomycota*.

Quantitative results on the success of crosses are usually not reported in *Ascomycota*. For *in vitro* crosses, male and female gametes are usually mixed in Petri dishes, where the formation of asci and ascospores is observed. Data in publications report the formation, viability and fertility of ascospores, without quantitative information. We thus considered reproductive isolation in *Ascomycota* as a discrete measure where 1, 0.75, 0.50, 0.25 and 0 indicate, respectively, lack of ascus formation, asci without ascospores, abnormal ascospores, viable but sterile ascospores and fertile ascospores. Pre-mating reproductive isolation is thus indicated by an RI = 1. The other categories (0, 0.25, 0.50 and 0.75) refer to different levels of post-mating isolation. As the measure of post-zygotic isolation in Coyne & Orr (1989), the classes of reproductive isolation we scored in *Ascomycota* are discrete. However, our index corresponds to the sequential developmental stages of the post-mating isolation rather than to the possibility of obtaining viable and fertile hybrids of both sexes with the two possible reciprocal crosses, as was carried out by Coyne & Orr (1989). The distinction between sexes in the success of interspecific crosses is indeed not commonly made in fungi.

Most of the data obtained from the literature are best suited for comparing patterns of reproductive isolation at the species level (for example, species *x* and *y* are sympatric and display a degree of reproductive isolation of  $RI = u$ ), rather than at the population level (for example, species *x* population *a* and species *y* population *a* are sympatric and  $RI = u$ , but species *x* population *a* and species *y* population *b* are allopatric and  $RI = v$ ). For only two species complexes, *Neurospora* and *Heterobasidion*, were data clearly available at the population level, and these indicated stronger reproductive isolation in sympatry than in allopatry. In the *Heterobasidion* species complex, one species pair exhibited a mean RIs = 0.87 when the strains had the same geographic origin and a mean RIa = 0.17 when they had different geographic origins (Capretti *et al.*, 1990; Stenlid & Karlsson, 1991). We performed the statistical analyses (see below) considering this species pair in three different ways: (1) excluding this species pair; (2) considering this species pair as sympatric with  $RI = 0.52$  (i.e. the mean  $RI = (RIs + RIa)/2$ ); and (3) including this species pair both as sympatric with  $RI = 0.87$  and as allopatric with  $RI = 0.17$ . The three ways of considering this species pair led to very similar results. We only present the results with exclusion of this pair. In the *Neurospora* species

complex, Dettman *et al.* (2003) identified a higher level of reproductive isolation among sympatric strains than among allopatric. The difference in the success of crosses between sympatric and allopatric strains was, however, quantitative, which did not change the class of reproductive isolation of the *Ascomycota* species pairs as we scored it.

### Genetic distances

We used ITS sequences available in GenBank to compute genetic distances between sibling species pairs. Sequences were available for 133 species, distributed in 25 of the 33 fungal complexes used in our study. Alignments were performed using Bioedit v6.0.7 (Tom Hall, Isis pharmaceuticals Inc., Carlsbad, CA, USA) and corrected by hand when necessary. Genetic distances between species were calculated using Mega 3.1 (Kumar *et al.*, 2004) using two models, Kimura-2-parameter (K2P) and *p*-distance. Distances obtained with these models were very similar; we therefore present our results using only the K2P distance.

### Data analyses

Because different measures of reproductive isolation had to be used in *Ascomycota* and *Basidiomycota*, and because previous reviews had suggested that intersterility may evolve more easily in *Basidiomycota* than in *Ascomycota* (Burnett, 2003; Kohn, 2005), analyses were performed separately on the two groups. The single species complex of *Basidiomycota* not belonging to *Homobasidiomycota* in our data, *M. violaceum*, had a life cycle more close to that of *Ascomycota* for some aspects and its measures of reproductive isolation also corresponded to different phenomena (see above). We therefore performed analyses on *Ascomycota* and *Homobasidiomycota* separately.

