



HAL
open science

To Self or Not to Self? Absence of Mate Choice despite Costly Outcrossing in the Fungus *Podospora Anserina*

Ivain Martinossi-Allibert, Sandra Lorena Ament-Velásquez, Sven J. Saupe,
Hanna Johannesson

► To cite this version:

Ivain Martinossi-Allibert, Sandra Lorena Ament-Velásquez, Sven J. Saupe, Hanna Johannesson. To Self or Not to Self? Absence of Mate Choice despite Costly Outcrossing in the Fungus *Podospora Anserina*. *Journal of Evolutionary Biology*, 2023, 36 (1), pp.238–250. 10.1111/jeb.14108. hal-04833217

HAL Id: hal-04833217

<https://cnrs.hal.science/hal-04833217v1>

Submitted on 12 Dec 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

RESEARCH ARTICLE

To self or not to self? Absence of mate choice despite costly outcrossing in the fungus *Podospora anserina*

Ivain Martinossi-Allibert^{1,2,3}  | Sandra Lorena Ament-Velázquez⁴ | Sven J. Saupe² | Hanna Johannesson¹

¹Systematic Biology, Department of Organismal Biology, Uppsala University, Uppsala, Sweden

²Institut de Biochimie et de Génétique Cellulaire, UMR 5095 CNRS, Université de Bordeaux, Bordeaux CEDEX, France

³Department of Biology, Realfagbygget, Norwegian University of Science and Technology, Trondheim, Norway

⁴Division of Population Genetics, Department of Zoology, Stockholm University, Stockholm, Sweden

Correspondence

Ivain Martinossi-Allibert, Systematic Biology, Department of Organismal Biology, Uppsala University, 752 36 Uppsala, Sweden.
Email: imartinossi@gmail.com

Funding information

Vetenskapsrådet, Grant/Award Number: 2019-06294; CNRS and University of Bordeaux, Grant/Award Number: IBGC UMR 5095

Abstract

Fungi have a large potential for flexibility in their mode of sexual reproduction, resulting in mating systems ranging from haploid selfing to outcrossing. However, we know little about which mating strategies are used in nature, and why, even in well-studied model organisms. Here, we explored the fitness consequences of alternative mating strategies in the ascomycete fungus *Podospora anserina*. We measured and compared fitness proxies of nine genotypes in either diploid selfing or outcrossing events, over two generations, and with or without environmental stress. We showed that fitness was consistently lower in outcrossing events, irrespective of the environment. The cost of outcrossing was partly attributed to non-self recognition genes with pleiotropic effects on fertility. We then predicted that when presented with options to either self or outcross, individuals would perform mate choice in favour of the reproductive strategy that yields higher fitness. Contrary to our prediction, individuals did not seem to avoid outcrossing when a choice was offered, in spite of the fitness cost incurred. Our results suggest that, although functionally diploid, *P. anserina* does not benefit from outcrossing in most cases. We outline different explanations for the apparent lack of mate choice in face of high fitness costs associated with outcrossing, including a new perspective on the pleiotropic effect of non-self recognition genes.

KEYWORDS

ascomycetes, fungi, mate choice, mating strategy, outcrossing, *Podospora*, reproductive strategy, selfing, sexual reproduction

1 | INTRODUCTION

Modes of reproduction are central to evolutionary processes (Hartfield & Keightley, 2012; Otto, 2021). A majority of sexually reproducing organisms are simultaneous hermaphrodites, including most plants and fungi and a large proportion of animals (Beekman et al., 2016; Jarne & Charlesworth, 1993), and may consequently be

able to engage in either self-fertilization (selfing) or outcrossing mating strategies. Selfing avoids the energy expenditures of mate search and sexual interactions, such as potential conflict and parasite transmission, but may come at the cost of inbreeding depression for diploid organisms (Charlesworth & Charlesworth, 1987). Outcrossing, on the other hand, entails the mixing of genetic information from different individuals, which is expected to increase offspring quality

[Correction added on 28 November 2022, after first online publication: Supporting information file has been corrected.]

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2022 The Authors. *Journal of Evolutionary Biology* published by John Wiley & Sons Ltd on behalf of European Society for Evolutionary Biology.

(Jarne & Charlesworth, 1993; Tregenza & Wedell, 2000). Such benefits can be more visible in stressful or competitive environments, where generating new genetic combinations is more likely to pay off (e.g., Morran et al., 2009). Outcrossing also allows for mate choice, which can be defined broadly as the preferential allocation of resources to certain types of reproductive events (Edward, 2015; Kempenaers, 2007). Mate choice may be adaptive by providing direct benefits, e.g. resources, or indirect benefits in the form of either genetic variation (“good genes”) or genetic complementarity (Neff & Pitcher, 2005; Tregenza & Wedell, 2000). Under this broad definition, a large variety of mechanisms can be termed mate choice, from behaviours involving complex cognition to cryptic prezygotic incompatibilities and postzygotic differential investment, as long as they allow for selective allocation of reproductive resources (Neff & Pitcher, 2005; Rogers & Greig, 2009; Smith & Greig, 2010). For hermaphrodite species, the frequency of outcrossing and mate choice likely depends on a balance of fitness costs and benefits, modulated by environmental conditions and population parameters such as mate availability and genetic diversity.

The study of evolution of selfing and outcrossing mating strategies has long been focused on plants and animals (Barrett, 2003; Bell, 1982; Charnov, 1982; Jarne & Auld, 2006). Yet, the fungal kingdom displays a great diversity of modes of reproduction and mating strategies to be explored from an evolutionary perspective (Billiard et al., 2012; Nieuwenhuis & James, 2016). Among the sexual species, a large proportion have the ability to reproduce by selfing. Homothallic fungi lack any mechanism preventing self-fertilization and many of them can thus either outcross, perform diploid selfing (the fusion of meiotic products from the same diploid genome), or haploid selfing (the fusion of genetically identical haploids) (Billiard et al., 2012). Heterothallic fungi carry mating types that prevent haploid selfing, but can still reproduce through outcrossing and diploid selfing. Finally, pseudohomothallic species carry genetically different haploid nuclei of opposite mating type in the same mycelium, which precludes haploid selfing but allows for automixis, a type of selfing that occurs between siblings of the same meiosis event and is more likely to maintain heterozygosity (Billiard et al., 2012). The general ability to self in fungi could have alternative explanations. First, species with a dominantly haploid life cycle could suffer little from inbreeding depression (but see Leslie & Raju, 1985 for an example of inbreeding depression in *Neurospora crassa*), making selfing an efficient reproductive strategy that saves the cost of mate searching and mate choice. Second, in species with very large dispersal ranges, the occurrence of selfing is unlikely and population densities low, which could make maximizing compatibility with potential partners a priority over self-incompatibility (Billiard et al., 2012). Despite these specific characteristics of fungal reproductive systems, little is known of the frequency at which fungi engage in different types of mating strategies and why (Nieuwenhuis & James, 2016).

Here, we investigate the mating strategy of the pseudohomothallic filamentous ascomycete *Podospira anserina*. It is a hermaphroditic species where the mycelium carries both female and male haploid reproductive structures (reminiscent of a monoecious system in

plants), referred to as protoperithecia and microconidia, respectively. In spite of being pseudohomothallic, it is not known whether selfing is the dominant mating strategy in *P. anserina*. On the one hand, *P. anserina* is known to successfully self under laboratory conditions, and outcrossing can be expected to incur costs. In particular, genetic incompatibilities may arise because of pleiotropic effects of non-self recognition loci, the *het* loci. The *het* loci have a primary role of vegetative incompatibility, by preventing the fusion of genetically different mycelia during vegetative growth through a mechanism of cell death (Saupe, 2000). However, the role of *het* loci is not limited to vegetative growth as some, in particular the *het-v* locus, can also be involved in sexual incompatibilities (Ament-Velásquez et al., 2022; Bernet, 1967; van der Gaag, 2005). Selfish genetic elements which are present at intermediate frequencies in populations of *P. anserina*, the *Spok* genes (Vogan et al., 2019), can also reduce fertility by causing meiotic drive during outcrosses and are sometimes accompanied by large deleterious genomic regions (Vogan et al., 2021). On the other hand, we know that different genotypes co-occur in the field on the same resource patch and outcrossing has been documented (Ament-Velásquez et al., 2022; Hartmann et al., 2021). Moreover, individuals produce a low but consistent proportion of monokaryotic spores (Rizet, 1939; van der Gaag, 2005), carrying only one mating type and thus destined to outcross. Because of its dikaryotic vegetative stage, meaning that it functionally expresses two different haploid genomes, *P. anserina* could be sensitive to inbreeding depression. Taken together, these clues suggest that outcrossing could sometimes but not always be a beneficial strategy. If that is the case, we expect that *P. anserina* should be able to assess potential mating partners and express preferences, or in other words, to perform mate choice. Mate choice and competition for mating have traditionally been studied in animals with separate sexes, where pre-copulatory sexual selection can lead to the evolution of conspicuous morphologies and behaviours (Andersson, 1994). However, mate choice can potentially occur in any population where individuals engage in outcrossing, including hermaphrodite sessile animals, plants and fungi, through the means of gamete-level mechanisms leading to preferential allocation (Beekman et al., 2016).

