The link between sexual and vegetative (in)compatibility in fungal speciation

Hanna Johannesson

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The capacity to discriminate self from non-self (allorecognition)

is necessary for fundamental life processes…

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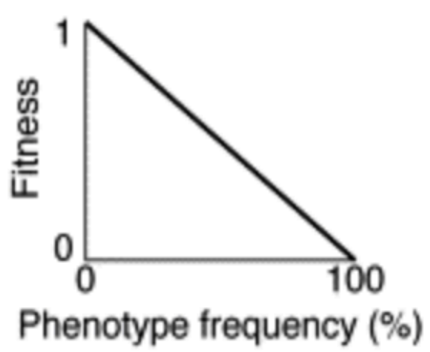
..such as pathogen recognition, choice of mating partner and vegetative growth

Allorecognition is achieved by the products of highly polymorphic genes

Allorecognition is achieved by the products of highly polymorphic genes

often found under negative frequency dependent (balancing) selection



and display signatures of rapid evolution

The significance of allorecognition for speciation

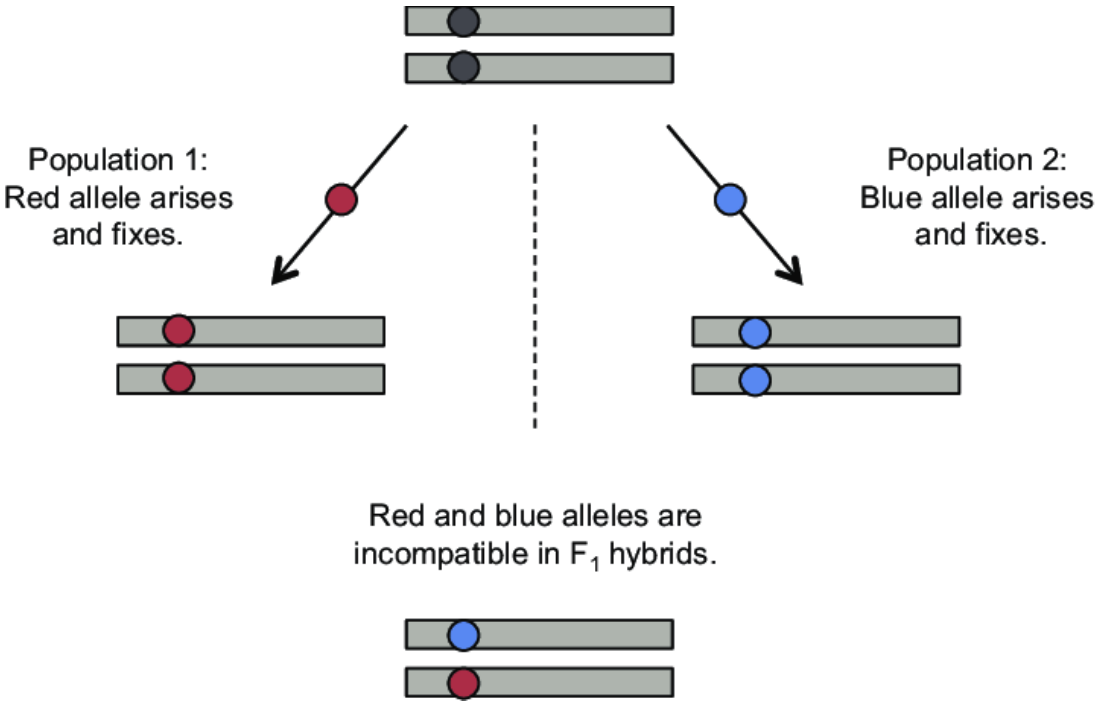
The significance of allorecognition for speciation

Indirect effects

The significance of allorecognition for speciation

Indirect effects

Rapid evolution may lead to genetic incompatibilities between diverging populations



Bateson-Dobzhansky-Muller (BDM) model for the evolution of reproductive isolation

The significance of allorecognition for speciation

Indirect effects

Rapid evolution may lead to genetic incompatibilities between diverging populations

For example, mismatch of immunity genes results in a lethal autoimmune reaction in plants

REVIEWS

Hybrid necrosis: autoimmunity as a potential gene-flow barrier in plant species

Kirsten Bomblies and Detlef Weigel

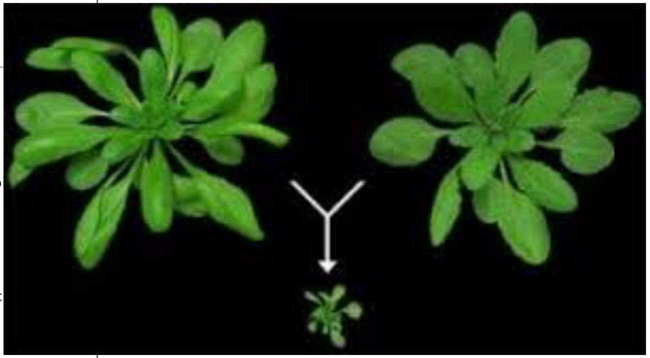
Abstract | Ecological factors, hybrid sterility and differences in ploidy levels are well known for contributing to gene-flow barriers in plants. Another common postzygotic incompatibility, hybrid necrosis, has received comparatively little attention in the evolutionary genetics literature. Hybrid necrosis is associated with a suite of phenotyp characteristics that are similar to those elicited in response to various environmental stresses, including pathogen attack. The genetic architecture is generally simple, and complies with the Bateson–Dobzhansky–Muller model for hybrid incompatibility between species. We survey the extensive literature on this topic and present the hypothesis that hybrid necrosis can result from autoimmunity, perhaps as a pleiotropic effect of evolution of genes that are involved in pathogen response.

Prezygotic barriers
Gene-flow barriers or incompatibilities that act to prevent successful fertilization.

Postzygotic barriers
Gene-flow barriers or

Why species exist as separate entities and how they form is a vibrant topic in evolutionary biology. It has long been recognized that species hybrids, if they are able to form at all, often fail owing to embryonic lethality, adult inviability or inferiority, or sterility. Considerable progress has been made in both plants and animals in unravelling

literature on pollinator preferences, local environmental adaptation, pollen recognition and other forces that reduce the chance of mating success (for example, REF. 13 and the references therein). Many examples of postzygotic gene-flow barriers are also known, and in several taxa postzygotic barriers might be at least as com-



The significance of allorecognition for speciation

Direct effects

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Direct effects

By interaction between different forms of allorecognition (pleiotropy)

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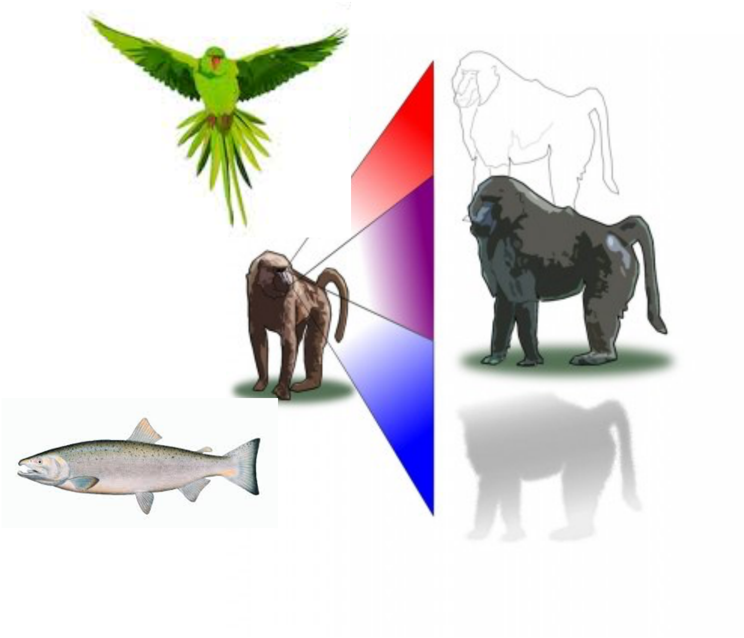
For example, an overlap between the MHC and mate choice in vertebrates

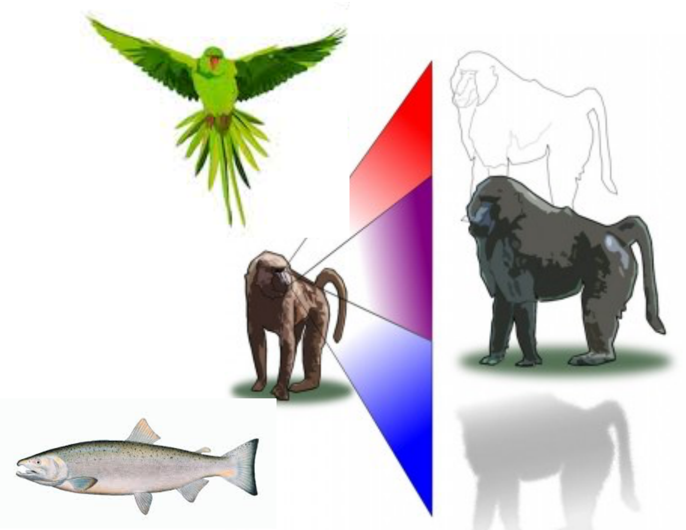


The significance of allorecognition for speciation

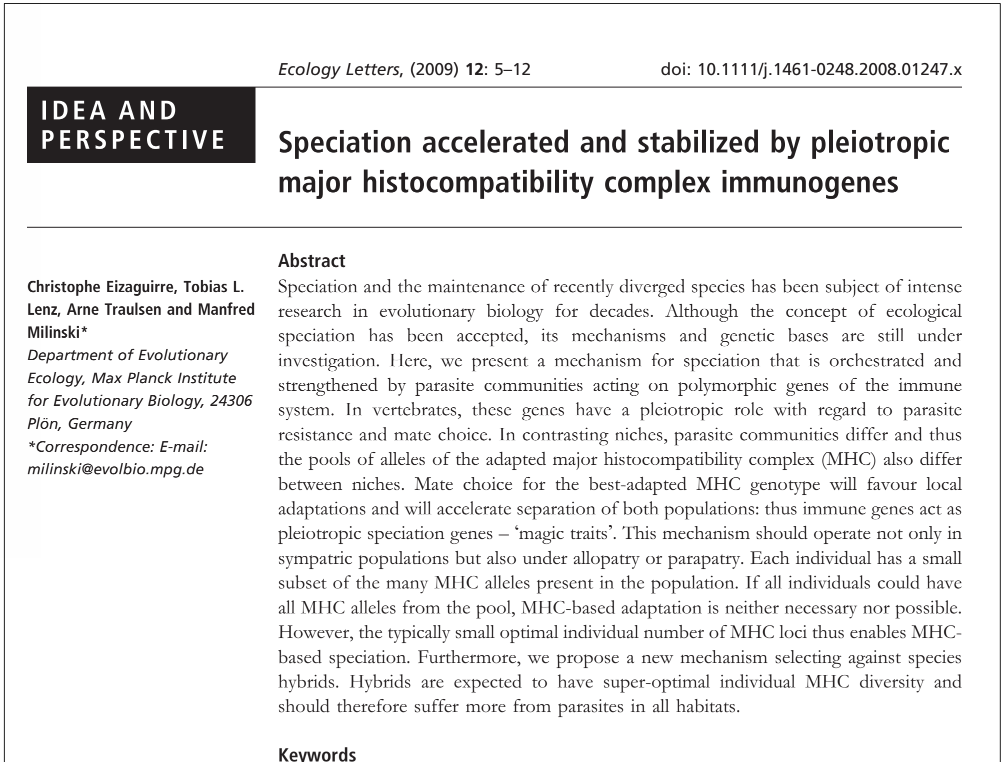
Direct effects

By interaction between different forms of allorecognition (pleiotropy)

For example, an overlap between the MHC and mate choice in vertebrates



In contrasting niches,
parasite communities differ
-- and hence,
the pools of alleles of the
adapted MHC also differ



Ecology Letters, (2009) **12**: 5–12 doi: 10.1111/j.1461-0248.2008.01247.x

IDEA AND PERSPECTIVE

Speciation accelerated and stabilized by pleiotropic major histocompatibility complex immunogenes