Statistical analyses were performed using JMP (SAS Institute Inc., SAS Campus Drive, Cary, NC, USA) on each of two data sets to compare patterns of *in vitro* reproductive isolation between species living in sympatry and allopatry. The first data set considered the results of the crossing experiments performed between all the species pairs as the statistical units, each pair being classified as either allopatric or sympatric, and being assigned a measure of reproductive isolation and a genetic distance when available. This data set including all species pairs, however, presents the potential problem of having many points that are not phylogenetically independent. We therefore used a second data set that considered the species complexes as the statistical units. For each species complex, the mean degree of reproductive isolation ( $\pm$ SEM) and the mean genetic distance ( $\pm$ SEM) of the species pairs were computed, in sympatry and/or allopatry. However, although some of the species pairs within species complex are not phylogenetically independent, we believe that the evolution of reproductive

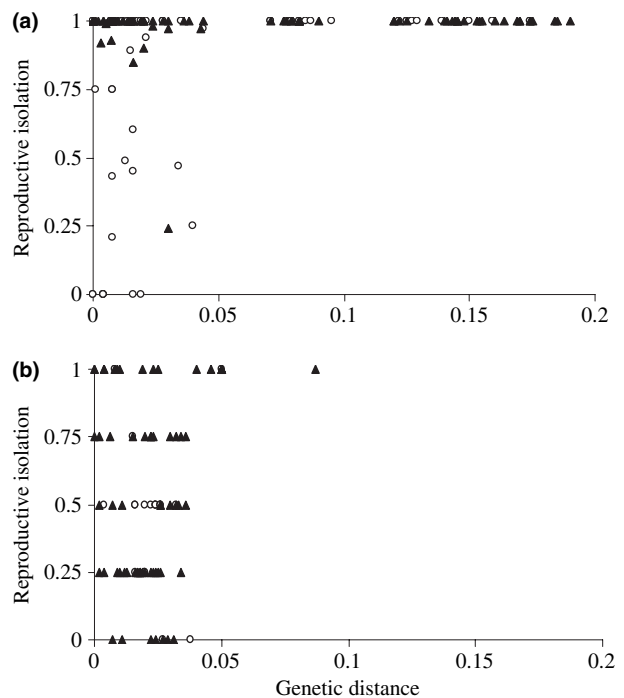
barriers could be considered as independent events, even within a species complex. Indeed, reproductive isolation has to evolve *de novo* for each speciation event and is not expected to be directly influenced by the type of reproductive barrier that evolved in the preceding speciation event. Evidence for the independent evolution of reproductive barriers is provided by the different types of reproductive barriers that are found within single species complexes.

Levels of reproductive isolation corresponded to discrete values in *Ascomycota* and to continuous values in *Homobasidiomycota*. To obtain similar statistical powers when performing statistical analyses within *Ascomycota* and within *Homobasidiomycota*, we transformed the reproductive isolation values obtained for *Homobasidiomycota* into discrete values prior to analyses. Reproductive isolation values of 0–0.24, 0.25–0.49, 0.50–0.74, 0.75–0.94 and 0.95–1.00 were, respectively, put in the classes 0, 0.25, 0.5, 0.75 and 1.

Nonparametric Wilcoxon rank tests were performed to assess whether there was a difference in degree of reproductive isolation between sympatric and allopatric species of *Ascomycota* on the one hand and *Homobasidiomycota* on the other hand. These tests were performed using both the data available for all species pairs and for the species pairs displaying a genetic distance <0.05 (see Results). For both *Ascomycota* and *Homobasidiomycota*, *t*-tests were performed to assess whether there was a difference in genetic distance between allopatric and sympatric species pairs. Finally, Spearman rank correlation tests were performed to investigate the relationship between reproductive isolation and genetic distance, in sympatry and allopatry, within *Ascomycota* and within *Homobasidiomycota*, using the data available both for all species pairs and for the species pairs with a genetic distance <0.05.

## Results

We obtained data on crossing experiments among sibling species for 16 species complexes of *Homobasidiomycota* and 16 species complexes of *Ascomycota* (Table 1). We could estimate genetic distances between sibling species within nine of the *Homobasidiomycota* species complexes and 14 of the *Ascomycota* species complexes (Table 1). The mean genetic distance among *Homobasidiomycota* species pairs ( $\text{dist}_H = 0.054 \pm 0.005$ ) was greater than that among *Ascomycota* species pairs ( $\text{dist}_A = 0.023 \pm 0.002$ ) (Fig. 1). To perform the analyses on species complexes of similar ages for *Homobasidiomycota* and *Ascomycota*, we used a restricted data set including only species pairs with a genetic distance below 0.05 (Fig. 2). In this restricted data set, genetic distances were not significantly different between allopatric and sympatric species pairs or species complexes, both within *Ascomycota* and *Homobasidiomycota* (Table 2). There was therefore no age bias in allopatry vs. sympatry comparisons.