We compared the fitness payoffs of the selfing and outcrossing mating strategies in *P. anserina* in a laboratory experiment with an ecologically relevant growth environment. We did this by measuring fitness proxies (time to reproduction, spore count and spore germination probability) of independent selfing and outcrossing reproductive events for nine strains with different genetic backgrounds. We included in our design strains carrying the two known alleles of the *het-v* locus, as this locus is known to have the strongest effect on fertility of crosses (Ament-Velásquez et al., 2022). Assays were performed over two generations, and F2 assays included stressful conditions simulating competition with another species. In a parallel experiment, we assessed individuals in their propensity to perform mate choice between compatible partners (i.e. of a different mating type), by offering simultaneously the possibility for selfing or outcrossing. We made the prediction that if one reproductive strategy was more beneficial,

reproductive investment should be biased towards that strategy in mate choice settings.

2 | METHODS

2.1 | Study population

Podospora anserina is a coprophilous filamentous ascomycete. Its spores and mycelium are mainly dikaryotic, meaning that they contain haploid nuclei of opposite mating type. *P. anserina* also naturally produces a low percentage of monokaryotic spores (carrying only one type of haploid genome, and thus only one mating type). Monokaryons can also develop into a mycelium but need a mycelium of the opposite mating type to complete the reproductive cycle (see Figure S1 for a simplified life cycle diagram).

Strains (or genotypes) of *P. anserina* used in this experiment originate from the Wageningen collection and were collected in Wageningen, the Netherlands between 1991 and 2016 (see Table S2 for the collection year of each strain). Since sampling, they have been maintained in the form of dikaryotic spores at -80°C in 40% glycerol solution. It is known that *het* genes, which govern non-self recognition at the vegetative stage, can show pleiotropic effects such as lowered fertility in crosses (Bernet, 1967). In particular, the *het-v* locus may be a marker of reproductively isolated groups (Ament-Velásquez, 2022). For this reason, we balanced our design with respect to the *het-v* genotype of the strains (see Table S2 for the *het* genotype of the strains). Other *het* loci also show variation among our investigated strains, which is expected in natural populations, where more than 95% of outcrossing events are expected to occur between strains with different *het* alleles (van der Gaag, 2005). Other known *het* loci (e.g., *het-z*, *het-c*, *het-d*) could also cause weaker pleiotropic effects on sexual reproduction. In May 2021, dikaryotic spores from 10 strains (Wa3, Wa4, Wa13, Wa15, Wa48, Wa50, Wa63, Wa68, Wa124 and Wa142; retrieved from the glycerol stock) were placed on germination medium. After a few days of growth, mycelium transplants were made on regular culture medium to obtain fresh spores from the selfing of each dikaryon. Monokaryotic spores from each strain were isolated and germinated. They were then tested for mating type by confrontation with reference monokaryons of known mating type. For each strain, one monokaryotic isolate of each mating type (+, -) was used in the experiment. The composition of the different growth media is given in Supporting Information S3.

[Correction added on 28 November 2022, after first online publication: The citation year for the Ament-Velásquez et al reference has been revised from 2020 to 2022 throughout the article.]

2.2 | Experimental design

Wa63 was designated as the reference strain, and it was subsequently used as an outcrossing partner for the nine other strains

during Outcrossing and Mate Choice assays. The experimental design is described graphically in Figure 1. During the first generation of the experiment (F1), isolated monokaryons (see Study population) were confronted in three different types of mating events: Selfing, Outcrossing and Mate Choice (see Figure 1). Fitness proxies were measured for each mating event. A selfing event results from a confrontation between monokaryotic mycelia of opposite mating types and originating from the same dikaryotic strain. An outcrossing event results from a confrontation between a monokaryotic mycelium of type (+) from one of the strains, and a monokaryotic mycelium of mating type (-) from the reference strain (Wa63). Finally, a Mate Choice assay is a confrontation between a monokaryotic mycelium of one of the non-reference strains, type (+), and two compatible partners type (-); one originating from the same non-reference strain and the other from the reference strain Wa63. The Mate Choice assay offered the opportunity for the type (+) mycelium to choose between outcrossing and selfing reproductive strategies.

For F1, Selfing assays were set up for each of the 10 strains (including the reference) and replicated four times per strain, over two repeated experimental batches started 3 days apart (for a total of eight assays per strain per type of assay). Outcrossing and Mate Choice assays were replicated at the same level, for each of the nine non-reference strains. At the end of F1, spores originating from the Selfing and Outcrossing assays were carried over to the second generation of the experiment (F2). Spores from the Mate Choice assays were not used in F2 due to the impossibility to determine whether a given spore originated from selfing or outcrossing events.

During F2, dikaryotic mycelia (carrying both parental genomes, with compatible mating types and thus self-fertile) originating from the F1 Selfing and F1 Outcrossing assays were allowed to self in isolation, and fitness proxies were measured again. For each of the 10 selfing strains and nine outcrossing strains, four different F1 spores were germinated, and the resulting mycelia included in F2. Each mycelium was assayed on two different media, a regular reproduction medium (same as F1) and a stress medium (medium composition in Supporting Information S3).

2.3 | Fitness assays

Monokaryotic confrontations of F1 were realized between three mycelia originating from monokaryotic cultures (see Study population). The focal mycelium was of type (+) and was presented in all assays with two mating partners of type (-) (Figure 1). Implants of standardized size (cut out with a sterilized metal tube) were placed centrally and evenly spaced using a placement pattern in a 5 cm petri dish containing reproduction medium. Dikaryotic selfings of F2 were realized by placing one dikaryotic mycelium implant in the center of a 5 cm petri dish containing either reproduction medium or stress medium. For both F1 and F2, assays were randomized by assigning a random ID number to each dish. All dishes were kept at 26°C in an incubator, with a 12h/12h light cycle. For both F1 and F2, fitness proxies measured were: time to reproduction (time in

