



Génomique des populations et adaptation des champignons pathogènes responsables de la maladie hollandaise de l'orme

Thèse

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Génomique des populations et adaptation des champignons pathogènes responsables de la maladie hollandaise de l'orme

Thèse de doctorat

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Résumé

La Maladie Hollandaise de l'Orme (MHO) est causée par des champignons du genre *Ophiostoma*. Ceux-ci sont responsables de la mort de plusieurs centaines de milliers d'ormes adultes en Europe ainsi qu'en Amérique du Nord, modifiant de manière drastiques les paysages forestiers et urbains. L'étude de la MHO a permis de caractériser deux espèces différentes, *O. ulmi* et *O. novo-ulmi*, qui présentent des phénotypes différents en terme de virulence et de croissance. L'analyse de données de séquençage à haut débit (génomique) associée à l'utilisation de données phénotypiques s'est répandue ces dernières décennies dans le domaine de la phytopathologie et permet de comprendre plus en détails la structure des populations ainsi que les gènes et mécanismes impliqués dans l'adaptation chez les champignons pathogènes.

Dans le premier chapitre, nous comparons les caractéristiques évolutives des champignons phytopathogènes des cultures et des forêts. Nous contrastons l'impact des différents degrés de domestication et de gestion des milieux agricoles et forestiers sur ces populations de pathogènes. Les milieux agricoles et les forêts présentent des caractéristiques très différentes, comme le temps de génération ou le niveau de domestication. Cependant, nous trouvons que les mécanismes modelant les populations de pathogènes restent similaires, comme l'hybridation, les sauts d'hôtes, la sélection, la spécialisation et l'expansion clonale. Dans un second temps nous faisons un bilan des méthodes et techniques disponibles pour la gestion et l'amélioration des plantes de ces systèmes afin de prévenir ou lutter contre de futures épidémies.

Dans le second chapitre, nous avons utilisé des données de génomiques pour examiner la structure génétique des populations des champignons responsables de la Maladie Hollandaise de l'Orme (MHO) *Ophiostoma ulmi* et *Ophiostoma novo-ulmi*. Nous quantifions et caractérisons la diversité génétique au sein des quatre lignées génétiques, ainsi que la divergence et la phylogénie entre chaque taxon. Nous décrivons le rôle de l'hybridation et de l'introgression dans l'histoire évolutive de ces pathogènes comme étant le mécanisme principal générant de la diversité génétique. La production de données phénotypiques nous permet également de caractériser l'impact de l'introgression sur l'adaptation de ces espèces.

Dans le troisième chapitre, nous avons utilisé une approche « GWAS » (*Genome Wide Analysis Study*) pour révéler les marqueurs impliqués dans l'adaptation à la température et à un

composé de défense de l'hôte chez *O. ulmi* et *O. novo-ulmi*. Nous trouvons d'importants gènes et familles de gènes associés avec les phénotypes de croissance et de virulence comme des transporteurs, des cytochromes, des protéines de choc thermique ou des protéines impliquées dans le système d'incompatibilité végétative qui pourraient jouer un rôle dans la protection contre les virus.

Abstract

Dutch Elm Disease (DED) is a highly destructive tree disease caused by fungi from the *Ophiostoma* genus. These fungi are responsible for the deaths of hundreds of thousands of mature elm trees both in Europe and in North America. Studies on DED allowed the characterization of two distinct species, *O. ulmi* and *O. novo-ulmi*, that exhibit different virulence and growth phenotypes. Global pathogen genomics data including population genomics and high-quality reference assemblies are crucial for understanding the evolution and adaptation of pathogens.

In a first chapter, we review crops and forest pathosystems with remarkably different characteristics, such as generation time and the level of domestication. They also have different management systems for disease control which is more intensive in crops than forest trees. By comparing and contrasting results from pathogen population genomic studies done on widely different agricultural and forest production systems, we can improve our understanding of pathogen evolution and adaptation to different selective pressures. We find that despite these differences, similar processes such as hybridization, host jumps, selection, specialization, and clonal expansion are shaping the pathogen populations in both crops and forest trees. We propose some solutions to reduce these impacts and to lower the probability of global pathogen outbreaks so that we can envision better management strategies to sustain global food production as well as ecosystem services.