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**Correspondence: E-mail: milinski@evolbio.mpg.de*

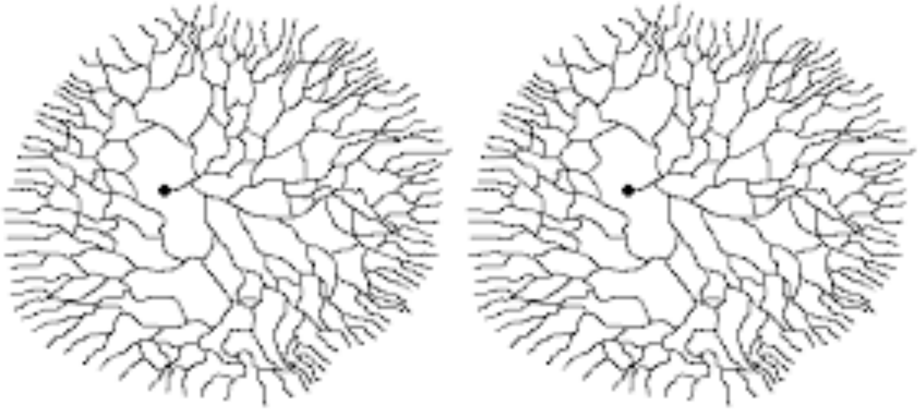
Abstract

Speciation and the maintenance of recently diverged species has been subject of intense research in evolutionary biology for decades. Although the concept of ecological speciation has been accepted, its mechanisms and genetic bases are still under investigation. Here, we present a mechanism for speciation that is orchestrated and strengthened by parasite communities acting on polymorphic genes of the immune system. In vertebrates, these genes have a pleiotropic role with regard to parasite resistance and mate choice. In contrasting niches, parasite communities differ and thus the pools of alleles of the adapted major histocompatibility complex (MHC) also differ between niches. Mate choice for the best-adapted MHC genotype will favour local adaptations and will accelerate separation of both populations: thus immune genes act as pleiotropic speciation genes – 'magic traits'. This mechanism should operate not only in sympatric populations but also under allopatry or parapatry. Each individual has a small subset of the many MHC alleles present in the population. If all individuals could have all MHC alleles from the pool, MHC-based adaptation is neither necessary nor possible. However, the typically small optimal individual number of MHC loci thus enables MHC-based speciation. Furthermore, we propose a new mechanism selecting against species hybrids. Hybrids are expected to have super-optimal individual MHC diversity and should therefore suffer more from parasites in all habitats.

Keywords

The significance of allorecognition for speciation

In colonial organisms – pleiotropy between vegetative and sexual incompatibility may lead to the equivalence between allo-groups and reproductively isolated groups



The significance of allorecognition for speciation

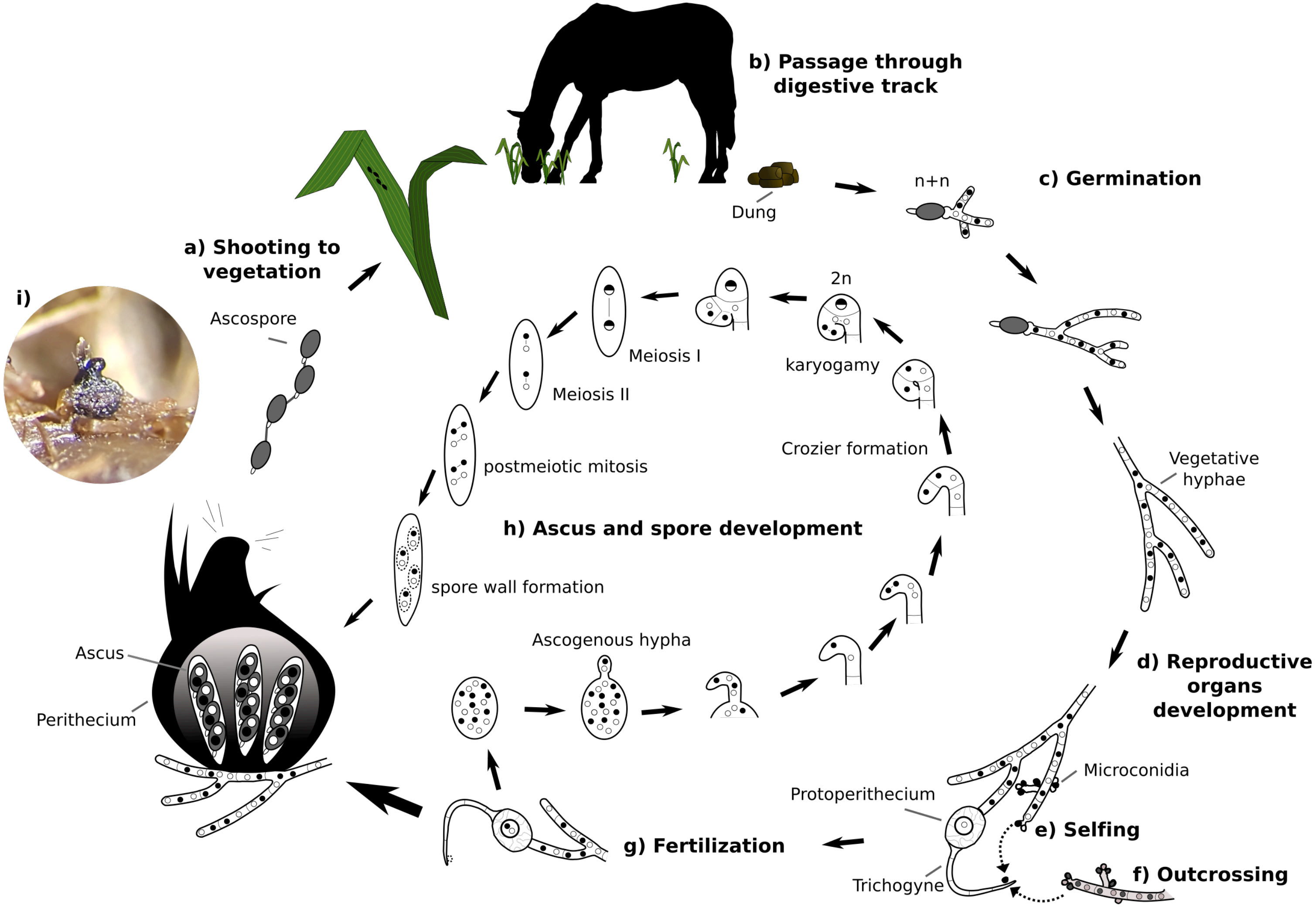
Sandra Lorena Ament Velasquez
Ivain Martinossi
Aaron Vogan

Collaboration with
Sven Saupe (Bordeaux)
Fons Debets (Wageningen)

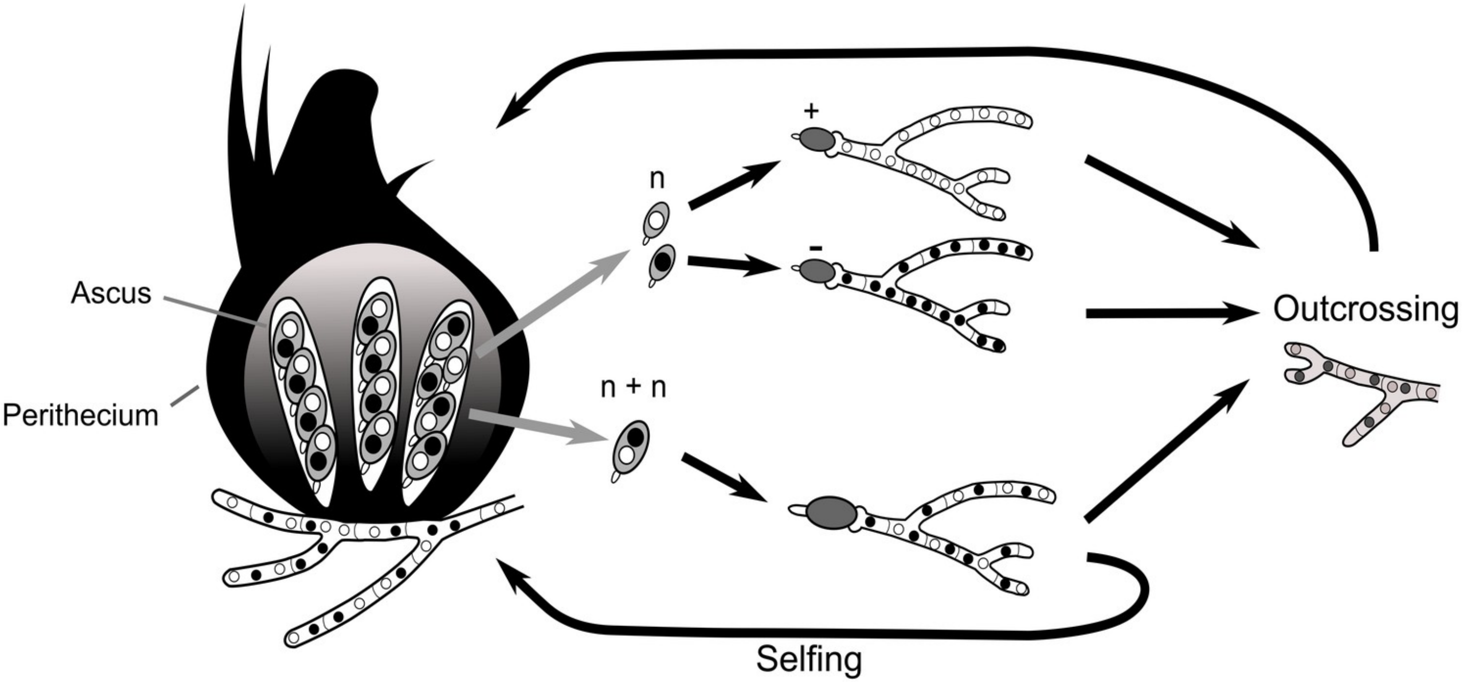
Our ultimate questions:

Can pleiotropy of allorecognition systems cause speciation in fungi? And if so, how?

Podospora anserina – the model system



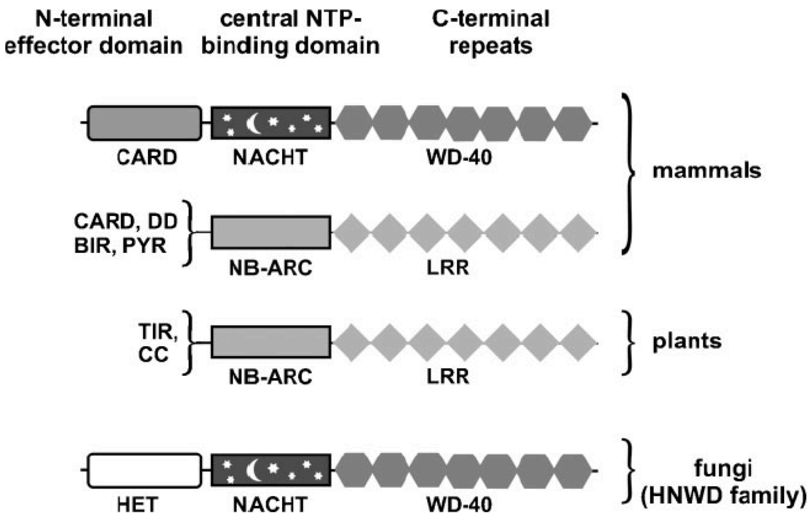
Podospora anserina – highly selfing with opportunities for outcrossing



Vogan et al., eLife 2019

Pleiotropic effects of allorecognition systems in *P. anserina*

Pleiotropic effects of allorecognition systems in *P. anserina*



Sven Saupe, University of Bordeaux

DOI 10.1002/bies.200900085 **Hypothesis**

Fungal incompatibility: Evolutionary origin in pathogen defense?