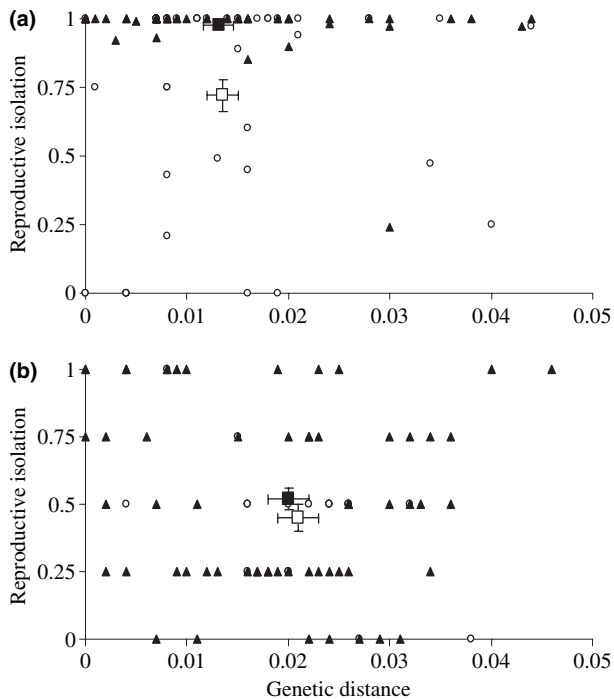


**Fig. 1** Reproductive isolation between species pairs as a function of genetic distance. Plot of the degree of reproductive isolation as a function of genetic distance (K2P), between all species pairs; in (a) *Homobasidiomycota*, (b) *Ascomycota*, with indication of the geographical situation: black triangles for allopatry and white circles for sympatry.

## Reproductive character displacement in *Homobasidiomycota*

Reproductive isolation data were available for 310 species pairs within 16 complexes of *Homobasidiomycota* (Table 3, Figs 1–3). Complete or nearly complete reproductive isolation was found between virtually all sympatric species pairs: 170 pairs showed complete reproductive isolation ( $RI = 1$ ) and seven pairs showed nearly complete reproductive isolation ( $RI$  between 0.84 and 0.97). A single sympatric species pair, from the genus *Flammulina*, exhibited high compatibility ( $RI = 0.24$ ). Among allopatric species pairs, 93 pairs presented complete reproductive isolation ( $RI = 1$ ), three pairs showed strong isolation ( $RI$  between 0.84 and 0.97), 17 pairs had intermediate levels of compatibility ( $RI$  between 0.21 and 0.75) and 19 pairs showed full compatibility ( $RI = 0$ ).

Reproductive isolation was found to be significantly stronger in sympatry than in allopatry for *Homobasidiomycota*, considering either the species pairs or the species complexes as the statistical units (Table 2). Genetic distances between species pairs could be estimated for 165 species pairs. All pairs with a genetic distance higher than 0.05 presented total incompatibility ( $RI = 1$ ,  $n = 60$ , Fig. 1). Considering only the species pairs with a genetic



**Fig. 2** Reproductive isolation between species pairs as a function of genetic distance, for genetic distances lower than 0.05. Plot of the degree of reproductive isolation as a function of genetic distance (K2P), between all species pairs having a genetic distance below 0.05; (a) *Homobasidiomycota*, (b) *Ascomycota*, with indication of the geographical situation: black triangles for allopatry and white circles for sympatry. Mean values and standard errors are represented separately for allopatry (black square) and sympatry (white square).

distance below 0.05, reproductive isolation was still significantly stronger in sympatry than in allopatry (Table 3).

### Lack of reproductive character displacement in *Ascomycota*

Results of crossing experiments were available for 120 species pairs within 16 complexes of *Ascomycota* (Table 3, Figs 1–3). Among sympatric species pairs, only 24 pairs showed complete reproductive isolation (RI = 1),

whereas 64 showed only post-zygotic isolation (23 pairs with RI = 0.25, 23 pairs with RI = 0.5 and 18 pairs with RI = 0.75) and six pairs presented full compatibility (RI = 0).

Among allopatric species pairs, five pairs showed complete reproductive isolation (RI = 1), 20 pairs showed post-zygotic isolation (three pairs with RI = 0.25, 15 pairs with RI = 0.5 and two pairs with RI = 0.75) and two pairs had full compatibility (RI = 0).