In a second chapter, we investigate how hybridization and the resulting introgression can drive the success of DED fungi via the rapid acquisition of adaptive traits. Using whole-genome sequences and growth phenotyping of a worldwide collection of isolates, we show that introgression has been the main driver of genomic diversity and that it impacted fitness-related traits. Introgressions contain genes involved in host-pathogen interactions and reproduction. Introgressed isolates have enhanced growth rate at high temperature and produce different necrosis sizes on an in vivo model for pathogenicity. In addition, lineages diverge in many pathogenicity-associated genes and exhibit differential mycelial growth in the presence of a proxy of a host defence compound, implying an important role of host trees in the molecular and functional differentiation of these pathogens.

In the third chapter, we performed the identification of *O. ulmi* and *O. novo-ulmi* genes potentially associated with virulence and growth using Genome-Wide Association (GWA) analysis. We measured necrosis size induced on apples as a proxy for fungal virulence and measured growth rates at three different temperatures and two different media. We found several candidate genes for virulence, such as a CFEM domain containing protein and a HC-toxin efflux carrier. For growth, we identify several important gene families such as ABC and MFS transporters, cytochromes, transcription factors and proteins from the vegetative incompatibility complex.

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Liste des abréviations, sigles, acronymes

Français

ADN : Acide désoxyribonucléique

ARN : Acide ribonucléique

CEF : Centre d'Étude de la Forêt

MHO : Maladie Hollandaise de l'Orme

ONU : *Ophiostoma novo-ulmi*

OU : *Ophiostoma ulmi*

TGH : Transfert de Gène Horizontal

Anglais

ABC transporter : ATP-Binding Cassette transporter

AM2SG : North American second vc supergroup

AMSG : North American vc supergroup

CAZy : Carbohydrate enzyme

CNV : Copy Number Variation

CRISPR : Clustered Regularly Interspaced Short Palindromic Repeats

DAPC : Discriminant Analysis in Principal Component

DED : Dutch Elm Disease

DNA : Desoxyribonucleic acid

DNB : Dothistroma Needle Blight

EAN : European *O. novo ulmi* race

EUSG : European vc supergroup

F1 : First generation hybrid

FAO : Food and Agriculture Organization

GE : Genome Editing

GEA : Genotype Environment Association

GMO : Genetically Modified Organism

GWAS : Genome-Wide Association Study

IAES : Invasive Alien Exotic Species

INDEL : Insertion deletion

IR : Introgressed Region

MAT : Mating type

MCMC : Markov Chain Monte Carlo

MDR : Multiple Disease Resistance

MEA : Malt Extract Agar

MYA : Million Years Ago

NAN : North American *O. novo ulmi* race

PCA : Principal Component Analysis

QTL : Quantitative Trait Loci

RAPD : Random Amplified Polymorphic DNA

RNA : Ribonucleic acid

RNAi : RNA interference

ROH : Runs Of Homozygosity

SNC : Swiss Needle Cast

SNP : Single Nucleotide Polymorphism

TE : Transposable Element

vc : vegetative compatibility

À ma grand-mère Christiane

*« Nothing in biology makes sense except in the
light of evolution » Theodosius Dobzhansky*

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Avant-propos

L'article intitulé « Evolution and adaptation of forest and crop pathogens in the Anthropocene » est parue dans la revue *Phytopathology* le 18 décembre 2020. J'ai le statut de co-premier auteur sur cet article. J'ai participé à la conception du manuscrit avec les autres coauteurs, les Dr. Feau, Dr. Brar, Dr. Gill, Dr. Schwessinger et le Pr. Hamelin. J'ai réalisé la rédaction du manuscrit en collaboration avec le Dr. Feau, également co-premier auteur. Le Pr. Hamelin a supervisé la conception et la rédaction du manuscrit.