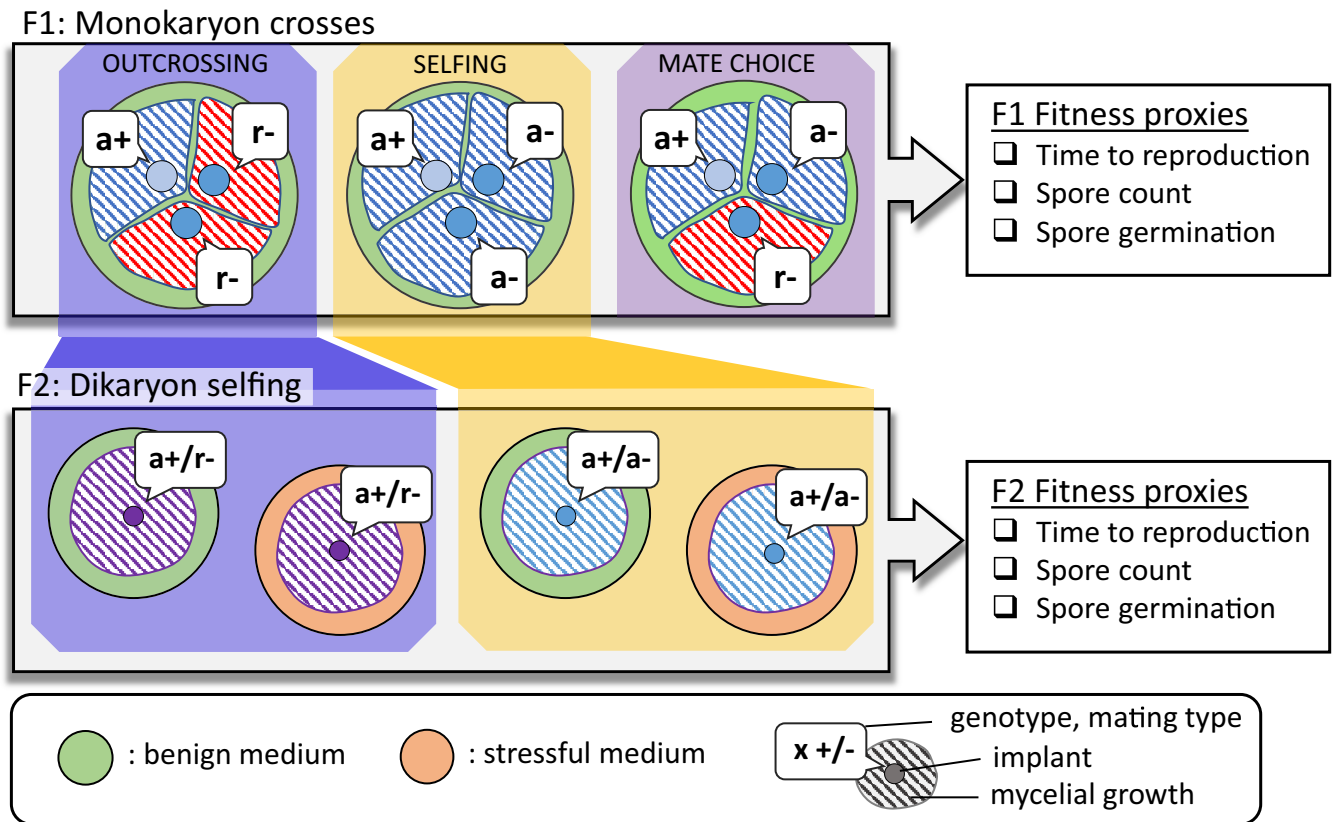


FIGURE 1 Experimental design. Mating in F1 occurred between monokaryons carrying opposite mating types (+/-) that came into contact through mycelial growth. Small full circles represent implants of mycelium deposited on the growth medium and diagonal stripes represent new mycelial growth. Letters indicate the genotype: (a) for the focal strain and (r) for the reference strain. Selfing assays included monokaryons originating from the same strain but carrying opposite mating types. In F2, each mycelium carried the two parental haploid genotypes from F1 and was thus a dikaryon able to self. Fitness proxies at F2 were assayed in both benign and stressful environments.

days between inoculation and first spore shot), number of spores shot (within 48 h of first recorded spore), and germination rate of the spores produced.

Time to reproduction was estimated by inspecting three times daily (10, 14, 17 h) the lids of petri dishes under a stereomicroscope for the first spores shot (spores are likely not shot during nighttime, see pilot data in Supporting Information S4).

2.3.1 | Spore count

Once the first spores had been recorded, assays were left 48 h to shoot spores before the lids were removed for spore collection (most of the spores are shot within this time span making it a good proxy for total spore count, see pilot data in Supporting Information S5). Spore collection on the lid of petri dishes was realized by pipetting 1200 μ l of spore harvesting solution (see Supporting Information S3) on the lid. The solution was then collected in a 1.5 ml Eppendorf and sonicated (Soniprep 150, 9 μ m amplitude, 15 s). Spore count was estimated from the number of spores in five drops of 5 μ l each of the solution, counted under the stereomicroscope just after agitation. If spore count was high, a 10 \times dilution in harvesting solution was made to allow accurate counting.

2.3.2 | Germination rate

Twenty-four hours after the first spores were observed, an agar cover was placed over the petri dish for 30 min during daytime, in order to collect spores for germination assays and F2. Agar covers with spores were stored at 4°C until needed for germination assays (not more than 5 days). Spore germination rate was estimated by the proportion of spores showing growth after 48 h on germination medium. Twenty spores per assay were sampled from agar covers using a needle and placed on germination medium, evenly spaced in a 9 cm petri dish containing germination medium. In total, germination was checked for 160 spores per strain per assay type for F1 (total 4640 spores) and 80 per strain per F1 assay type per medium for F2 (total 3040 spores).

2.3.3 | Fruiting bodies

During F1 assays only, the number of fruiting bodies (or perithecia) located at the confrontation between the different monokaryons was recorded (Figure 1). Perithecia found on the border between the focal type (+) individual and each potential partner of type (-) were counted independently, allowing to estimate the relative allocation

to each reproductive strategy in the Mate Choice assays. The number of perithecia was taken as an estimate of the fertility of a cross, which could include elements of genetic compatibility and elements of relative energy investment in a reproductive event.

2.4 | Statistical analysis

F1 fitness proxies were analysed in a dataset with only Selfing and Outcrossing assay types (i.e., excluding Mate Choice assays). Each fitness proxy was analysed in a linear mixed model with *het-v* genotype (V or V1), type of assay (Selfing or Outcrossing) and their interaction as fixed effects. Strain identity and strain crossed by experimental batch were added as random effects.

F2 fitness proxies were analysed in a linear mixed model with type of F1 parental assay (Selfing or Outcrossing), F2 assay environment (Normal or Stress), *het-v* genotype, the interaction of *het-v* and F1 assay, and the three-way interaction of *het-v*, F1 assay and F2 environment, as fixed effects. Strain identity and strain crossed with F1 parental assay were added as random effects.

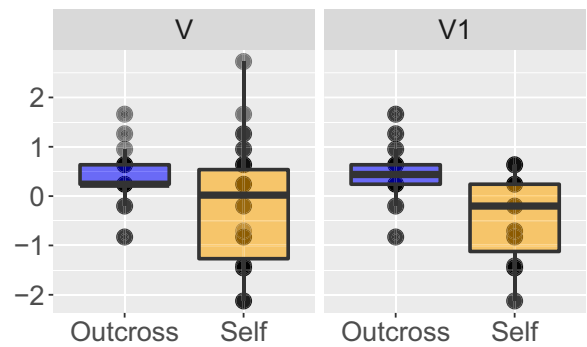
For F1 and F2 fitness proxies, spore germination probability was analysed in a generalized linear model with a binomial error distribution, while spore count and time to reproduction were analysed in models with a normal error distribution.

Outcrossing propensity was estimated from perithecia numbers. Perithecia were used as a proxy for reproductive investment into each potential partner, because they are localized at the border between two cross-fertilizing mycelia and can thus be directly attributed to parents in the Mate choice assays (whereas spores, once ejected, cannot be traced back). Outcrossing propensity was calculated as the relative difference (divided by the mean) in number of perithecia between outcrossing and selfing mating events, so a positive score indicates a preference or a higher fertility in outcrossing and vice versa. The *expected outcrossing propensity under no mate choice* was calculated as the relative difference in perithecia numbers between the mean score of each genotype in independent F1 Selfing and Outcrossing assays. It was used as a null expectation of what would happen in a Mate choice assay if mate choice does not occur. The *realized outcrossing propensity* was calculated in the same fashion but from the mean score of F1 Mate Choice assays, in which focal mycelia were in contact with a selfing and outcrossing partner at the same time (see Figure 1, experimental design). Comparing the expected outcrossing propensity under no mate choice to the realized outcrossing propensity in Mate Choice assays allowed us to test whether allocation to reproductive partners changed when mate choice was possible.

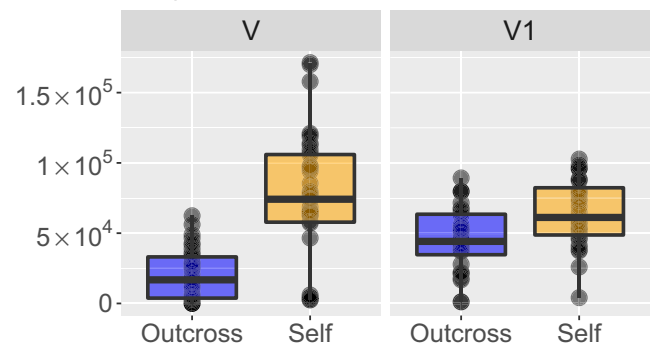
The effect of the *het-v* and *het-Z* genotypes on realized outcrossing propensity was analysed with a linear model. Realized outcrossing propensity was the response variable and *het-v* and *het-Z* genotypes (V or V1 and Z1 or Z2), as well as their interactions, were fixed effects in the model.

All analyses were run with the lme4 package version 1.1.28 (Bates et al., 2015) for R (R Core Team, 2021).