There was no significant difference between sympatric and allopatric pairs of *Ascomycota* in the degree of reproductive isolation, considering either the species pairs or the species complexes as the statistical units (Table 3). Genetic distances were obtained for 89 species pairs. The 10 pairs with a genetic distance higher than 0.05 showed complete reproductive isolation (RI = 1). Considering only the pairs with a genetic distance below 0.05, there was still no significant difference in the degree of reproductive isolation between sympatric and allopatric species pairs (Table 3).

### Reproductive isolation as a function of genetic distance

We investigated whether the degree of reproductive isolation and genetic distance were correlated, in sympatry and in allopatry, for both *Ascomycota* and *Homobasidiomycota*. We only found a significant correlation among allopatric species pairs of *Homobasidiomycota* in the complete data set (Table 4).

## Discussion

### Reproductive character displacement

Our results suggest that there are contrasting patterns of reproductive character displacement in fungi. *Homobasidiomycota* showed evidence for reproductive character displacement, with stronger reproductive isolation in sympatry than in allopatry, but *Ascomycota* did not. This was observed for similar genetic distances of *Ascomycota* and *Homobasidiomycota* species pairs, and the species complexes had similar genetic distances in allopatry and in sympatry in the two groups. This indicates that the difference between *Ascomycota* and *Homobasidiomycota* in

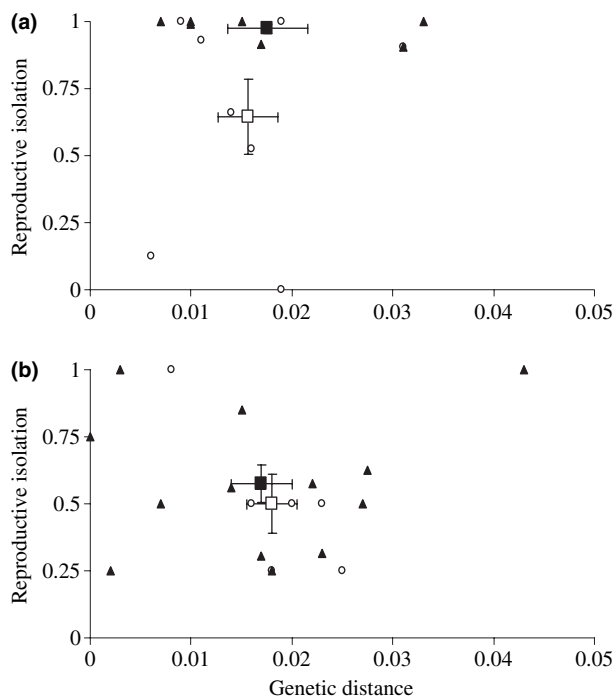
**Table 2** Results of the *t*-test for assessing whether genetic distances are different between allopatric and sympatric species pairs or species complexes, within *Ascomycota* and within *Homobasidiomycota* respectively.

		Genetic distance		<i>t</i>	d.f.	<i>P</i> -value
		In sympatry	In allopatry			
<i>Ascomycota</i>	Complexes	0.017 ± 0.012	0.018 ± 0.006	0.288	17	0.78
	Pairs	0.019 ± 0.011	0.021 ± 0.008	0.923	77	0.36
<i>Homobasidiomycota</i>	Complexes	0.018 ± 0.010	0.016 ± 0.008	0.414	13	0.69
	Pairs	0.013 ± 0.002	0.014 ± 0.002	0.178	103	0.86

**Table 3** Results of the nonparametric Wilcoxon rank test to assess the difference in reproductive isolation between sympatric and allopatric species complexes or species pairs, within *Ascomycota* and within *Homobasidiomycota*.

		Ns	Na	RIs	RIa	Z	P-value
(A)							
<i>Ascomycota</i>	Pairs	94	27	0.58 ± 0.03	0.55 ± 0.05	0.45	0.65
	Complexes	16	9	0.68 ± 0.06	0.60 ± 0.10	0.91	0.36
<i>Homobasidiomycota</i>	Pairs	178	132	0.99 ± 0.01	0.79 ± 0.03	6.66	<0.0001
	Complexes	16	14	0.99 ± 0.01	0.70 ± 0.08	3.52	0.0004
(B)							
<i>Ascomycota</i>	Pairs	60	19	0.52 ± 0.04	0.45 ± 0.05	0.62	0.54
	Complexes	13	6	0.58 ± 0.07	0.50 ± 0.11	0.85	0.40
<i>Homobasidiomycota</i>	Pairs	58	47	0.98 ± 0.01	0.72 ± 0.06	4.20	<0.0001
	Complexes	7	8	0.97 ± 0.02	0.64 ± 0.14	2.04	0.04

Numbers of sympatric (Ns) and allopatric (Na) units, average reproductive isolation in sympatry (RIs) and allopatry (RIa) ± standard error, absolute Z scores, and P-values are indicated (A) on the complete data set; (B) on species pairs with genetic distances below 0.05.