L'article intitulé « Hybridization and introgression drive genome evolution of Dutch elm disease pathogens » a été publiée dans la revue *Nature Ecology and Evolution* le 2 mars 2020. J'ai le statut de co-premier auteur sur cet article. J'ai réalisé la sélection des isolats à séquencer, leur mise en culture, les extractions d'ADN, les contrôles qualité et la préparation de l'envoi à la plateforme de séquençage. J'ai également réalisé le nettoyage des données de séquençage, une partie des analyses génomiques (phylogénie, structure des populations), l'analyse des données de phénotypage et la rédaction du manuscrit. Le Dr. Anna Fijarczyk, également co-premier auteur, a réalisé des analyses génomiques (hybridation, introgression, enrichissement, diversité) et la rédaction du manuscrit. J'ai réalisé le phénotypage des isolats en collaboration avec le Dr. Guillaume Charron et Jérôme Chapuis. Les Dr. Hélène Martin et Dr. Julien Prunier ont participé aux analyses génomiques (structure des populations, nombre de copies). Le Pr. Louis Bernier, le Dr. Philippe Tanguay et le Pr. Richard Hamelin ont participé au design de l'étude et la révision du manuscrit. L'ensemble des analyses et de la rédaction a été supervisé par le Pr. Christian Landry.

Le chapitre intitulé « Adaptation in Dutch elm disease pathogens » est traité comme un chapitre traditionnel, mais rédigé en anglais et selon le même format que les autres articles inclus dans la thèse. Dans ce travail, j'ai réalisé le choix et la mise en culture des isolats, les extractions d'ADN, les préparations de librairies ainsi que la préparation des échantillons pour l'envoi à la plateforme de séquençage. Les données phénotypiques utilisées proviennent du travail réalisé pour le chapitre 2 et ont été produites avec l'aide du Dr. Guillaume Charron et de Jérôme Chapuis. J'ai réalisé les analyses génomiques et la rédaction du manuscrit. Le Dr Anna Fijarczyk ainsi que le Pr Louis Bernier ont aidé à l'interprétation des résultats et ont commenté le texte.

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Introduction

Les dernières décennies ont vu une augmentation dans le nombre de pathogènes menaçant les écosystèmes forestiers et agronomiques ainsi que la santé des humains et des autres animaux (voir Simler-Williamson et al. 2019 pour les écosystèmes forestiers, Paini et al. 2016 pour les systèmes agronomiques et Brown et al. 2012 pour les humains). Un pathogène, du grec *páthos* («souffrance») et *génos* («naissance») est un organisme (virus, bactérie, eucaryote uni ou pluricellulaire) capable d'engendrer des effets néfastes sur un autre organisme, l'hôte. Certains *mycètes*, ou champignons, ont développé une multitude de stratégies pour coloniser les plantes, résultant en interactions bénéfiques comme la symbiose mycorhizienne, ou bien en étant fatale pour l'hôte comme dans le cas des agents pathogènes fongiques qui tuent leur hôte afin d'extraire les nutriments des tissus morts.

Les champignons pathogènes, appartenant principalement aux clades des basidiomycètes et des ascomycètes, sont capables de coloniser une grande variété d'hôtes et dans une multitude d'environnements différents. En agriculture, les dégâts causés par les champignons pathogènes se chiffrent chaque année à plusieurs millions de dollars (Fisher et al. 2012 et Savary et al. 2019). Cela pourrait être en partie attribuable au fait que les écosystèmes agricoles sous aménagement intensif possèdent très peu de diversité biologique (spécifique et génétique) comparé aux écosystèmes forestiers naturels. Ainsi, les pressions de sélections et les interactions hôte-pathogène seraient donc différentes entre ces deux types d'écosystèmes (voir Chapitre 1 pour une revue complète). Malgré cette diversité des populations naturelles, les champignons pathogènes restent tout de même une menace pour les milieux forestiers.