(a) Time to reproduction in days (mean scaled)



(b) Spore count



(c) Spore germination probability

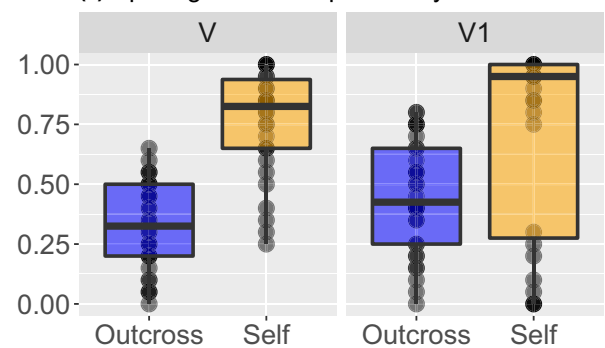


FIGURE 2 F1 fitness proxies measured in outcrossing and selfing monokaryon crosses. Time to reproduction (a), spore count (b) and spore germination probability (c) were measured for F1 monokaryon crosses. Five strains of the *het-v* genotype V (different from the reference genotype) and four strains of genotype V1 (same as the reference genotype) were assayed. Each measurement was replicated eight times per strain over two experimental batches. Figure S6 shows the same data detailed at the level of each strain.

3 | RESULTS

3.1 | Selfing and outcrossing fitness proxies at F1

Outcrossing assays yielded lower fitness than selfing assays with respect to all three fitness proxies for most of the strains. More specifically, Selfing and Outcrossing assays (assay type) resulted in differences in the time to reproduction with outcrossing assays showing a significantly delayed reproduction of about 24 h as

compared with selfing assays (Figure 2a, Table 1). Strains carrying the V or V1 allele of *het-v* did not differ in their time to reproduction, and no interactions were detected, indicating that the effect of assay type was not different across *het-v* genotypes. For the analysis of time to reproduction, strain Wa4 was excluded from the data as it did not reproduce in outcrossing assays.

Spore count was lowered in outcrossing assays as compared with selfing assays (Figure 2b, Table 1). A *post-hoc* test confirmed that outcrossing spore count was significantly lower than selfing spore count within both *het-v* groups (Tukey's *post-hoc*, V group: estimate = -54528, df = 124, p -value < 0.001; V1 group: estimate = -18228, df = 124, p -value = 0.0011). The significant interaction of *het-v* genotype and assay type showed that the reduction of spore count was significantly greater in group V (different group than the reference genotype) as compared with group V1. Finally, strains appeared to differ from each other in their overall spore count (Figure S6b).

Spore germination probability was significantly reduced for outcrossing assays (Figure 2c, Table 1). It also appeared to differ among strains, with in particular Wa124 showing an overall very low spore germination probability, and a higher germination probability for outcrossing than for selfing assays, a trend opposed to the eight other strains tested (Figure S6c). Except for Wa124, strains of the V1 group showed an overall higher germination probability than those of the V group. Because of Wa124, selfing spore germination probability was lowered in the V1 group, resulting in a smaller reduction of germination probability when outcrossing in this group as compared with the V group. In fact, if Wa124 is excluded from the dataset, the reduction in germination probability due to outcrossing is on the contrary more important in the V1 group. Thus, the significant interaction between *het-v* genotype and assay type is relying heavily on the idiosyncratic behaviour of Wa124 and should probably be disregarded. For the analysis of spore germination probability, genotype Wa4 was excluded since no outcrossing spores were available, making the comparison of the two assay types impossible.

Overall, the three fitness proxies measured were consistently reduced for outcrossing assays as compared with selfing assays. Moreover, spore count was reduced more strongly and consistently among outcrossing strains of the V group as compared with strains of the V1 group. For the other two fitness proxies, timing to reproduction and spore germination probability, there was no strong

indication that the two *het-v* groups differed in the negative impact that outcrossing had as compared with selfing.

To note, individuals that did not successfully reproduce in outcrossing assays were included in the analysis of spore count as having produced zero spores, while they were excluded of the time to reproduction and germination probability analysis as missing values. The assays that failed to produce spores were all outcrossing assays of strain Wa4, a strain that already had a very low spore count in selfing assays and belonged to the V group.

3.2 | Selfing and outcrossing fitness proxies at F2

Consistent with F1, F2 fitness proxies were all generally lower for dikaryotic mycelia originating from F1 outcrossing than from F1 selfing. More specifically, time to reproduction at F2 was only affected by the F1 assay type from which the F2 originated, with dikaryons originating from F1 outcrossing assays having a reproduction delay of about 8 h on average as compared with dikaryons originating from selfing assays (Table 2, Figure 3a).

Spore count was not affected by F2 environmental conditions (Table 2). *het-v* genotype affected spore count significantly, with dikaryons of the V1 group having overall higher spore count, likely driven by the lesser reduction of spore count in outcrossing assays for V1 strains overall (Figure 3b). F1 assay type also affected spore count, with dikaryons originating from F1 outcrossing assays having lower spore count as compared with dikaryons originating from F1 selfing assays. More precisely, spore count was significantly reduced for dikaryons originating from F1 outcrossing assays only in the V group, but not in the V1 group (Tukey's *post-hoc*, V group: estimate = -80410, df = 7, p = 0.004, V1 group: estimate = 9329, df = 7, p = 0.667). This effect likely originates from two sources: (i) a generally lower reduction in spore count for dikaryons originating from outcrossing in the V1 group, and (ii) the surprisingly low spore count of Wa124 at F2 when originating from F1 selfing. The score of Wa124 lowers the average spore count of the V1 group (see Figure S7).

Spore germination probability at F2 was affected by F1 assay type, with dikaryons originating from F1 outcrossing producing overall spores with lower germination probability (Table 2, Figure 3c). Furthermore, a significant three-way interaction was detected, with the effect of F1 assay type on spore germination

TABLE 1 Anova table for mixed-effect models with F1 fitness proxies as response variables (time to reproduction: Type II, spore count and germination probability: Type III).

Response variable	Time to repro.			Spore count		Spore germination	
	df	χ^2	p -Value	χ^2	p -Value	χ^2	p -Value
Intercept	1			3.40	0.07	2.30	0.13
<i>het-v</i> genotype	1	0.22	0.64	2.61	0.11	0.21	0.65
Assay type (Self/Outcross)	1	82.47	<0.001	120	<0.001	216	<0.001
<i>het-v</i> type by assay type	1	0.10	0.75	23.3	<0.001	5.61	0.018

TABLE 2 Anova table for mixed-effect models with F2 fitness proxies as response variables (time to reproduction: Type II, spore count and germination probability: Type III).

Response variable	df	Time to repro		Spore count		Spore germination	
		χ^2	p-Value	χ^2	p-Value	χ^2	p-Value
Intercept	1			1.36	0.243	1.00	0.318
F2 environment	1	2.04	0.152	0.35	0.553	1.26	0.262
het-v genotype	1	0.35	0.552	4.14	0.042	0.63	0.429
F1 assay (Self/Outcross)	1	4.37	0.037	10.68	0.001	6.34	0.012
het-v: F1 assay	1	0.39	0.533	5.01	0.025	0.002	0.962
F2 Env: het-v: F1 assay	3	1.39	0.708	1.73	0.630	7.94	0.047

affected by an interaction of *het-v* group and F2 assay environment (Table 2, Figure 3c). This represents the only significant effect of the F2 assay environment. In details, the reduction of germination probability due to outcrossing at F1 was strongest in the stressful environment within the V group, but strongest in the benign environment for the V1 group (Tukey's *post-hoc*, comparing spore germination probability for dikaryons originating from outcrossing versus selfing at F1 in: benign environment, V group: estimate = -2.19, $p = 0.012$, stressful environment, V group: estimate = -2.98, $p = 0.001$, benign environment, V1 group: estimate = -2.13, $p = 0.018$, stressful environment, V1 group: estimate = -1.34, $p = 0.132$). This interaction suggests that there may be a reduced cost of outcrossing to spore germination probability under stress, but only if the genotypes match in terms of *het-v* group.

In summary, F2 dikaryons showed generally reduced fitness on the three proxies measured when originating from F1 outcrossing assays, consistent with results from F1 fitness proxies of monokaryon crosses. Spore count at F2 was reduced more strongly in dikaryons originating from F1 outcrossing assays in the V group as compared with the V1 group, which is also consistent with F1 results. This result indicates that fitness effects seen at F1 are not only due the specific setup of interacting monokaryons, but also arise once the dikaryon is formed. F2 dikaryons were assayed in two environments, benign and stressful, which did not appear to affect timing to reproduction or spore count. Only spore germination probability was affected by F2 environment in a complex interaction with *het-v* group and F1 assay type, suggesting that the cost of outcrossing on spore germination probability may be reduced under stress when outcrossing partners are of the same *het-v* group.