**Fig. 3** Reproductive isolation as a function of genetic distance within species complexes, for genetic distances lower than 0.05. Plot of the degree of reproductive isolation as a function of genetic distance (K2P), within complexes whose species pairs had a genetic distance below 0.05; in (a) *Homobasidiomycota*, (b) *Ascomycota*, with indication of the geographical situation: black triangles for allopatry and white circles for sympatry. Mean values and standard errors are represented separately for allopatry (black square) and sympatry (white square).

the pattern of reproductive isolation was not because of biases in ages of the species.

Data gathered in the literature may be subject to publication bias. For instance, one can imagine that existence of reproductive isolation can be reported more

**Table 4** Results of the Spearman rank correlation test between genetic distance and degree of reproductive isolation, among sympatric or allopatric species pairs or species complexes, within *Ascomycota* and within *Homobasidiomycota* respectively.

		Sympatry		Allopatry	
		$\rho$	P-value	$\rho$	P-value
(A)					
<i>Ascomycota</i>	Complexes	0.43	0.13	0.31	0.55
	Pairs	0.18	0.14	-0.19	0.42
<i>Homobasidiomycota</i>	Complexes	0.21	0.59	0.1	0.82
	Pairs	0.12	0.23	0.46	<0.0001
(B)					
<i>Ascomycota</i>	Complexes	0.07	0.82	-0.62	0.19
	Pairs	-0.12	0.35	-0.41	0.09
<i>Homobasidiomycota</i>	Complexes	-0.48	0.28	0.12	0.77
	Pairs	-0.01	0.52	0.23	0.12

(A) On the complete data set; (B) on species pairs with genetic distances below 0.05.

often than the lack of a reproductive barrier, especially between sympatric species pairs. However, the numerous examples of *Ascomycota* species pairs reported in the literature that are genetically isolated, but do not display any form of pre-mating reproductive isolation, or even any reproductive isolation at all, indicate that such a bias is minimum and there is no reason why it should be present in *Homobasidiomycota* but not in *Ascomycota*.

In *Homobasidiomycota*, reproductive isolation was stronger between sympatric species pairs than between allopatric species pairs, and this was due to enhanced pre-mating reproductive isolation due to sexual partner recognition. All but one of the sympatric species pairs indeed displayed very strong pre-mating reproductive isolation. This suggests the existence of a strong selective pressure for the evolution of pre-mating barriers between closely related species in sympatry, such as reinforcement in response to low hybrid fitness (Noor, 1999).

Alternatively, reproductive character displacement can result from other mechanisms, such as the extinction of one population or the fusion of insufficiently isolated incipient species after a secondary contact.

A pattern of reproductive character displacement has been shown to occur in several taxa, but studies generally focused on particular animal species pairs, by comparing reproductive isolation between sympatric and allopatric populations (Coyne & Orr, 2004, pp. 357–365). By contrast, we provide evidence for reproductive character displacement at the scale of a large taxonomic group. *Homobasidiomycota* are thus good models for studying speciation, in particular for studying the evolution of pre-mating reproductive isolation. However, they are virtually never considered in theoretical work or in reviews (see, for instance, Coyne & Orr, 2004). Many examples of fungal speciation have, however, been studied in detail (Burnett, 2003; Kohn, 2005; Dettman *et al.*, 2003; Le Gac *et al.*, 2007a).