Les forêts naturelles et urbaines sont d'importance vitale pour la société car elles produisent un grand nombre de services écosystémiques : séquestration de carbone, refuge pour la biodiversité, lutte contre l'imperméabilisation des sols, elles ont également une importance économique, participent à la santé des populations humaines et occupent une place de choix dans les activités récréatives et culturelles. Certaines maladies provoquées par les champignons pathogènes ont des effets délétères majeurs sur les arbres, tant en milieu naturel qu'urbain. La maladie du flétrissement du frêne ou la mort subite du chêne, ainsi que la maladie hollandaise de l'orme sont des maladies bien connues du grand public dans les régions affectées pour leurs effets considérables sur les paysages urbains et naturels. Par ailleurs, les changements climatiques

et les échanges globaux peuvent exacerber les effets des pathogènes indigènes, en rendant les hôtes plus vulnérables à cause de la sécheresse ou en favorisant la sporulation des et la dissémination des agents pathogènes grâce à une température et une humidité plus élevées par exemple (La Porta et al. 2012). Ils peuvent également faciliter l'établissement et la propagation de nouvelles espèces pathogènes hors de leur aire de répartition d'origine. Celles-ci sont alors appelées espèces envahissantes.

Il est primordial de comprendre les mécanismes évolutifs qui façonnent la diversité et la structure des populations de ces espèces invasives afin de pouvoir détecter, prévenir, contrôler et ultimement prédire l'évolution de ces populations. Dans la première section de cette introduction, nous développerons les concepts de la coévolution, l'évolution de la virulence, ainsi que les différents mécanismes évolutifs façonnant la diversité génétique des pathogènes (dérive, mutation, flux génique, système de reproduction) et nous finirons sur les spécificités des espèces invasives. Dans la seconde section, nous nous intéresserons à l'adaptation de ces espèces pathogènes à leur environnement, les mécanismes impliqués dans l'adaptation, ainsi que les méthodes utilisées pour étudier celle-ci. Enfin, nous nous intéresserons plus particulièrement à la maladie hollandaise de l'orme (MHO), bien connue en phytopathologie forestière, et sujet de cette thèse. Nous décrirons l'historique de cette maladie et feront un état des connaissances à propos de la génétique des populations et de la biologie des champignons responsables de la MHO.

Génétique des populations des agents pathogènes fongiques

La coévolution hôte pathogène et l'évolution de la virulence

L'évolution biologique se définit comme le changement des fréquences alléliques dans populations au cours du temps. Dans l'environnement, les hôtes et leurs agents pathogènes sont en contact permanent. L'interaction durable entre deux espèces différentes amène à l'exercice de pressions sélectives réciproques, qui façonnera la diversité génétique de chacune. Ce processus est appelé « coévolution ». Dans le cas des interactions hôte-agent pathogène, l'agent pathogène exerce une pression sélective sur son hôte car il diminue sa capacité à se reproduire. Lorsqu'il n'y a pas de phénomène d'extinction, ceci aboutit à l'évolution de stratégies d'évitement et de mécanismes de résistance chez l'hôte. Ces mécanismes agissent en retour comme une pression de sélection qui influencera la diversité génétique de l'agent pathogène et en modulant sa capacité

à rencontrer et infecter son hôte, soit son mode de dispersion et son succès reproducteur. Hôtes et agents pathogènes se retrouvent alors dans une véritable « course aux armements » évolutive.

En 1973, Leigh Van Valen formalise l'hypothèse selon laquelle la coévolution, donc l'influence des facteurs biotiques, est un moteur de l'évolution (Van Valen 1973). Cette hypothèse devient connue sous le nom d'hypothèse de la reine rouge (*Red Queen Hypothesis*), en référence au roman de Lewis Caroll, *De l'autre côté du miroir*. En effet, chaque espèce doit évoluer et s'adapter afin de pouvoir « rester dans la course » qui se déroule avec les espèces contre lesquelles elle est en compétition. Ainsi, dans une interaction hôte-agent pathogène, chaque fois qu'une des deux espèces acquiert un avantage sélectif, celui-ci modifie l'environnement de la seconde. Cette hypothèse s'est largement répandue dans la communauté scientifique et a fourni un cadre conceptuel permettant la compréhension du maintien de la reproduction sexuée chez les pathogènes malgré l'avantage de la reproduction asexuée (Jaenike 1978, Hamilton 1980).