*Mathieu Paoletti and Sven J. Saupe**

Laboratoire de Génétique Moléculaire des Champignons, Institut de Biochimie et de Génétique Cellulaires, UMR 5095 CNRS – Université de Bordeaux 2, 1 rue Camille St Saëns, 33077 Bordeaux cedex, France

In fungi, cell fusion between genetically unlike individuals triggers a cell death reaction known as the incompatibility reaction. In *Podospora anserina*, the genes controlling this process belong to a gene family encoding STAND proteins with an N-terminal cell death effector domain, a central NACHT domain and a C-terminal WD-repeat domain. These incompatibility genes are extremely polymorphic, subject to positive Darwinian selection and display a remarkable genetic plasticity allowing for constant diversification of the WD-repeat domain responsible for recognition of non-self. Remarkably, the architecture of these proteins is related to pathogen-recognition receptors ensuring innate immunity in plants and animals. Here, we hypothesize that these *P. anserina* incompatibility genes could be components of a yet-unidentified innate immune system of fungi. As already proposed in the case of plant hybrid necrosis or graft rejection in mammals, incompatibility could be a by-product of pathogen-driven divergence in host defense genes.

Keywords: allorecognition; fungi; innate immunity

Introduction

In all organisms the ability to protect oneself from pathogens is absolutely essential for survival. Pathogens impose a considerable selective pressure and, as a result, both prokaryotic and eukaryotic organisms have developed genetic arsenals of ever increasing complexity to cope with this constant and evolving threat.(1) To the biologist, and in common thought, the fungal branch of the eukaryota tree often lies in the shade of the more renowned plant and animal branches and, because many fungal species interfere in human affairs as nasty pathogens, they are not commonly envisioned as being themselves potential victims of pathogen attack. Still, like all organisms, fungi are potential hosts for microbial pathogens and have developed defense systems against competitors and pathogens, penicillin secretion being a simple and well-known example of such a system.(2) While molecular components of innate immunity are reasonably well characterized in plants and animals,(3–6) virtually nothing is known about the pathogen-recognition systems in fungi. What is known, however, is that fungi display genetic systems that lead to recognition and response to non-self during cell fusion between different individuals belonging to the same species.(7,8) These genes that lead to rejection of conspecific non-self are termed heterokaryon incompatibility genes.

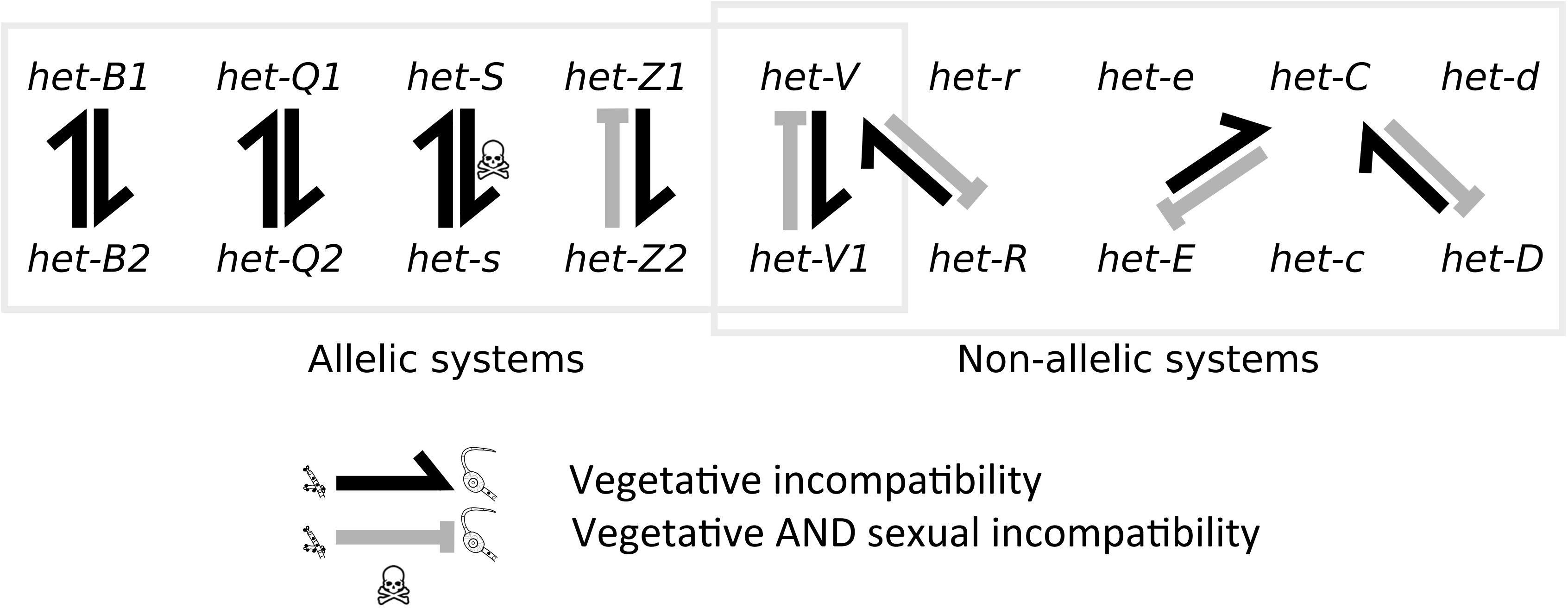
It is often proposed that fungi reject conspecific non-self to prevent various forms of conspecific parasitism or to limit horizontal transmission of infectious cytoplasmic elements.(9,10) We propose here a change in perspective regarding the biological significance of fungal incompatibility systems. We have noted the resemblance of the HNWD heterokaryon incompatibility gene family of the model species *Podospora anserina* with pathogen-recognition receptors (PRRs) in plant and animals, and propose that these genes might have a function in the recognition and response to heterospecific pathogenic non-self. In this view, fungal incompatibility genes that were originally experimentally characterized in the context of conspecific non-self recognition might actually have a function in pathogen recognition and represent a component of what could be a fungal innate immune system. Here we re-evaluate current knowledge on the genetics and cell biology of the incompatibility response in *P. anserina* and draw analogies between fungal incompatibility, hybrid necrosis in plants and sterile inflammatory response in mammals.

Non-self recognition in fungi

Filamentous fungi are constituted of partially separated and plurinucleated adjacent cells, so that the somatic apparatus of a fungus, called the mycelium, is a syncytial structure. To quickly colonize its growth medium, filaments within the growing colony will frequently fuse with each other through a process called anastomosis.(11) Anastomosis is not restricted to self fusion and can also occur between genetically different

Pleiotropic effects of allorecognition systems in *P. anserina*

Of the nine genetically identified *het* loci, six have pleiotropic effects on sexual function



het-B1 *het-Q1* *het-S* *het-Z1* *het-V* *het-r* *het-e* *het-C* *het-d*

het-B2 *het-Q2* *het-s* *het-Z2* *het-V1* *het-R* *het-E* *het-c* *het-D*

Allelic systems

Non-allelic systems

Vegetative incompatibility

Vegetative AND sexual incompatibility

Sequencing of the Wageningen collection of *Podospora anserina*

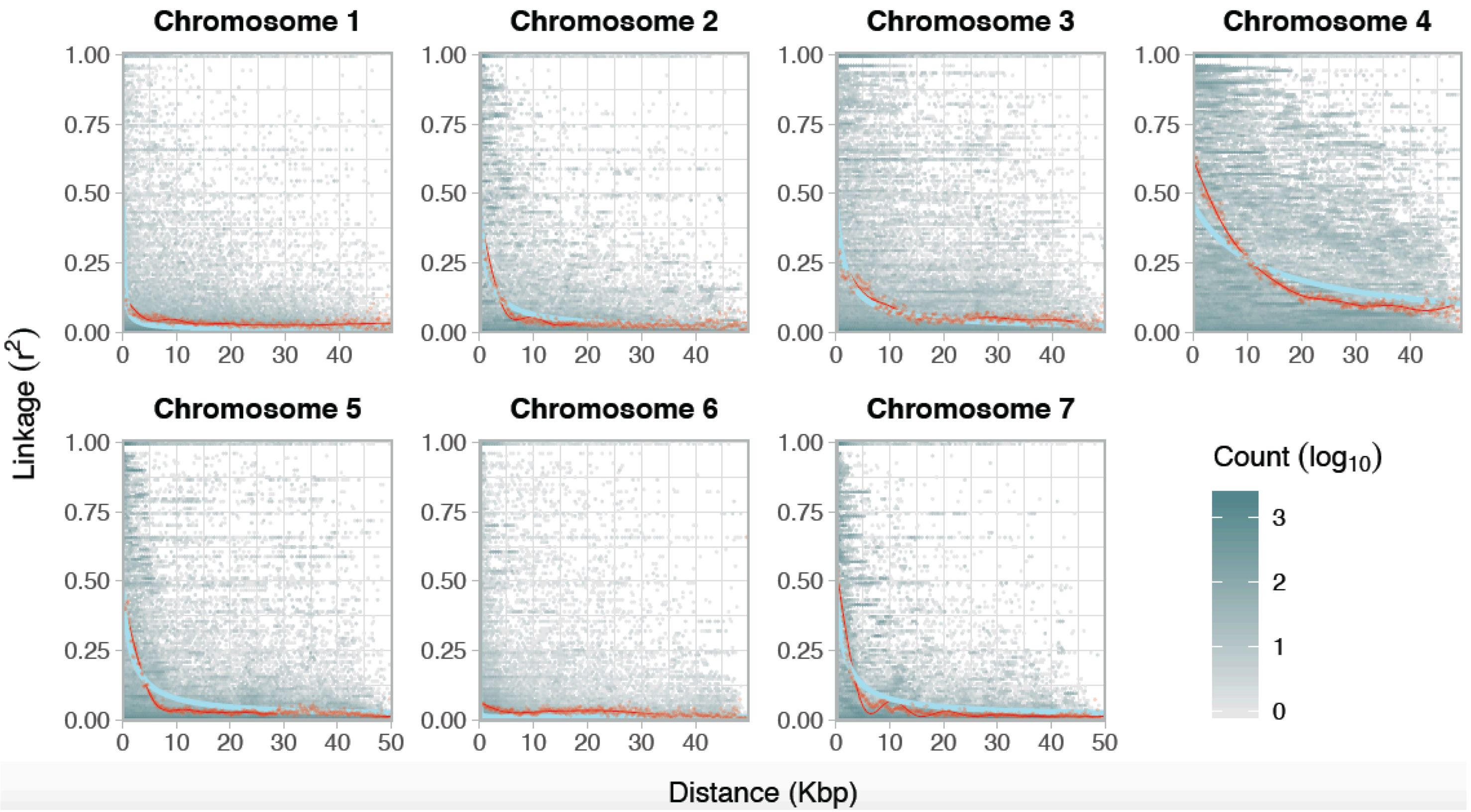
* 106 *P. anserina* strains spanning 25 years of sampling (1991-2016) around Wageningen, NL

(along with three French strains sampled in 1937, one sample from Ontario, Canada from 1944, and one Swiss strain from 1964.)