In contrast to *Homobasidiomycota*, we found no clear pattern of reproductive character displacement in *Ascomycota*. In most of the cases, both allopatric and sympatric species pairs had similarly low levels of reproductive isolation and the reproductive barriers were mostly post-mating. It is in fact intriguing to find in *Ascomycota* many cases of pairs of closely related species in sympatry that are not isolated by a strong pre-mating barrier. Theory usually predicts that such species pairs should fuse, undergo reinforcement or one of the species should go extinct (Noor, 1999). The lack of pre-mating isolation among sympatric pairs of *Ascomycota* may be due to a peculiarity of their life cycle. The mycelium of most of the *Homobasidiomycota* grows into the ground where it may encounter mycelia from other species. On the contrary, the mycelium of most of the *Ascomycota* grows within or on a specific substrate (a host) and because their gametes have very low dispersal abilities, *Ascomycota* can mate only where their parent mycelium is able to grow. As a result, species specialized on different hosts rarely have the opportunity to mate even in sympatry. Thus, such species may be functionally allopatric. A recent model based on such a life cycle predicts that in these parasites sibling species can be maintained in sympatry through strong specialization alone, even in the absence of pre-mating isolation due to recognition (Giraud, 2006; Giraud *et al.*, 2006). Most of the *Ascomycota* species complexes included in this study were in fact plant pathogens (12 of 16). Further, the above mechanism of ecological speciation can be generalized to nonparasite *Ascomycota* species specialized in different habitats. We believe that the contrasted pattern found here, i.e. reproductive character displacement in *Homobasidiomycota* but not in *Ascomycota*, is consistent with the mechanism of speciation by mere specialization described by Giraud *et al.* (2006) in which specialization pleiotropically generates both adaptation and reproductive isolation.

Alternative explanations for the maintenance of sibling species in the same geographic area without evolution of strong pre-mating barriers include: (1) the possibility that sexual reproduction is not frequent in nature, impeding strong selection for enhanced pre-mating isolation and fusion of insufficiently isolated populations; (2) the possibility that the sibling species only share a restricted geographic contact zone (parapatry). In this case, the local selective pressure in the contact zone may not be able to overcome the gene flow from nonsympatric areas.

### Tempo of speciation

In addition to comparing the degree of reproductive isolation among sympatric and allopatric fungal species, we also looked at the evolution of reproductive isolation with genetic divergence. Reproductive isolation among allopatric incipient species is expected to rise gradually and slowly as a result of independent mutation, genetic drift and indirect effects of natural selection driving local adaptation (Coyne & Orr, 2004, pp. 83–110). Positive correlations between reproductive isolation and genetic distance, commonly taken as a surrogate for time, have in fact been reported among several allopatric taxa, such as insects (Coyne & Orr, 1989; Presgraves, 2002; Christianson *et al.*, 2005), frogs (Sasa *et al.*, 1998), birds (Price & Bouvier, 2002; Tubaro & Lijtmaer, 2002; Lijtmaer *et al.*, 2003), fish (Mendelson, 2003; Russell, 2003; Bolnick & Near, 2005) and angiosperms (Moyle *et al.*, 2004). Here, we found a significant correlation between reproductive isolation and genetic distance among allopatric species in *Homobasidiomycota* considering all species pairs and the whole range of genetic distances. The lack of significant correlation between genetic distance and reproductive isolation in *Ascomycota* seems to be because of the lower level of divergence within these species complexes (Fig. 1). Indeed, only 10 species pairs of *Ascomycota* had a genetic distance higher than 0.05, and they all had reached complete pre-mating isolation. We considered only *Homobasidiomycota* species pairs with a genetic distance below 0.05, the test was not significant either.

Few studies have examined the time course of speciation in sympatry. Here, we did not find significant correlations between genetic distance and reproductive isolation among sympatric species in either *Ascomycota* or *Homobasidiomycota*. The reason may be once again different in the two groups. In *Homobasidiomycota*, reproductive isolation seems to have evolved rapidly in sympatry, with almost all species pairs completely isolated, so that the degree of reproductive isolation cannot increase further with time. In *Ascomycota*, reproductive isolation was weak in sympatry as well as in allopatry and the lack of correlation between reproductive isolation and time is certainly due here again to a young age of the species complexes considered. This again shows the difference in *Ascomycota* and *Homobasidiomycota* for the evolution of reproductive isolation between sympatric species.

## Conclusions

This study is, to our knowledge, the first one to report that large taxonomic groups of organisms can behave completely differently as regards the evolution of reproductive isolation. It seems that only species with a strong premating isolation due to sexual partner recognition can coexist in sympatry within *Homobasidiomycota*, whereas sympatric sibling species often coexist without premating isolation within *Ascomycota*. This suggests that some phylogeny-dependent life-history trait may strongly influence the evolution of reproductive isolation between closely related species. Identifying those life-history traits that influence the evolutionary processes responsible for these different patterns requires comparative studies between phylogenetically independent taxa, having a broad assortment of life-history traits.

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