

et al. 2011). Conjugation occurs preferentially within the meiotic tetrad (automixis) that comprises the teliospore, the transmissible stage of *Microbotryum* (Hood and Antonovics 2000; Giraud et al. 2008b; Granberg et al. 2008). This form of selfing is promoted by the development of the meiotic products in a multicellular basidium (e.g., promycelium), where neighboring cells readily conjugate. The fungus may also undergo autogamy or outcrossing, primarily through the production and mating of haploid yeast-like cells (sporidia) (Hood and Antonovics 2000; Giraud 2004; Hood and Antonovics 2004; Giraud et al. 2005, 2008b; Schäfer et al. 2010; Gladieux et al. 2011). Hybridization with other fungal species occurs through this sporidial mating as well (Le Gac et al. 2007b; Gladieux et al. 2011). Meiosis and syngamy, through either intrapromycelial mating and/or sporidial mating, occur following deposition of hundreds of diploid teliospores of *Microbotryum* upon the surface of a new host plant (Schäfer et al. 2010). Some combination of automixis, autogamy, outcrossing, and hybridization results in the formation of infectious dikaryons on the plant surface. The numerous sibling progeny then compete to occupy the host meristem, the seat of *Microbotryum* infection which is limited to colonization by a single diploid individual (Audran and Batcho 1982; López-Villavicencio et al. 2007; Schäfer et al. 2010). These life-history traits raise the question of the degree to which developmentally promoted selfing and the production of multiple progeny may serve as a barrier to interspecific gene flow between sympatric *Microbotryum* species.

In this study, we assessed the significance of the combination of selfing and the sibling competition arena as a mechanism of reproductive isolation between closely related species of *Microbotryum*. Artificial inoculations of host plants were designed to compare rates of overall infection and of hybrid infection in the presence and absence of selfing or the sibling competition arena. First, we tested the effect of developmentally promoted selfing by intrapromycelial mating on gene flow: we asked if fewer hybrids are formed under inoculation with diploid teliospores, undergoing mostly automixis, as opposed to inoculation with cultures of haploid sporidia. Second, we tested the effect of the sibling competition arena by comparing hybrid infection rates when hybridization was forced to situations where competition with nonhybrids was allowed. Under competition, the rate of hybrid infection is expected to be lower than that based solely upon the noncompetitive fitness differentials of hybrids and nonhybrids.

Materials and Methods

MODEL SYSTEM

Fungi of the genus *Microbotryum* (Basidiomycetes: Microbotryales) cause anther-smut disease on plant hosts of the Caryophyllaceae. Host-specific lineages have recently been delineated into species, where the criterion of concordance be-

tween multiple gene genealogies demonstrated a lack of gene flow (Kemler et al. 2006; Le Gac et al. 2007a; Lutz et al. 2008; Denchev et al. 2009). The fungus replaces host pollen with dark fungal spores and is transmitted between hosts via pollinators. As previously described, intrapromycelial or intratetrad mating (automixis) is the dominant life-history strategy for *Microbotryum* species (Hood and Antonovics 2000, 2004; Schäfer et al. 2010), although rates of automixis vary between species and populations (Giraud et al. 2005; Granberg et al. 2008). Following mating, dikaryotic hyphae form and invade host tissues, with infecting strains ultimately establishing in a limited region of the host meristems (Audran and Batcho 1982; Schäfer et al. 2010). Upon flowering, diploid teliospores are formed and expressed in the anthers, for disease transmission via pollinators.

Natural hybrids have rarely been observed (Gladieux et al. 2011), although Devier et al. (2010) proposed that historic hybridization events between moderately distant species were significant in generating several *Microbotryum* species. Hybridization between the closely related species *M. lychnidis-dioicae* and *M. silenes-dioicae* has been most commonly studied. Their respective hosts, *Silene latifolia* and *S. dioica*, are frequently sympatric (Van Putten et al. 2005; Refrégier et al. 2010), and hybrids of the two fungal species are viable, fertile, and infectious in a laboratory setting (Van Putten et al. 2003; Le Gac et al. 2007b; de Vienne et al. 2009). In natural populations, however, evidence for hybridization is limited. Gladieux et al. (2011) reported only 15 hybrids out of 1028 pathogen individuals based upon microsatellite characterization, suggesting strong reproductive isolation in the field between these closely related, host-specific pathogen species. Ecological isolation, through specialization of habitat or pollinator, may play some role, but it is far from complete (Goulson and Jerrim 1997; Van Putten et al. 2007). Assortative mating in the form of preference for conjugation with conspecific sporidial gametes could not be detected, even between closely related species occurring in sympatry (Le Gac et al. 2007b; Refrégier et al. 2010). This finding holds under simultaneous exposure to both conspecific and heterospecific gametes such that hybridization is optional rather than forced (Refrégier et al. 2010). Selfing has therefore been proposed as the primary barrier to interspecific gene flow (Giraud et al. 2008b).

PREPARATION OF FUNGAL STRAINS

The *Microbotryum* collections used as inoculum, as well as the original location of their collection, are identified in Table 2. Collections were sampled from natural populations of six host species, *S. latifolia*, *S. dioica*, *S. nutans*, *S. vulgaris*, *S. paradoxa*, and *Lychnis flos-cuculi*. These fungal species are known respectively as *M. lychnidis-dioicae* (MvSI), *M. silenes-dioicae* (MvSd), *M. violaceum sensu stricto* (MvSn), *M. lagerheimii* (MvSv1), *M. violaceum sensu lato* (MvSp), and *M. violaceum*