Fons Debets, Wageningen Lab of Genetics

Podospora anserina – highly selfing with opportunities for outcrossing

LD reaches 0.2 at distances below 3.5 kb



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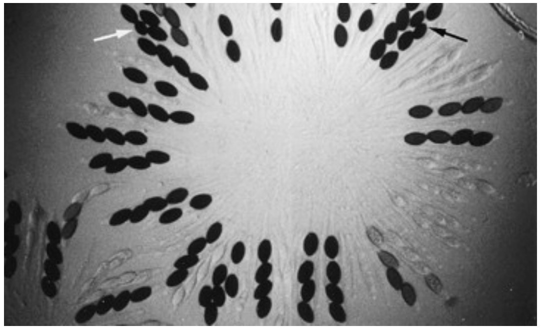
Podospora anserina – highly selfing with opportunities for outcrossing

Sampling in nature shows spore killing (heterozygosity)



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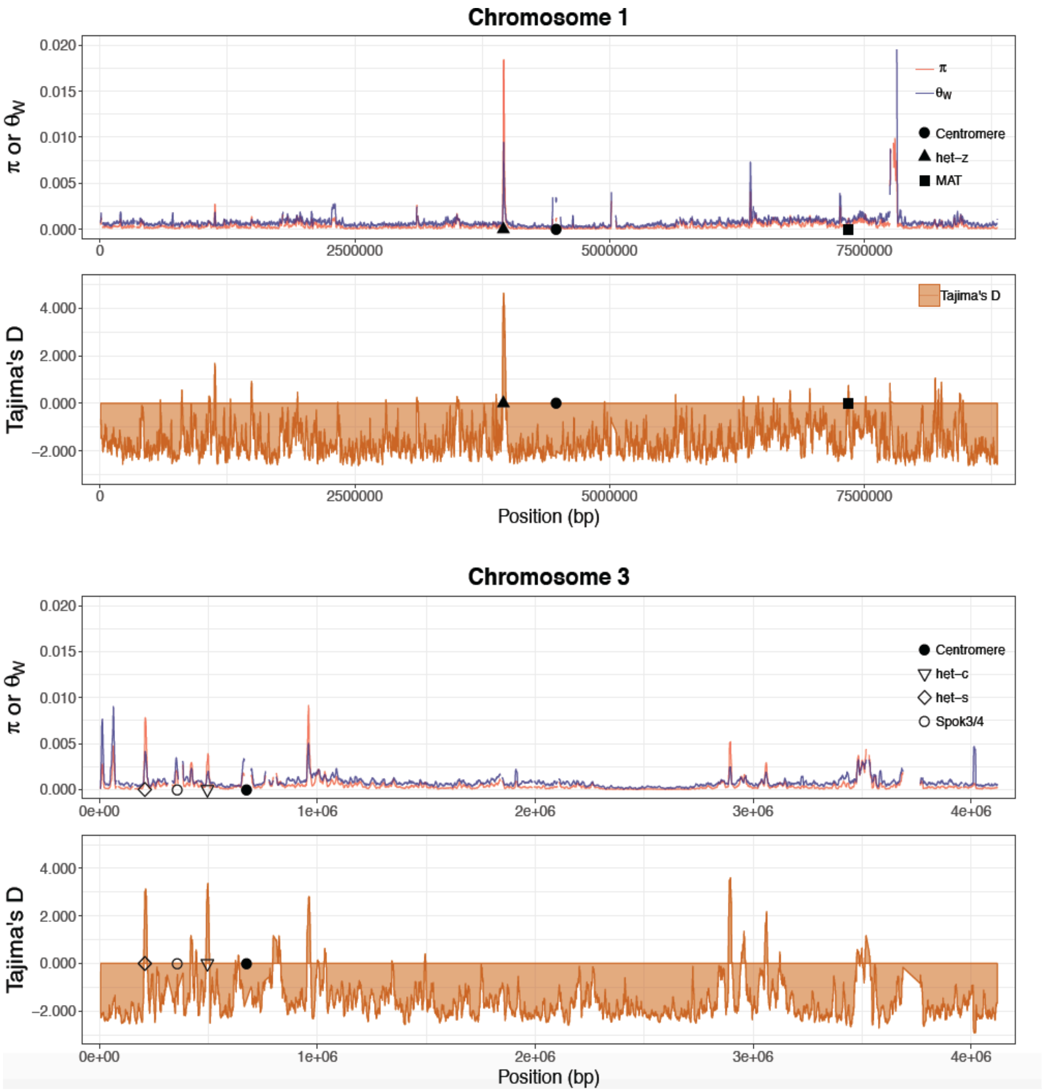
Low genetic variation with signatures of balancing selection



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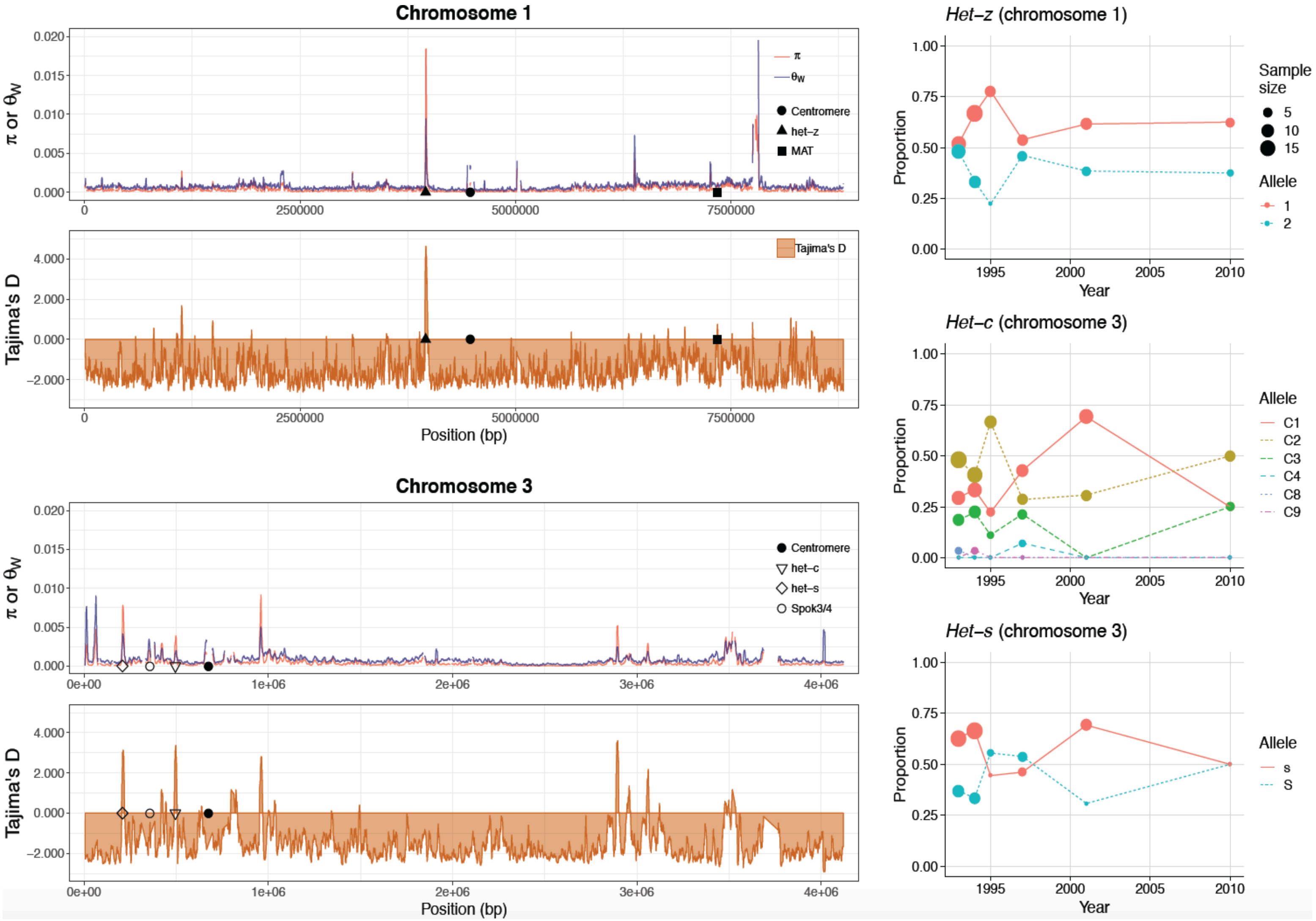

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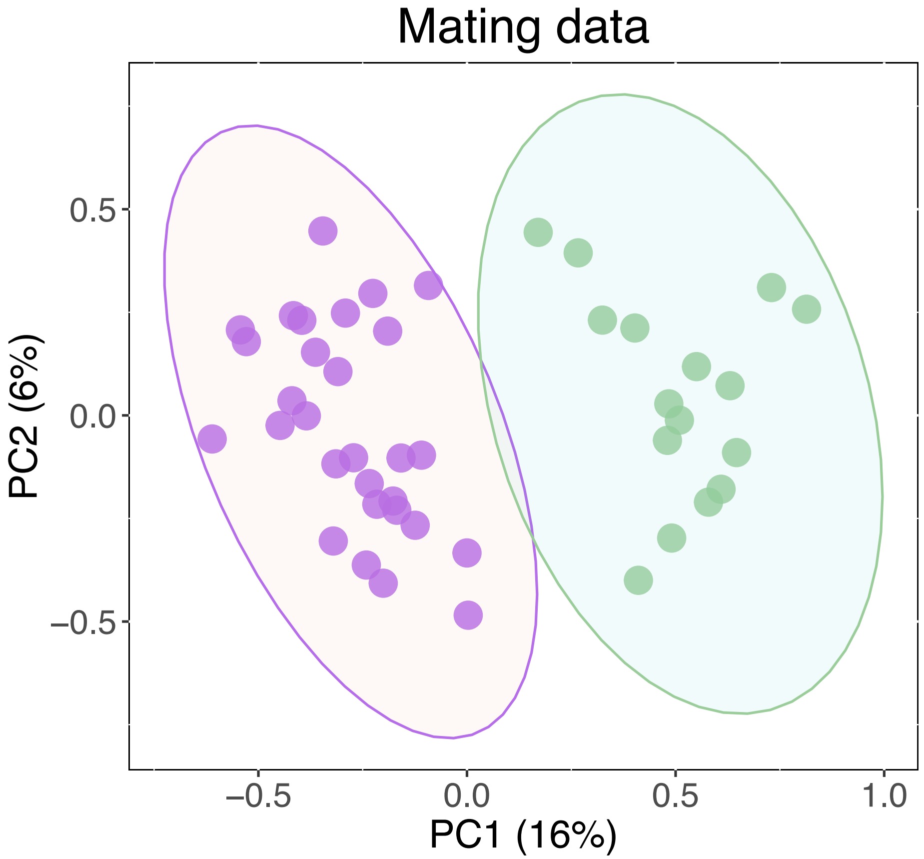
Analyses of mating success defines two groups in Wageningen



Analysis of data gathered by van der Gaag, 2005
Groups identified by the Partitioning Around Medioids (PAM) clustering method

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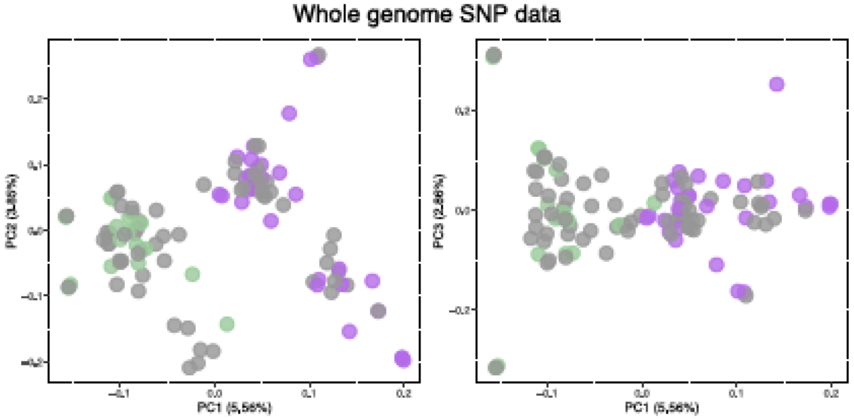
Mating groups supported by PCA analyses of SNP-data