3.3 | Propensity to outcross in a mate choice setting

The number of perithecia (fruiting bodies) produced in F1 selfing and outcrossing assays by each genotype were used to calculate an expectation of the proportion of outcrossing perithecia produced in selfing and outcrossing if mate choice does not occur (see Figure 1 for experimental design details). This expected outcrossing propensity was the relative difference in average number of perithecia

developed in each type of assay (difference outcrossing minus selfing divided by the mean). It was calculated based on four replicates per genotype within each experimental batch. Realized outcrossing propensity was the same relative difference in number of selfing and outcrossing perithecia but calculated for Mate Choice assays (again with four replicates per genotype and batch). Most strains showed negative values of expected outcrossing propensity (Figure 4a), which means that they had a higher fertility in selfing assays as compared with outcrossing assays in number of perithecia. Wa15 and Wa50, however, showed values that were positive (for one batch) or close to zero. Similarly, realized outcrossing propensity in mate choice settings yielded negative values for most strains (Figure 4a), indicating a higher number of perithecia developed in contact with the selfing partner, except for Wa15 and Wa50. The relationship between expected outcrossing propensity and realized outcrossing propensity is a relatively strong correlation ($R^2 = 0.79$): no single strain departs from the central diagonal line in Figure 4a consistently across experimental batches. This means that the expected outcrossing propensity under no mate choice calculated from independent Selfing and Outcrossing assays was a very good predictor of the realized outcrossing propensity, supporting the absence of mate choice in *P. anserina*. Figure 4a also shows that the *het-v* genotype predicts well the level of outcrossing propensity, as strains of the same genotype as the reference strain Wa63 generally show higher expected and realized outcrossing propensity.

Figure 4b details further how the *het-v/het-z* allelic combinations of each strain affected the level of realized outcrossing propensity, as both loci are known to have a role in sexual incompatibility. Again, the effect of having a different *het-v* genotype from the reference is clear. *Het-Z* appears to have an effect within the V1 group (*het-v* compatible with the reference), but that effect is not clear within the V group, although more replication in the V:Z2 group would have been needed to test for that effect (Table 3).

4 | DISCUSSION

In the ascomycete *P. anserina*, selfing and outcrossing are two reproductive strategies that occur in the wild. We wondered what the fitness consequences of these two strategies are and we addressed this question by contrasting estimates of three fitness proxies from the

two reproductive strategies over two generations. At F1, monokaryons were confronted in selfing and outcrossing events, and their respective offspring were then assessed as selfing dikaryons at F2.

4.1 | Outcrossing fitness is consistently lower in *P. anserina*

The fitness assays carried out over two generations showed consistently lower fitness proxies for outcrossing events as compared with selfing. This finding was surprising: because of the dikaryotic vegetative stage of *P. anserina* that carries haploid nuclei of two different genotypes in the same cytoplasm, some benefits of outcrossing could have been expected similar to what can be found in diploid organisms (Charlesworth & Willis, 2009). Benefits of outcrossing are expected from the masking of recessive deleterious mutations and may not be apparent if genotypes are highly lab-adapted or tested in conditions with *ad libitum* resources, or else if the relevant fitness proxies are not recorded. We planned our experiment to avoid such pitfalls and therefore argue that our results are robust. Specifically, we used wild collected strains, measured fitness using three major proxies (development time, fertility and offspring survival) and with a growth medium that mimicked the natural conditions of *P. anserina*, by including horse dung and limiting nutrient availability. In addition, F2 individuals were also assayed on a stress medium with the prediction that heterozygous individuals would face challenges better, again due to the masking of recessive mutations. Stress was induced by the presence of nourseothricin, which is a compound produced by *Streptomyces* bacteria in competition, to which *P. anserina* is sensitive. However, this stress treatment had no effect on fitness proxies apart from an interaction with *het-v* genotype and spore germination probability, suggesting that the dosage of nourseothricin may not have been high enough. At the level of individual strains, fitness proxies in outcrossing were always lower than or equal to proxies in selfing but for one notable exception: the spore germination probability of strain Wa124 at F1, which was extremely low in selfing conditions (*ca* 5% germination) and recovered slightly with outcrossing (*ca* 15%). Taken in combination, our results thus strongly support a generally lowered outcrossing fitness in *P. anserina*. It is known that the genomes of *P. anserina* sampled from the Wageningen population show little genetic diversity with the exception of a few hotspots associated with non-self recognition loci, the *het* genes (Ament-Velázquez et al., 2022). It is thus possible that, because of low genetic diversity at most loci, there can be no positive effect of outcrossing caused by heterosis, except in extreme cases where a novel deleterious mutation could be masked. Instead, the main aspect of genetic differences between strains induces outcrossing costs due to incompatibility. The case of Wa124, for which very low spore germination probability improved under outcrossing, could be an instance of masking of a deleterious mutation. An additional cost to outcrossing could arise in natural populations due to meiotic drive by the *Spok* genes present at intermediate frequencies in populations of *P. anserina* (Vogan et al., 2019). In the present experiment,

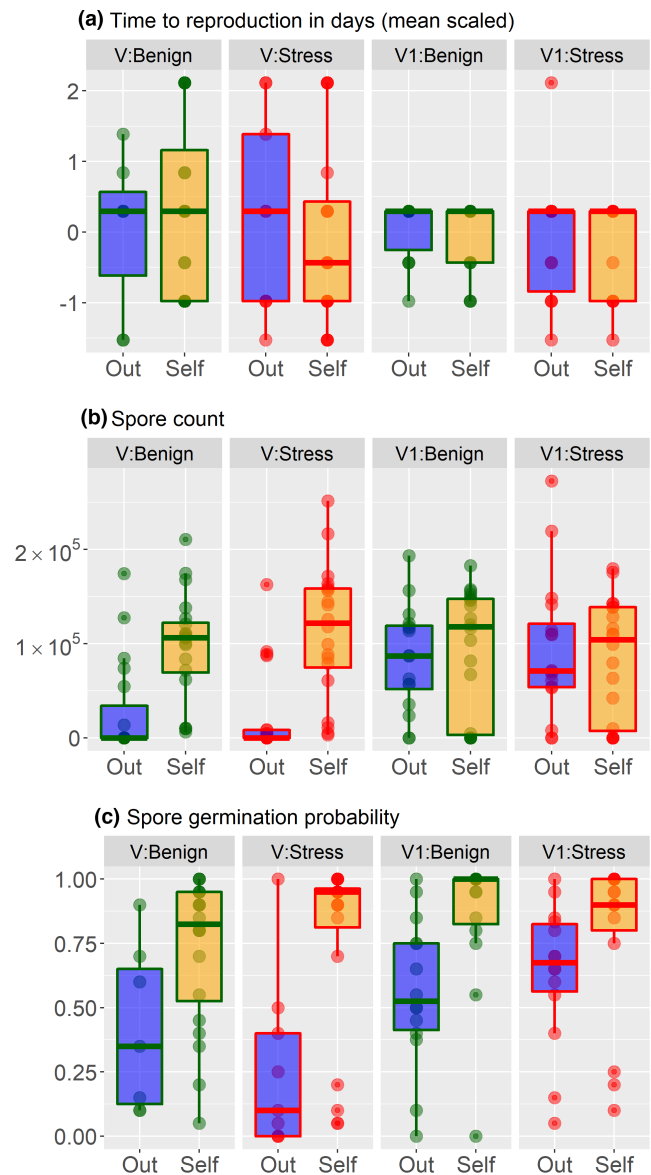


FIGURE 3 F2 fitness proxies measured in two environments for selfing dikaryons originating from either F1 selfing or outcrossing events. Time to reproduction (a), spore count (b) and spore germination probability (c) are measured for selfing F2 dikaryons in either benign conditions, or under stressful conditions induced by the addition of nourseothricin. Five strains of *het-v* genotype V (different from the reference genotype) and four strains of genotype V1 (same as the reference genotype) are assayed. Each measurement was replicated four times per strain per environment. Figure S7 shows the same data detailed at the level of each strain.

we ensured that all strains had the same *Spok* status, so this effect did not interfere with our measurements. However, it would be interesting to include the effect of *Spok* genes on outcrossing fitness and mate choice in future studies.