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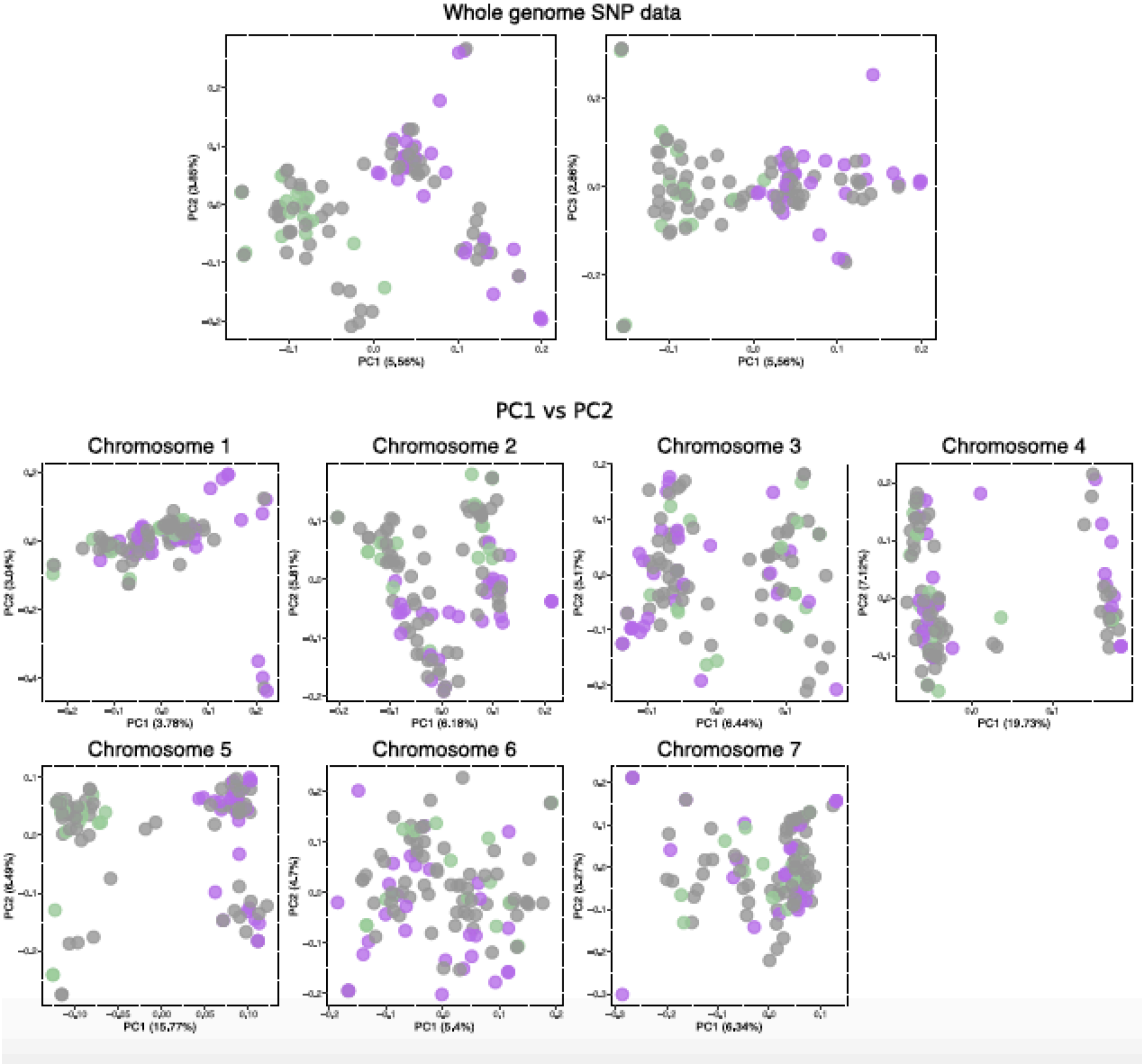

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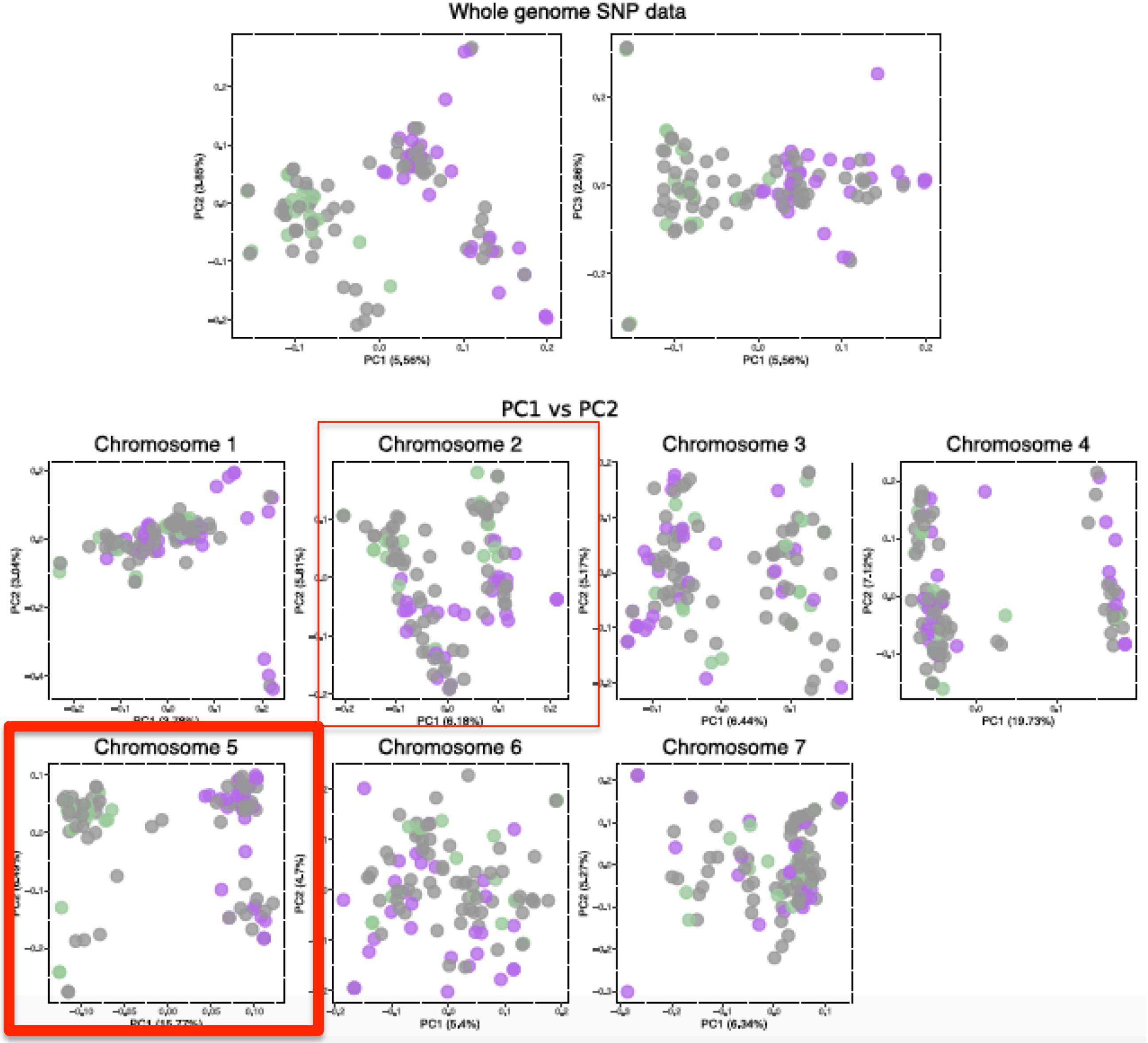
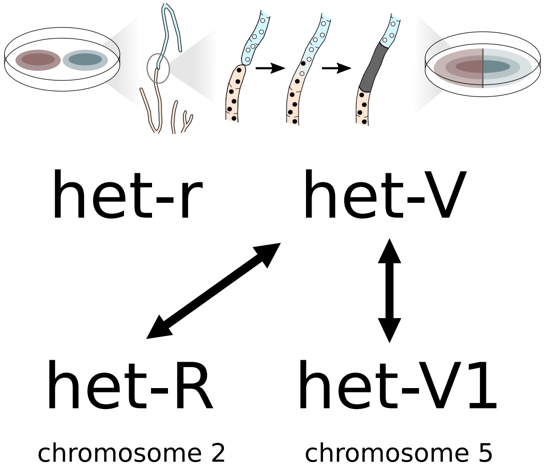


het-r and *het-v* are causal candidates behind the mating groups' isolation

Vegetative incompatibility



het-r

het-V

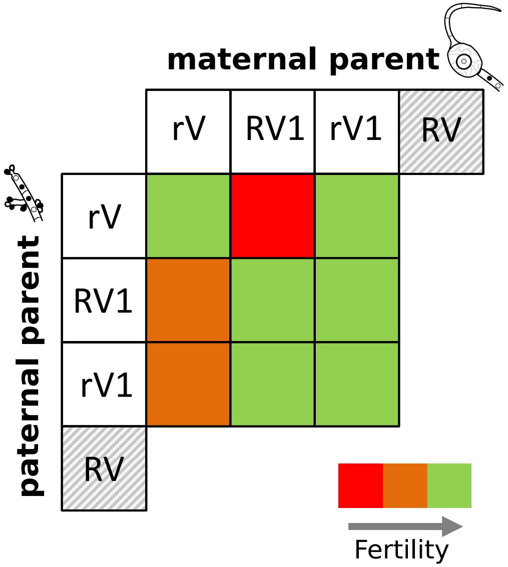
het-R

het-V1

chromosome 2

chromosome 5

Sexual incompatibility



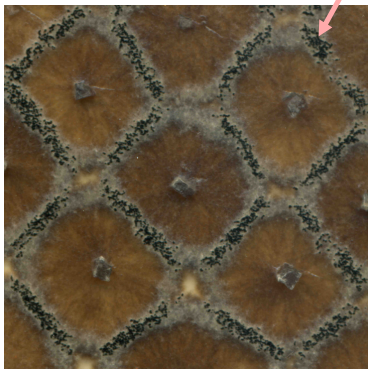
maternal parent

paternal parent

	rV	RV1	rV1	RV
rV	🟩	🟥	🟩	
RV1	🟧	🟩	🟩	
rV1	🟧	🟩	🟩	
RV				

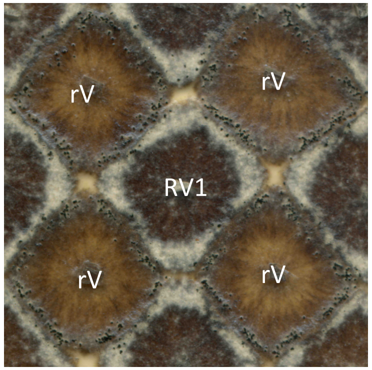
🟥🟧🟩 → Fertility

rV x rV

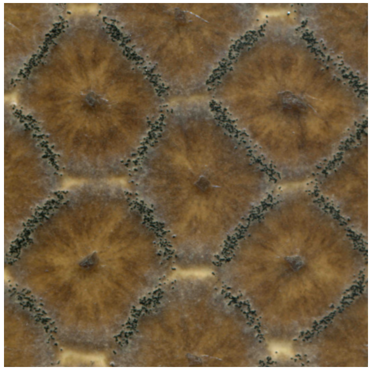


Fruiting bodies

RV1 x rV



RV1 x RV1



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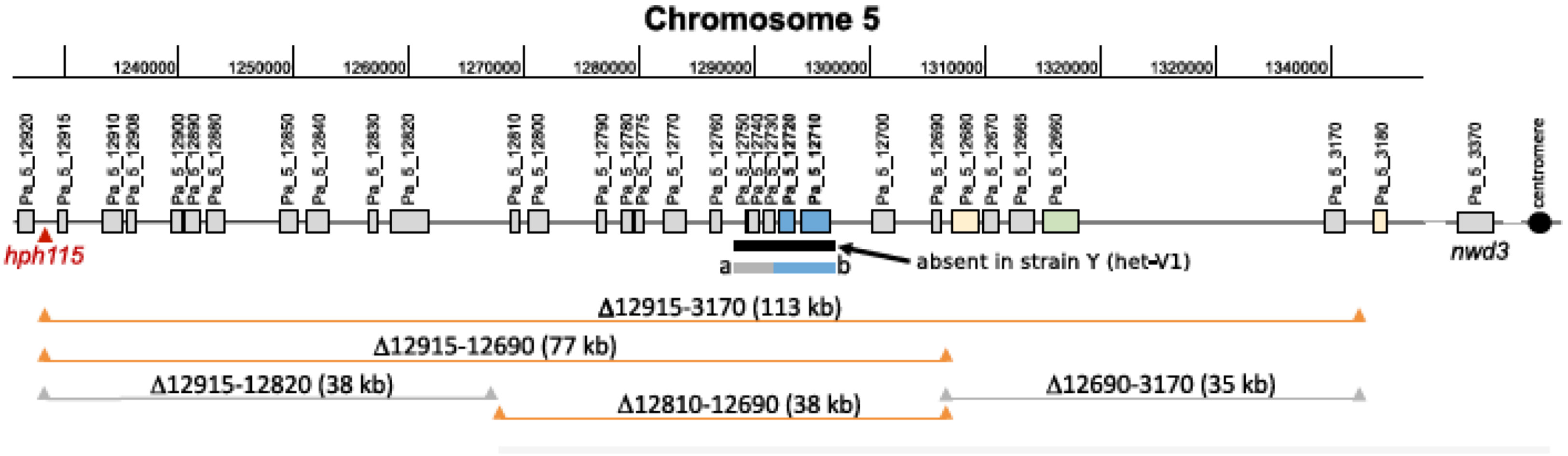
Molecular characterization of *het-v*

Positional and complementation cloning reveals the genetic basis of *het-v*



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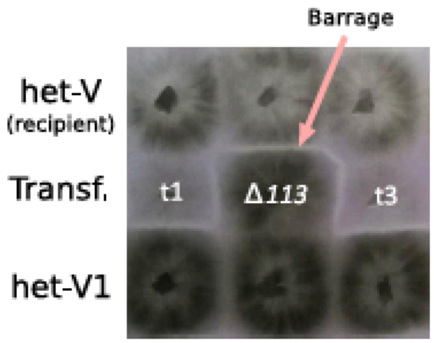
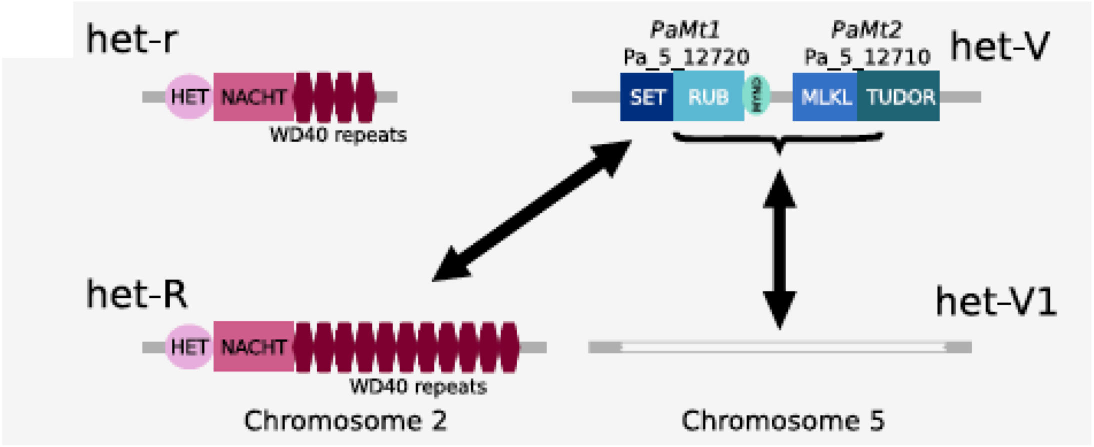
Molecular characterization of *het-v*

Site-directed mutants of PaMt1 confirm a methyltransferase activity





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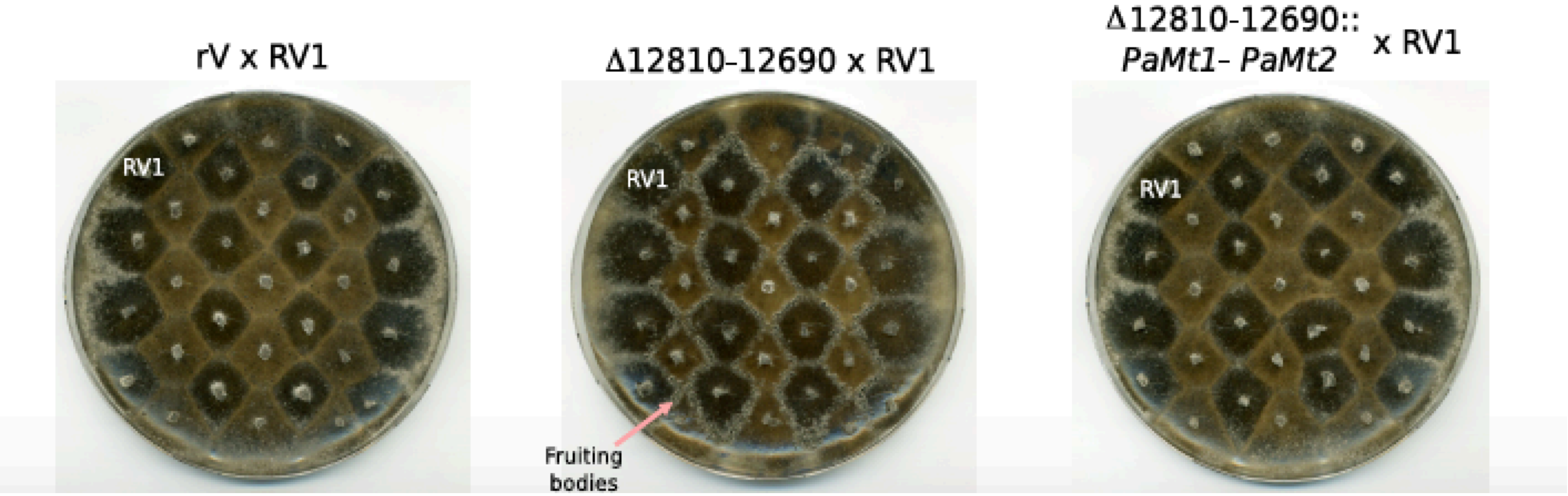
Molecular characterization of *het-v*

The deletion of PaMt1 and PaMt2 restores fertility with *het-R* strains

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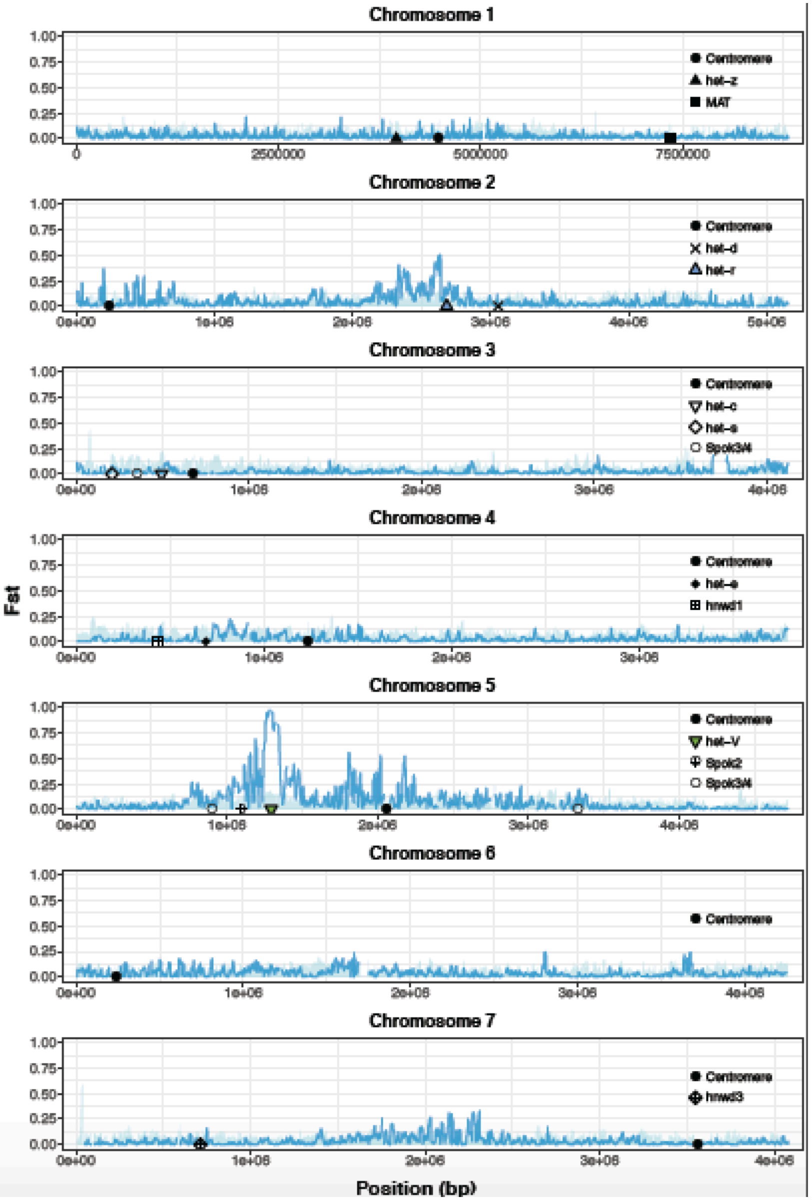
het-r and *het-v* are causal candidates behind the mating groups' isolation



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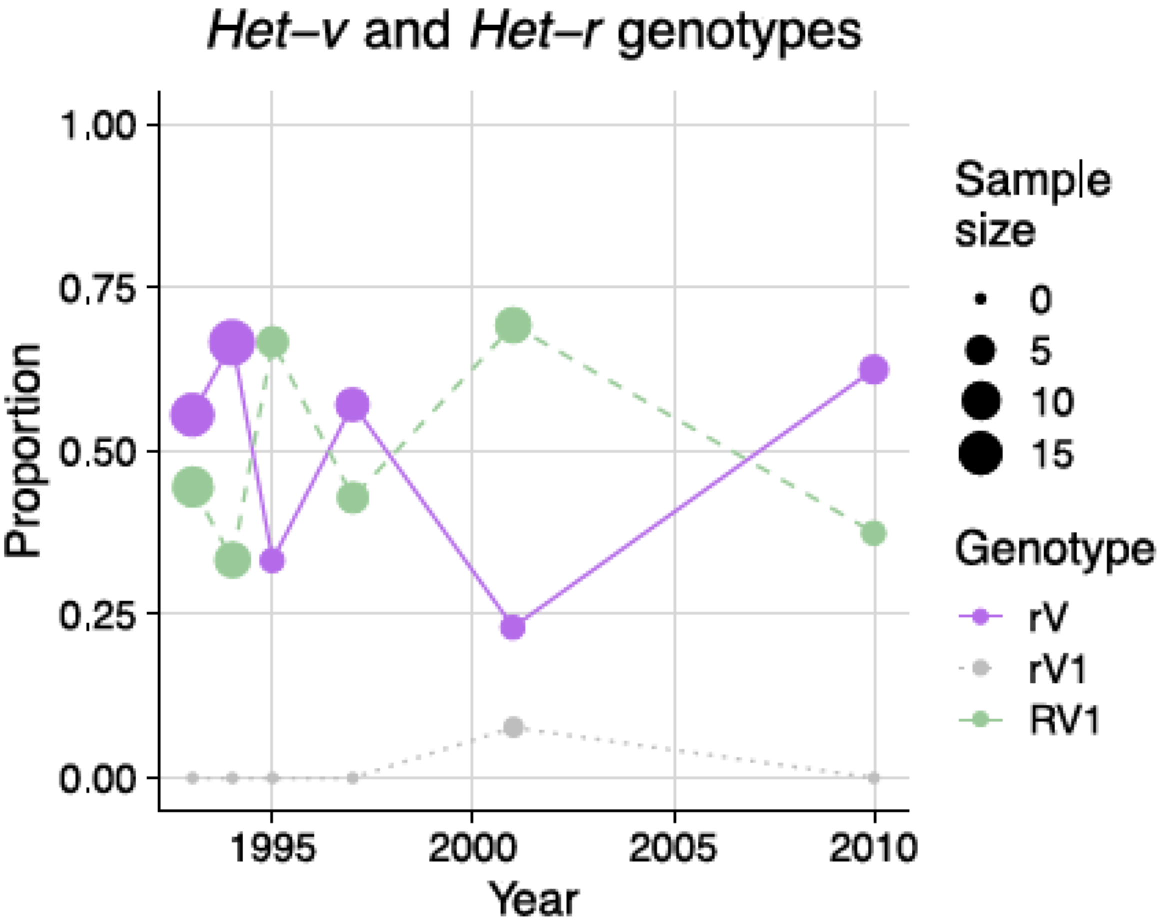
The rV and RV1 genotypes define the mating groups in nature