A potential explanation for low outcrossing fitness that we want to eliminate could have been the presence of cryptic species of *Podospira* among our strains; we present to that effect a phylogenetic tree including 8 of the 10 strains used in the present study in relation to other strains of *P. anserina* collected in Wageningen and

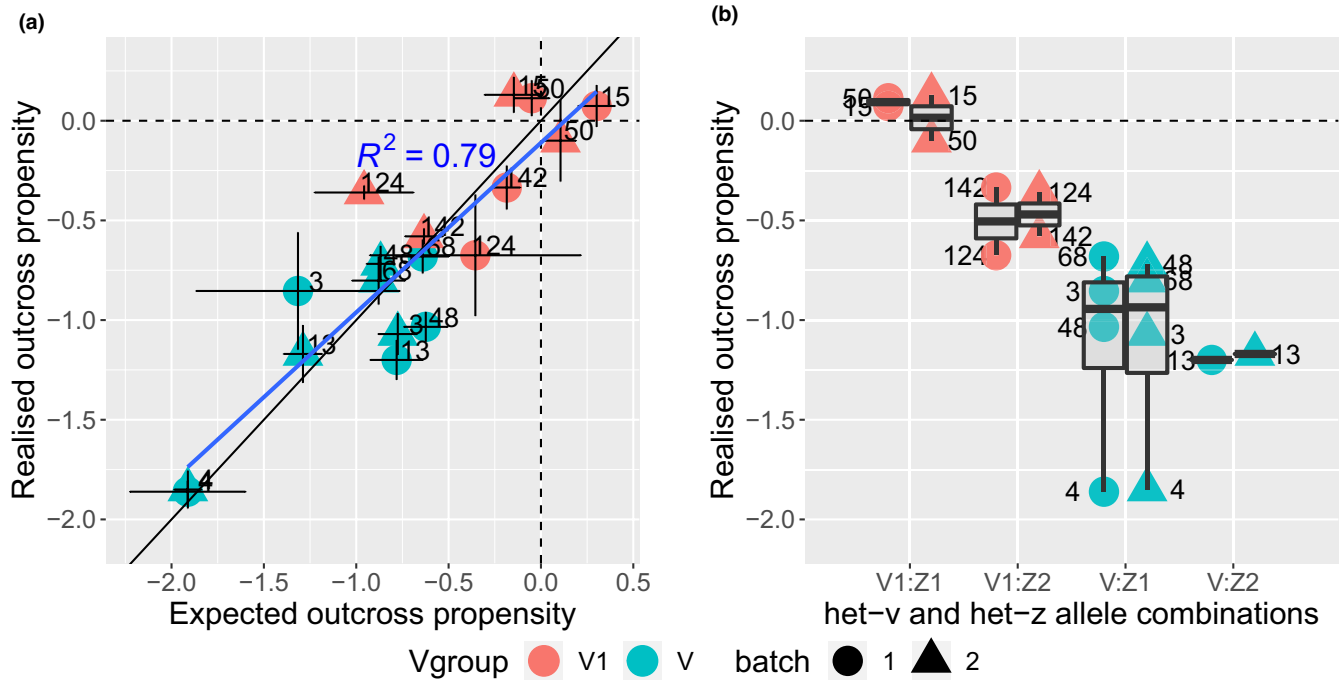


FIGURE 4 Predictors of outcrossing propensity in Mate Choice assays. In (a), expected outcrossing propensity under no mate choice, calculated as the relative difference of perithecia number between independent Outcrossing and Selfing assays, is plotted against realized outcrossing propensity, calculated as the relative difference of perithecia number between outcrossing and selfing perithecia in Mate Choice assays. The vertical (resp. horizontal) dashed line represents the limit between expected (resp. realized) preference for outcrossing (positive values) and selfing (negative values). The black diagonal line indicates equality between realized outcrossing propensity and the expectation under no mate choice. The blue line represents the linear regression between the two variables. Error bars are standard deviations calculated from four replicates per dot. In (b), realized outcrossing propensity is given for each allelic combination of the sexual incompatibility loci: *het-v* and the locus *het-Z*, also known to have fertility effects. The genotype of the reference strain for outcrossing assays, Wa63, is V1:Z1. Each dot represents four replicated measures, with the shape of the dot indicating experimental batch and the accompanying number the identity of the strain.

TABLE 3 Type II Anova table for a mixed-effect model with realized outcrossing propensity as the response variable.

Effect	χ^2	df	p-Value
V genotype	32.8	1	<0.001
Z genotype	3.26	1	0.093
V genotype by Z genotype	1.55	1	0.233

to the *P. comata* and *P. pauciseta* species (Supporting Information S8). The two remaining strains were not sequenced, but we note that under our culture conditions *P. comata* is morphologically distinct from *P. anserina* (Vogan et al., 2021), and that *P. pauciseta* was never sampled in the Wageningen population.

[Correction added on 28 November 2022, after first online publication: 'sub-species' has been replaced with 'species' in the "A potential explanation for ... *P. pauciseta* species." sentence.]

4.2 | Non-self recognition gene contributes to outcrossing cost

The role of *het* genes in non-self recognition in *P. anserina* has been studied for many decades (Pinan-Lucarré et al., 2007; Rizet, 1953).

The primary function of such genes is thought to be to prevent mycelial fusion of different genotypes during vegetative growth. Fusion within a mycelium is beneficial as it optimizes nutrient flow, but fusion between mycelia of different genotypes exposes them to risks of parasitism and pathogen transmission (e.g. Biella et al., 2002). Moreover, some *het* genes and in particular *het-v* have known pleiotropic effects during reproduction that have been reported as lowered fertility during crosses estimated in perithecia numbers (Bernet, 1967). Here, we investigated further the effect of the *het-v* genotype on different components of fitness during crosses: there was no indication that *het-v* genotype mattered for time to reproduction and spore germination probability at F1, which were lower for all outcrossing strains. In contrast, spore count (fertility) at F1 was less reduced in outcrossing events if strains shared the same *het-v* genotype. This effect was also present at F2, where dikaryons that originated from F1 outcross only showed reduced fertility if they were heterokaryotic for *het-v* (V/V1 genotype). By measuring several components of fitness over two generations, we were able to confirm that the effect of *het-v* on fertility is relevant to wild collected strains. Moreover, we found that the negative effects are not restricted to aborted perithecia, as was previously thought, but can also result in perithecia with lowered spore count (Figure S6d). By showing a generalized fitness cost to outcrossing, including but not

limited to the effect of *het-v*, our results encourage a more detailed study of *het* gene polymorphism throughout the life cycle.

In light of these results, we propose an explanation for the observation that only some *het* genes have prezygotic effects, i.e., show a reduced fertility by lowering fertilization rates (Bernet, 1965). *het* genes achieve their function of non-self recognition between vegetative mycelia by the expression of different proteins triggering an incompatibility reaction leading to cell death (Pinan-Lucarré et al., 2007). During outcrosses, *het* genes may lead to the production of self-incompatible offspring, but at variable frequencies depending on their genetic architecture. More specifically, most allelic *het* systems (involving two alleles at the same locus) also undergo first division segregation at meiosis (Esser, 1974), which result in few self-incompatible offspring, while non-allelic systems (involving different loci) and allelic systems with second division segregation produce many self-incompatible offspring. It appears that the *het* genes with an architecture that would result in highest proportions of self-incompatible offspring, such as the allelic *het-v* and *het-z* and the non-allelic *het-c*, *d*, *e* and *r* are also the ones that cause prezygotic effects (Bernet, 1965). This observation suggests that prezygotic effect occur where reproductive effort would be invested into inviable offspring and raises the possibility that such mechanisms could avoid the fitness costs of incompatible crosses. In this view, prezygotic effects of *het* loci should not be seen as pleiotropic costs of non-self recognition loci, but rather as potentially adaptive features directing reproductive allocation. In that sense, *het* genes could contribute to prezygotic isolation, an important step in the divergence of sympatric populations (Kirkpatrick & Ravigné, 2002). In the ascomycete *Neurospora*, reinforcement has been described through lowered fertility between sympatric sister species (Turner et al., 2010), and similar reproductive barriers could very well arise in *Podospora* as part of a speciation process (Ament-Velásquez, 2022).