The rV1 genotype is significantly less frequent than expected by random mating

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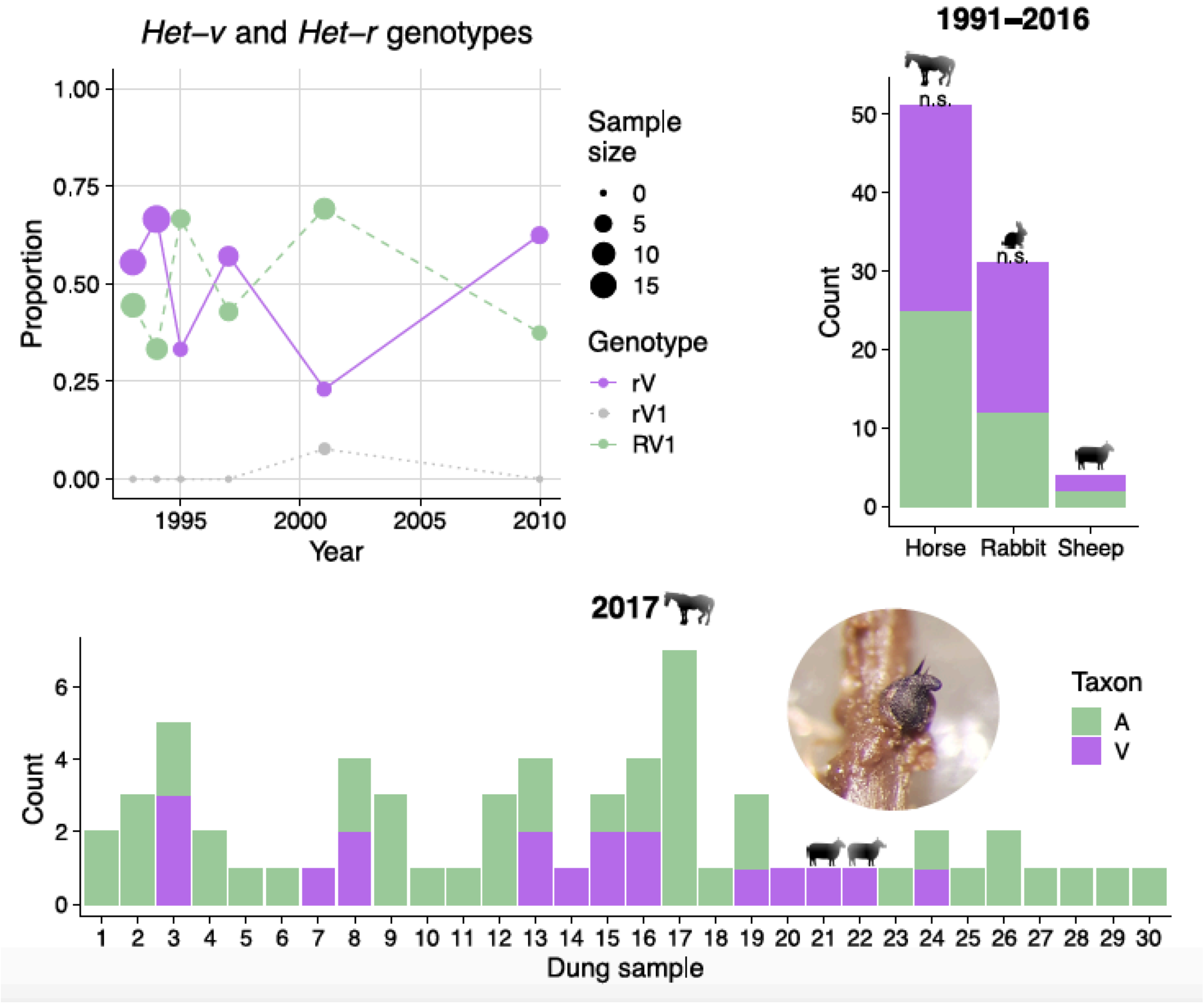
The mating groups coexist in their substrate – no ecological isolation



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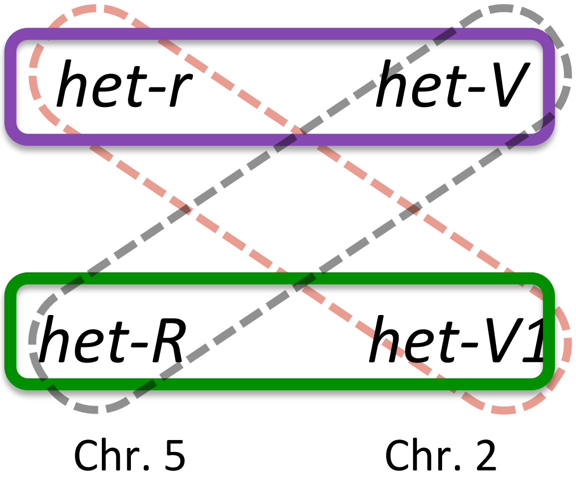


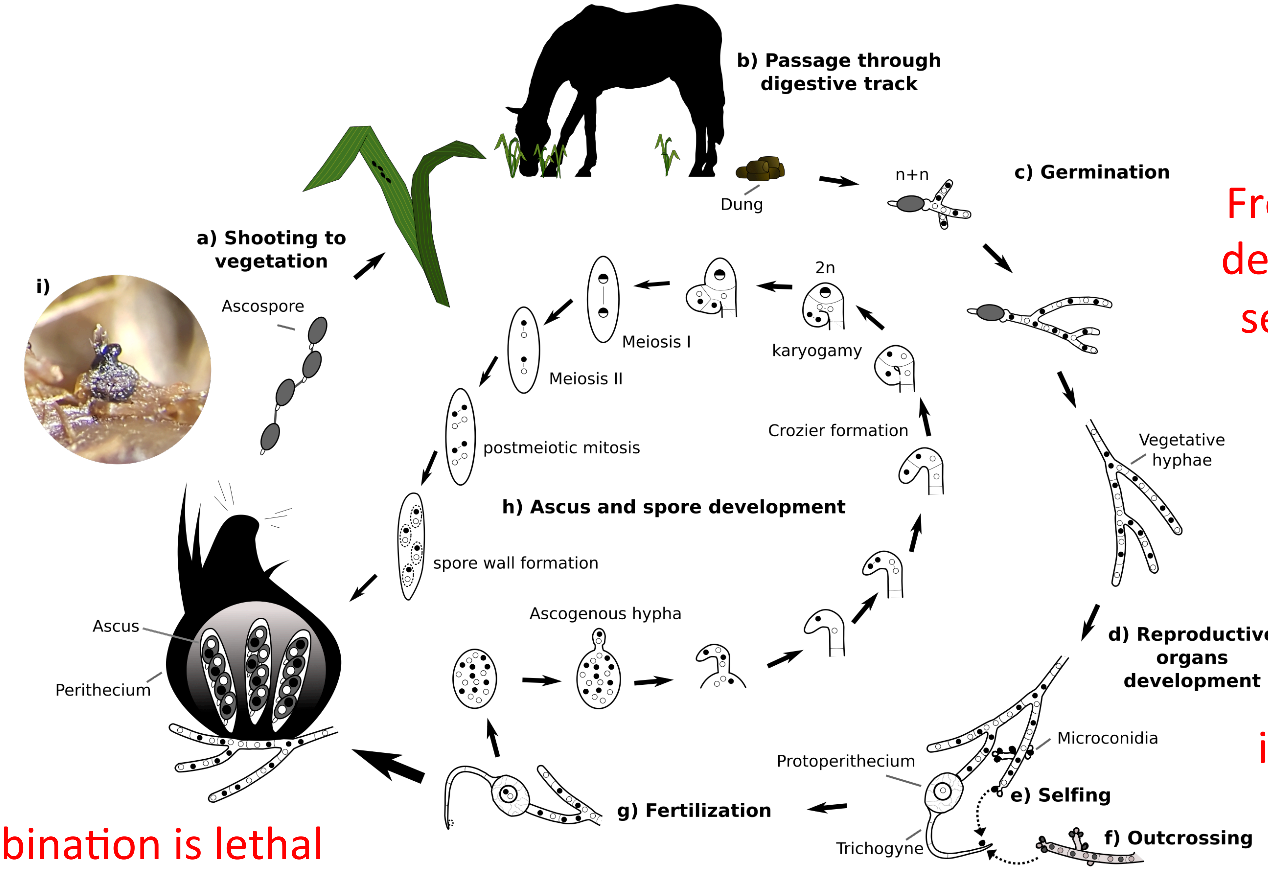
We only see two genotypes in nature:
rV and **RV1**

Okay RV is lethal so that's easy...
but where is rV1?

Ivain Martinossi, postdoc

Sandra Lorena Ament et al., Manuscript

Can we explain what we see with what we know about the *Podospora* life cycle?



Frequency dependent selection

Sexual incompatibillity

VR combination is lethal

Variable selfing rate

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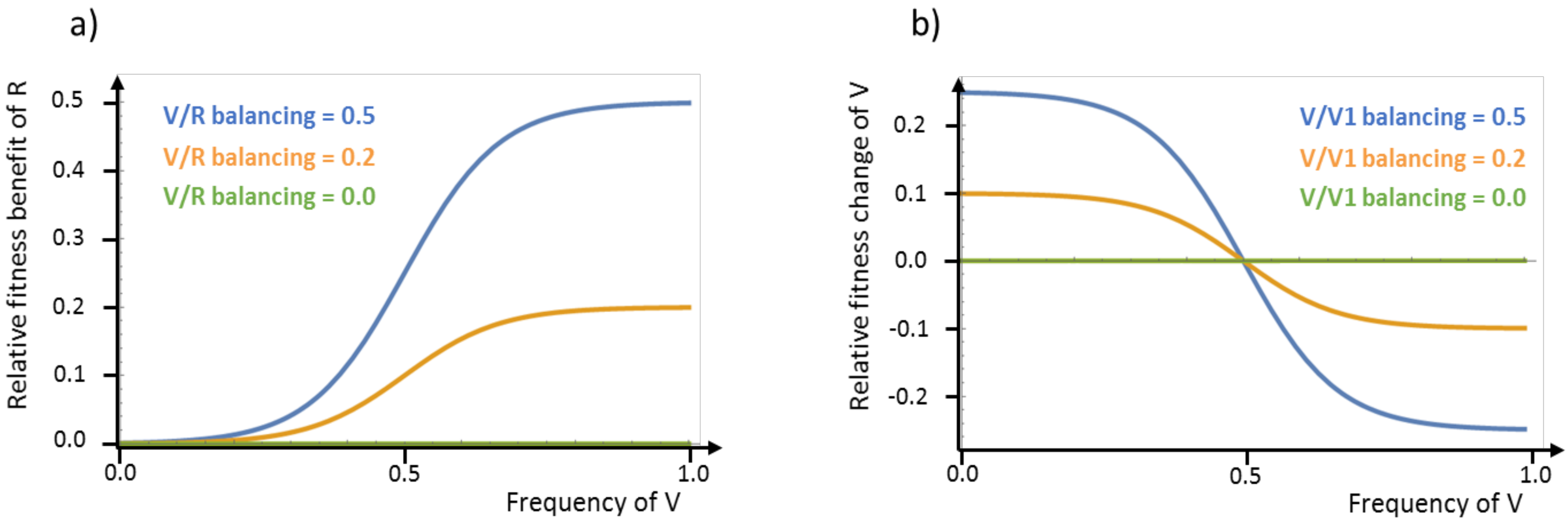
Individual-based simulation shows how allorecognition genes can lead to the formation of two mating groups

Individual-based simulation shows how allorecognition genes can lead to the formation of two mating groups

Fitness effects of genotypes in the model

- RV genotype is lethal
- R, V and V1 can provide a fitness benefit because of vegetative incompatibility. They are frequency dependent.



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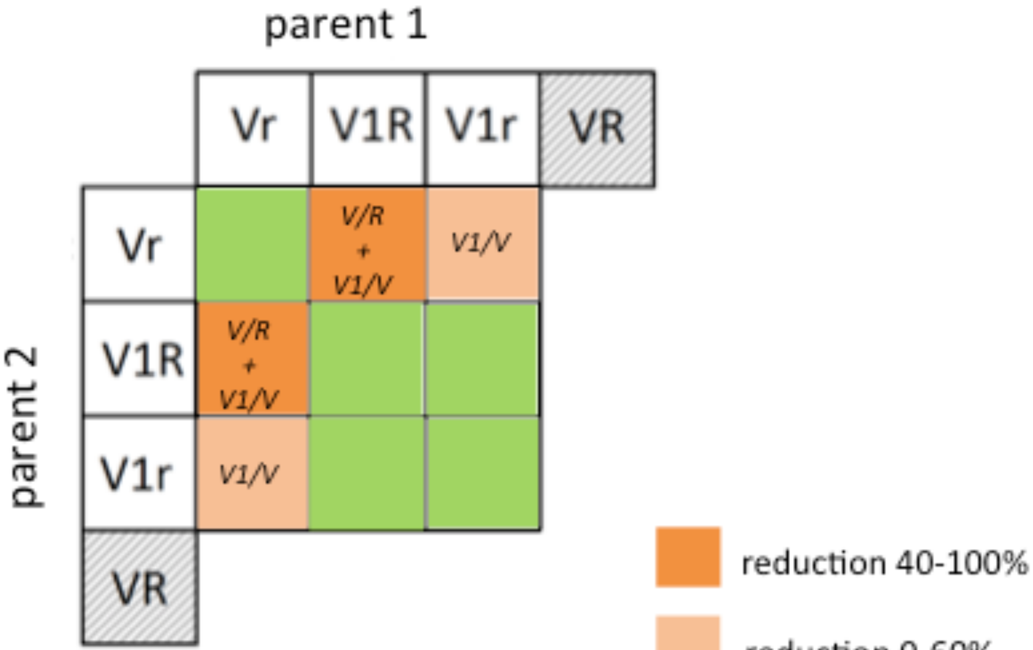
Individual-based simulation shows how allorecognition genes can lead to the formation of two mating groups

Sexual incompatibility in the model

- V/R interaction effect, reduces offspring survival by 40%. Constant.
- V/V1 effect, reduces survival by a variable amount, 0-60%



parent 1

parent 2	Vr	V1R	V1r	VR
Vr	🟩	V/R + V1/V	V1/V	
V1R	V/R + V1/V	🟩	🟩	
V1r	V1/V	🟩	🟩	
VR				

🟧 reduction 40-100%

reduction 0-60%

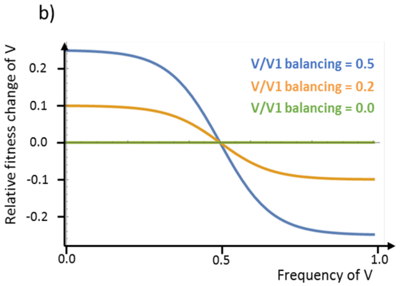
Sandra Lorena Ament et al., Manuscript

Individual-based simulation shows how allorecognition genes can lead to the formation of two mating groups

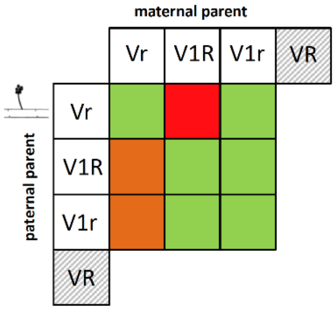
Scenario of the model

- Invasion of rV in a RV1 population (or RV1 in a rV population)
- 1 000 individuals (2 000 genotypes)
- Introduce the invading genotype, wait 300 generations, sample genotype frequencies, repeat.
- Change **parameters** and see what happens….

Balancing selection:
-V/R
-V/V1



Sexual incompatibility:
-V/V1

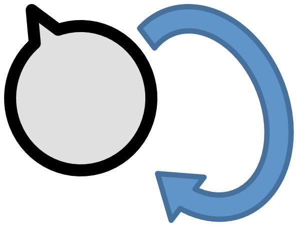


maternal parent

paternal parent

	Vr	V1R	V1r	VR
Vr	🟩	🟥	🟩	
V1R	🟧	🟩	🟩	
V1r	🟧	🟩	🟩	
VR				

Selfing rate

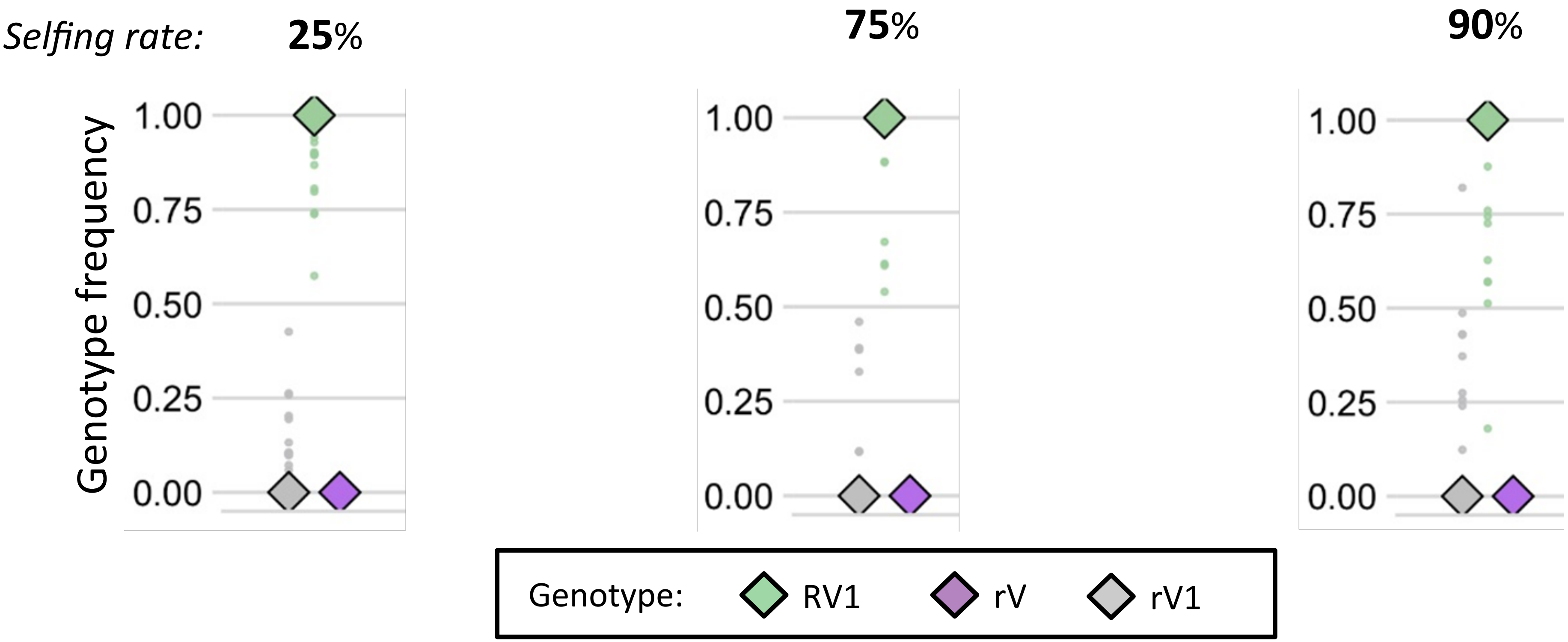


Sandra Lorena Ament et al., Manuscript

Individual-based simulation shows how allorecognition genes can lead to the formation of two mating groups

Outcome of the model

- No balancing selection
- RV lethality, R/V sexual incompatibility



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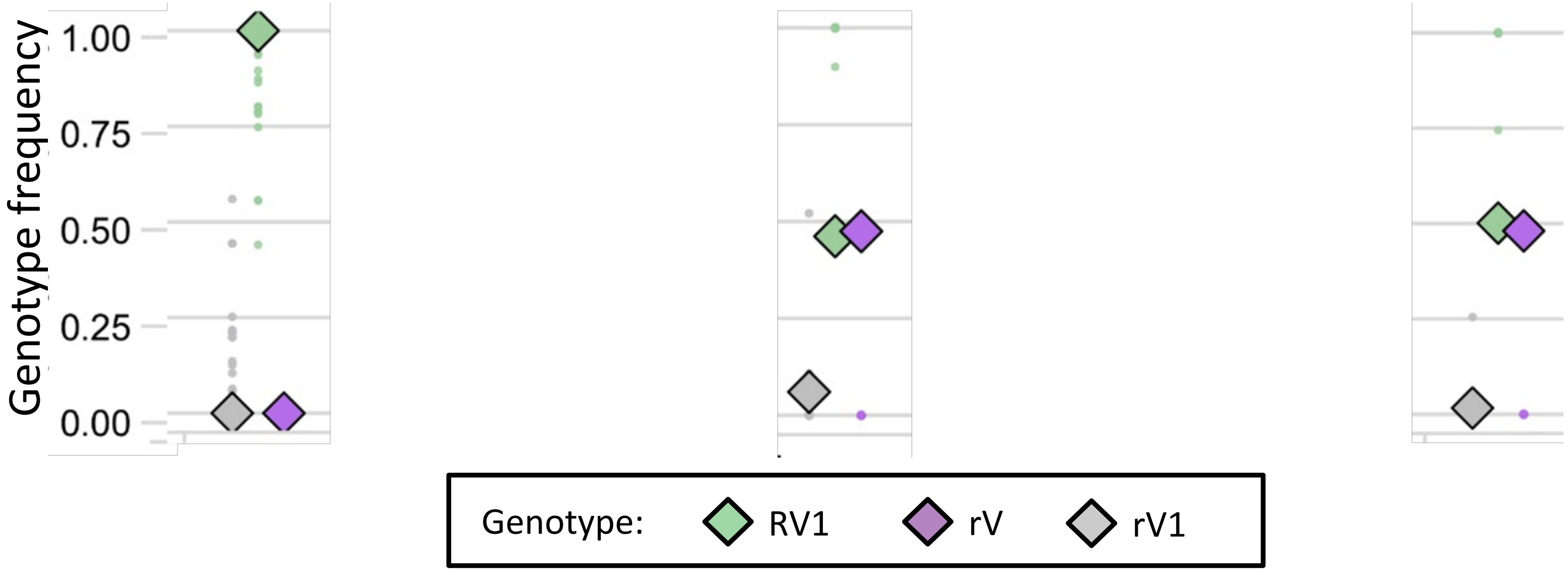
Individual-based simulation shows how allorecognition genes can lead to the formation of two mating groups

Outcome of the model

- Balancing selection, sexual incompatibility, RV lethality

Selfing rate: **25%** **75%** **90%**



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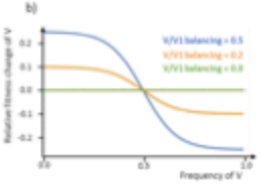
Take home from the model

Selfing rate → **Makes invasion of rV *possible* by reducing the cost of outcrossing with RV1**

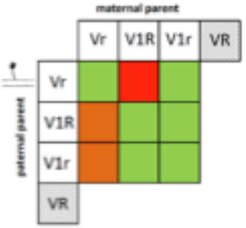


Balancing selection
-V/R
-V/V1

→ **Both encourage the invasion of V in a RV1 population**

→ **Balances V/R, encourages strongly the RV1/rV coexistence**

→ **Balances V/V1, can result in rV/rV1 population**



maternal parent

paternal parent

	Vr	V1R	V1r	VR
Vr	🟩	🟥	🟩	
V1R	🟧	🟩	🟩	
V1r	🟧	🟩	🟩	
VR				

Sexual incompatibility → **Reduces the outcrossing success of rV1**

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Conclusions

- Pleiotropy between vegetative and sexual incompatibility may cause speciation
- Selfing is needed for the maintenance of mating groups in *P. anserina*
- A common mode of fungal speciation?

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