4.3 | No mate choice detected in *P. anserina*

We had predicted that mycelia presented with two reproductive partners would allocate more energy to the reproductive event yielding highest fitness. Mate Choice assays were conducted at F1 by confronting monokaryons, which is likely not the most common way mycelia encounter in nature. However, using monokaryons was necessary to ensure that selfing and outcrossing partners were given the same access to compatible reproductive structures (gametes). Contrary to our expectation, we could detect no difference between the expected and realized outcrossing propensity, indicating that the number of perithecia allocated to different partners was not affected by their availability. The evolution of mate choice mechanisms will ultimately depend on the balance between costs and benefits of such mechanisms weighted by the frequency of outcrossing events. Consequently, a trivial explanation for the absence of mate choice is that the prevalence of outcrossing in nature is so low that mate choice mechanisms are not adaptive and thus simply do not occur in *P. anserina*. Ecological conditions limiting the possibilities for outcrossing (e.g., limit to dispersal of spores) could lead to

the absence of mate choice, and a cost of outcrossing due to genetic divergence between local populations (as in the case of the social spider *Stegodyphus dumicola*, Berger-Tal et al., 2014). In fact, the actual outcrossing rate of *P. anserina* is an unresolved question. The selective signatures of non-self recognition genes in *P. anserina* (Ament-Velásquez et al., 2022) suggest that mycelia of different genotypes must encounter frequently during vegetative growth. Nevertheless, the morphological advantage of selfing in dikaryons (shorter dispersion of reproductive structures) might make outcrossing frequency negligible even when confrontation of genotypes occur. However, genomic data show levels of Linkage Disequilibrium (LD) decay intermediate between those found in outcrossers and extreme selfers (Ament-Velásquez et al., 2022), suggesting that outcrossing does occur after all. There are also genetic mechanisms that naturally lead to the formation of monokaryons, which are then much more likely to outcross. For instance, 1%–5% of spores produced by most strains are monokaryotic and this level is increased by exposure to dihydrostreptomycin or other stressors (Rizet, 1942; van der Gaag, 2005). Additional monokaryotic mycelia may also result from the sectoring of self-incompatible dikaryons (themselves originating from a previous outcrossing event) (Bernet, 1965). The quantitative contribution of monokaryons and dikaryons to outcrossing rates remains unknown, but several lines of evidence suggest that outcrossing occurs in nature to some extent. One then needs to resolve the apparent paradox of the absence of detected mate choice in face of high fitness costs associated with outcrossing. We consider two more possibilities: first, that we failed to detect mate choice in response to mate availability because of a limitation of our experimental setup, and second, that mate choice occurs at other levels independent of mate availability.

In our experimental setup, the propensity to outcross was estimated by the number of perithecia found at the border between mycelia of different mating types. This method presents the advantage of allocating parentage with certainty in Mate Choice assays, so that perithecia can easily be attributed to either outcrossing or selfing reproductive events. However, it also limits our understanding of the resource allocation to one single fertility proxy. Although the proportion of perithecia counted in Mate Choice assays did not depart from the expectation derived from independent Selfing and Outcrossing assays, it is still possible that other energy allocations were modified in response to partner availability. For example, perithecia of the selfing type could have received proportionally more resources than outcrossing perithecia in Mate Choice assays. Since we know that Selfing assays showed generally higher fertility and higher spore germination probability, we can ask whether Mate Choice assays showed higher spore count or germination probability than expected. In Figure S9, we show correlations between outcrossing propensity of strains and gains in fitness proxies obtained in Mate Choice assays as compared with the expectation from independent Selfing and Outcrossing assays. These show that there is no gain obtained for spore count but suggest that there may be an increase in spore germination probability in Mate Choice assays for strains with a strong incompatibility with the reference strain ($r^2 = -0.23$ between spore germination gain and propensity to

outcross, if Strain Wa4 is excluded, see Figure S9). This weak trend suggests that resources could after all be reallocated during reproductive events when several partners are available, which would qualify as mate choice, but clearly warrants further investigation.

Another possibility is that allocation of reproductive resources does not respond plastically to partner availability, but still reflects a form of mate choice. The two scenarios that we discuss below do not involve the active recognition of partners and plastic response that are usually implied in the term “mate choice,” but they would qualify as preferential allocation of reproductive resources. First, we have seen that prezygotic incompatibilities determined by *het-v* may act as reproductive barriers to avoid the production of low fitness offspring. Generally, genetic incompatibilities are simply expected to incur fitness costs, but prezygotic incompatibilities may allow a reduction in such costs by avoiding the allocation of resources to the development of spores that would later be self-incompatible. However, to truly constitute a form of mate choice this mechanism would have to result in a fitness benefit, such as the recovery of spore germination probability described in the previous paragraph, which remains to be measured. Second, it is possible that the reproductive morphology of *P. anserina* in its dikaryotic form acts in several ways to strongly favour selfing, precluding the need for further reproductive allocation mechanisms in response to mate availability. Most selfing events in natural conditions would happen within a dikaryotic mycelium while outcrosses always happen at the contact zone between two growing mycelia. In that context, selfing is largely favoured due to the proximity of reproductive structures of opposing mating types within the same mycelium. The position of protoperithecia (female reproductive structures) on the mycelium could influence the opportunity for outcrossing to occur. In the laboratory, most strains show rings of perithecia far from the edge of the mycelium, which could be indicative of a morphology that evolved to favour fertilization of the mycelium's own female reproductive structures. At the same time, strain genotype as well as growth medium can alter the pattern of placement of protoperithecia (Boucherie & Bernet, 1980) meaning that there could be variation in this trait. The same is true of the production of microconidia (male gametes) that is also modulated by growth conditions and genotype (Arnaise et al., 2001; Coppin, 2002). Microconidia production could thus also contribute to the regulation of reproductive allocation. In that hypothesis, there could exist a regulation of reproductive strategies towards a nearly exclusive selfing in dikaryons, driven by the cost of outcrossing that we revealed here.

Our experiment eliminated the morphological aspect of selfing by using only monokaryons at F1 in order to make selfing and outcrossing comparable. However, in natural conditions, selfing would benefit from that morphological aspect, which may contribute to reproductive allocation decisions in *P. anserina*.

5 | CONCLUSION

We have shown that there is a general cost of outcrossing to fitness in wild collected strains of *P. anserina*, and detailed the contribution of a

non-self recognition locus to these costs. Our findings strengthen the view that *P. anserina* does not benefit from outcrossing despite being a functionally diploid organism that frequently encounters conspecifics. Although our experiment failed to detect mate choice in *P. anserina*, we suggest that mechanisms preventing costly outcrossing are likely to exist and propose that the reproductive morphology of *P. anserina* favouring selfing, as well as prezygotic effects of *het-v* (and potentially other *het* systems), could have evolved as part of such mechanisms. We thus propose that sexual incompatibility in *P. anserina* could be adaptive rather than a collateral cost of non-self recognition loci.

Despite these dispositions to selfing in dikaryons, there are genomic signatures of outcrossing in *P. anserina* (Ament-Velásquez et al., 2022; Hartmann et al., 2021), which remind us that benefits of outcrossing might still be detected under conditions that remain to be established (e.g. exposure to pathogens or competitors). Outcrossing rates could then be regulated for example by the production of monokaryotic spores.

Fungi have long been model organisms for the study of genetics, but they can also be useful tools for the understanding of costs and benefits of different mating systems, and on the propensity of fungi to engage in different reproductive strategies (see e.g. Giraud et al., 2005). Continuing to explore fungal mating systems will not only help us understand the evolution and population dynamics of these major actors of our ecosystems, but it is also likely to provide insights on the role of sexual reproduction as a determinant of genetic diversity.

AUTHOR CONTRIBUTIONS

Ivain Martinossi-Allibert: Conceptualization (lead); data curation (lead); formal analysis (lead); funding acquisition (lead); investigation (lead); methodology (equal); visualization (lead); writing – original draft (lead); writing – review and editing (equal). **S. Lorena Ament-Velásquez:** Formal analysis (supporting); methodology (supporting); software (supporting); validation (supporting); visualization (supporting); writing – review and editing (equal). **Sven J. Saupé:** Conceptualization (equal); funding acquisition (supporting); investigation (equal); methodology (equal); project administration (equal); supervision (equal); validation (equal); writing – original draft (supporting); writing – review and editing (equal). **Hanna Johannesson:** Conceptualization (equal); funding acquisition (equal); investigation (equal); methodology (equal); project administration (equal); supervision (lead); writing – original draft (equal); writing – review and editing (equal).

ACKNOWLEDGEMENTS

We thank recurrent funding from CNRS and University of Bordeaux to the SJS lab (IBGC UMR 5095), and grant Vetenskapsrådet 2019-06294 to I.M. We also thank Alexandra Granger-Farbos and Corinne Clavé for their expertise of *P. anserina*, their support and fruitful discussions throughout the experimental phases of the project.

CONFLICT OF INTEREST

None.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/jeb.14108>.

DATA AVAILABILITY STATEMENT

Raw data and R code that support the finding of this study are available on Dryad: <https://doi.org/10.5061/dryad.np5hqbzx6>.

ORCID

Ivain Martinossi-Allibert  <https://orcid.org/0000-0002-8332-8620>

REFERENCES

- Ament-Velázquez, S. L., Vogan, A. A., Granger-Farbos, A., Bastiaans, E., Martinossi-Allibert, I., Saupe, S. J., de Groot, S., Lascoux, M., Debets, A. J. M., Clavé, C., & Johannesson, H. (2022). Allorecognition genes drive reproductive isolation in *Podospora anserina*. *Nature Ecology & Evolution*, 6, 1–14.
- Andersson, M. (1994). *Sexual selection* (Vol. 72). Princeton University Press.
- Arnaise, S., Zickler, D., Poisier, C., & Debuchy, R. (2001). pah1: A homeobox gene involved in hyphal morphology and microconidiogenesis in the filamentous ascomycete *Podospora anserina*. *Molecular Microbiology*, 39, 54–64.
- Barrett, S. C. (2003). Mating strategies in flowering plants: The outcrossing–selfing paradigm and beyond. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences*, 358, 991–1004.
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Beekman, M., Nieuwenhuis, B., Ortiz-Barrientos, D., & Evans, J. P. (2016). Sexual selection in hermaphrodites, sperm and broadcast spawners, plants and fungi. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 371, 20150541.
- Bell, G. (1982). *The paradox of sexuality. The masterpiece of nature. The evolution and genetics of sexuality* (pp. 19–78). Routledge.
- Berger-Tal, R., Tuni, C., Lubin, Y., Smith, D., & Bilde, T. (2014). Fitness consequences of outcrossing in a social spider with an inbreeding mating system. *Evolution*, 68(2), 343–351.
- Bernet, J. (1965). *Mode d'action des gènes de "barrage" et relation entre l'incompatibilité cellulaire et l'incompatibilité sexuelle chez "Podospora anserina"*. Ph.D. dissertation, Masson & Cie, Éditeurs.
- Bernet, J. (1967). Incompatibility systems in *Podospora anserina*. *Comptes Rendus Hebdomadaires des Seances de L Academie des Sciences Serie D*, 265, 1330.
- Biella, S., Smith, M. L., Aist, J. R., Cortesi, P., & Milgroom, M. G. (2002). Programmed cell death correlates with virus transmission in a filamentous fungus. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 269, 2269–2276.
- Billiard, S., López-Villavicencio, M., Hood, M. E., & Giraud, T. (2012). Sex, outcrossing and mating types: Unsolved questions in fungi and beyond. *Journal of Evolutionary Biology*, 25, 1020–1038.
- Boucherie, H., & Bernet, J. (1980). Protoplasmic incompatibility in *Podospora anserina*: A possible function for incompatibility genes. *Genetics*, 96, 399–411.
- Charlesworth, D., & Charlesworth, B. (1987). Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics*, 18, 237–268.
- Charlesworth, D., & Willis, J. H. (2009). The genetics of inbreeding depression. *Nature Reviews Genetics*, 10, 783–796.
- Charnov, E. L. (1982). The theory of sex allocation. *Monographs in Population Biology*, 18, 1–355.
- Coppin, E. (2002). The fle1 gene encoding a C2H2 zinc finger protein co-ordinates male and female sexual differentiation in *Podospora anserina*. *Molecular Microbiology*, 43, 1255–1268.
- Edward, D. A. (2015). The description of mate choice. *Behavioral Ecology*, 26, 301–310.
- Esser, K. (1974). *Podospora anserina. I bacteria, bacteriophages, and fungi* (pp. 531–551). Springer.
- Giraud, T., Jonot, O., & Shykoff, J. A. (2005). Selfing propensity under choice conditions in a parasitic fungus, *Microbotryum violaceum*, and parameters influencing infection success in artificial inoculations. *International Journal of Plant Sciences*, 166(4), 649–657.
- Hartfield, M., & Keightley, P. D. (2012). Current hypotheses for the evolution of sex and recombination. *Integrative zoology*, 7, 192–209.
- Hartmann, F. E., Ament-Velázquez, S. L., Vogan, A. A., Gautier, V., Le Prieur, S., Berramdane, M., Snirc, A., Johannesson, H., Grognet, P., Malagnac, F., Silar, P., & Giraud, T. (2021). Size variation of the nonrecombining region on the mating-type chromosomes in the fungal *Podospora anserina* species complex. *Molecular Biology and Evolution*, 38(6), 2475–2492.
- Jarne, P., & Auld, J. R. (2006). Animals mix it up too: The distribution of self-fertilization among hermaphroditic animals. *Evolution*, 60, 1816–1824.
- Jarne, P., & Charlesworth, D. (1993). The evolution of the selfing rate in functionally hermaphroditic plants and animals. *Annual Review of Ecology and Systematics*, 24, 441–466.
- Kempnaers, B. (2007). Mate choice and genetic quality: A review of the heterozygosity theory. *Advances in the Study of Behavior*, 37, 189–278.
- Kirkpatrick, M., & Ravigné, V. (2002). Speciation by natural and sexual selection: Models and experiments. *The American Naturalist*, 159, S22–S35.
- Leslie, J. F., & Raju, N. B. (1985). Recessive mutations from natural populations of *Neurospora crassa* that are expressed in the sexual diplophase. *Genetics*, 111, 759–777.
- Morran, L. T., Parmenter, M. D., & Phillips, P. C. (2009). Mutation load and rapid adaptation favour outcrossing over self-fertilization. *Nature*, 462, 350–352.
- Neff, B. D., & Pitcher, T. E. (2005). Genetic quality and sexual selection: An integrated framework for good genes and compatible genes. *Molecular Ecology*, 14, 19–38.
- Nieuwenhuis, B. P., & James, T. Y. (2016). The frequency of sex in fungi. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 371, 20150540.
- Otto, S. P. (2021). Selective interference and the evolution of sex. *Journal of Heredity*, 112, 9–18.
- Pinan-Lucarré, B., Paoletti, M., & Clavé, C. (2007). Cell death by incompatibility in the fungus *Podospora*. *Seminars in Cancer Biology*, 17, 101–111.
- R Core Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. <https://www.R-project.org/>
- Rizet, G. (1939). De l'hérédité du caractère absence de pigment dans le mycélium d'un ascomycète du genre *Podospora*. *Comptes Rendus. Académie des Sciences*, 209, 771.
- Rizet, G. (1942). Sur l'obtention d'irrégularités dans les asques et les spores de Pyrénomycètes du genre *Podospora* par l'action de quelques anastésiques. *Bulletin. Société Linnéenne de Normandie*, 2, 123–130.
- Rizet, G. (1953). Sur la multiplicité des mécasismes génétiques conduisant à des barrages chez *Podospora anserina*. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences*, 237, 666–668.

- Rogers, D. W., & Greig, D. (2009). Experimental evolution of a sexually selected display in yeast. *Proceedings of the Royal Society B: Biological Sciences*, 276, 543–549.
- Saupe, S. J. (2000). Molecular genetics of heterokaryon incompatibility in filamentous ascomycetes. *Microbiology and Molecular Biology Reviews*, 64(3), 489–502.
- Smith, C., & Greig, D. (2010). The cost of sexual signaling in yeast. *Evolution*, 64, 3114–3122.
- Tregenza, T., & Wedell, N. (2000). Genetic compatibility, mate choice and patterns of parentage: Invited review. *Molecular Ecology*, 9, 1013–1027.
- Turner, E., Jacobson, D. J., & Taylor, J. W. (2010). Reinforced postmating reproductive isolation barriers in *Neurospora*, an Ascomycete microfungus. *Journal of Evolutionary Biology*, 23, 1642–1656.
- van der Gaag, M. (2005). *Genomic conflicts in Podospora anserina=Genomische conflicten in Podospora anserina*. Wageningen University and Research.
- Vogan, A. A., Ament-Velásquez, S. L., Bastiaans, E., Wallerman, O., Saupe, S. J., Suh, A., & Johannesson, H. (2021). The Enterprise, a massive transposon carrying Spok meiotic drive genes. *Genome Research*, 31, 789–798.
- Vogan, A. A., Ament-Velásquez, S. L., Granger-Farbos, A., Svedberg, J., Bastiaans, E., Debets, A. J., Coustou, V., Yvanne, H., Clavé, C., Saupe, S. J., & Johannesson, H. (2019). Combinations of Spok genes create multiple meiotic drivers in *Podospora*. *eLife*, 8, e46454.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Martinossi-Allibert, I., Ament-Velásquez, S. L., Saupe, S. J., & Johannesson, H. (2023). To self or not to self? Absence of mate choice despite costly outcrossing in the fungus *Podospora anserina*. *Journal of Evolutionary Biology*, 36, 238–250. <https://doi.org/10.1111/jeb.14108>