Un agent pathogène se distingue d'un non-pathogène car il provoque une altération de l'état physiologique normal d'un organisme, l'hôte, chez lequel on peut alors observer des symptômes (Andrivon 1993). Le terme virulence a différentes significations, qui dépendent du contexte et du domaine considérés. En microbiologie, un agent pathogène sera appelé virulent lorsqu'il provoquera la maladie chez les hôtes susceptibles, et avirulent dans le cas contraire, soit lorsqu'il est confronté à des hôtes résistants (Andrivon 1993). Selon cette définition, la virulence est donc un trait purement qualitatif. Pour les écologistes, la virulence inclut également une dimension quantitative et se définit comme la sévérité des symptômes induits chez l'hôte (ce qui correspond à l'agressivité chez les microbiologistes). Chez les biologistes de l'évolution, la virulence peut également être définie comme la réduction du succès reproducteur de l'hôte causée par l'infection d'un agent pathogène (Read 1994). En phytopathologie, la virulence est souvent mesurée par les « dégâts » causés par l'agent pathogène, comme la défoliation des branches ou l'obstruction des stomates par exemple. La virulence est dans tous les cas un trait complexe déterminé par une combinaison de facteurs impliquant l'agent pathogène, l'hôte et l'environnement. Dans le reste de cette thèse, nous utiliserons la définition la plus commune de la virulence, à savoir la capacité à infecter un hôte et l'amplitude des symptômes provoqués. La virulence est elle-même soumise à des pressions sélectives. En effet, la virulence la plus élevée n'est pas toujours le paramètre le plus optimal, car si l'agent pathogène tue son hôte trop rapidement, cela peut l'empêcher lui-même de compléter son cycle reproducteur. Les agents

pathogènes moins virulents seraient alors avantagés en maintenant l'hôte en vie plus longtemps, ce qui augmenterait leur succès reproducteur. Cette stratégie intuitive n'est cependant pas la règle absolue. Des modèles montrent que dans certains cas, la sélection naturelle désavantage les agents pathogènes avirulents au profit des virulents (Bull 1994). La virulence maximisant la fitness d'un agent pathogène dépend d'une combinaison de facteurs incluant le mode de transmission, le niveau de co-infection, les pressions sélectives, ainsi que les conditions au sein de l'hôte et entre les hôtes (Alizon et Lion 2011).

Les forces et les mécanismes façonnant la diversité et la structure des populations

La structure génétique est la quantification et la distribution de la diversité génétique entre et au sein des populations. La connaissance de cette structure génétique permet de reconstruire l'histoire évolutive des populations, soit de comprendre les différents processus et mécanismes qui l'ont façonnée. Selon McDonald et Linde (2002), cette connaissance permettrait également d'évaluer le potentiel évolutif futur des populations d'agents pathogènes. En effet, le système de reproduction, le flux génique, la taille efficace de population ainsi que le taux de mutation seraient des paramètres permettant d'évaluer le risque pour un agent pathogène d'envahir sa population hôte. Certaines forces évolutives comme la dérive génétique et la sélection naturelle réduisent la diversité génétique, alors que la mutation en crée et le flux génique peut en apporter depuis une autre population.

Dérive génétique et taille efficace

La dérive génétique provoque un changement des fréquences alléliques au cours du temps de manière aléatoire, ce qui est dû à l'échantillonnage aléatoire des gamètes lors de la reproduction. La dérive est d'autant plus forte (fixe plus vite les allèles) que la taille efficace de la population est faible. Ainsi, une population d'agents pathogènes possédant une taille efficace réduite verra ses allèles se fixer et sa diversité génétique s'éroder beaucoup plus vite (Allendorf 1986, Nei et Tajima 1981). Les populations possédant une taille efficace plus importante voient également plus de mutations apparaître à chaque génération, ce qui leur confère un réservoir de diversité génétique plus important.

Mutation

Les mutations sont la seule source de nouveauté génétique dans les populations. Une mutation dans un gène crée un nouvel allèle, ce qui chez les agents pathogènes peut amener à l'apparition de nouvelles souches virulentes, résistantes aux défenses de l'hôte ou aux pesticides. Par exemple, le gène CYP51 est impliqué dans la synthèse de composants lipidiques essentiels au fonctionnement cellulaire des champignons. Une mutation ponctuelle dans ce gène peut conférer une résistance aux azoles, groupe de composés chimiques qui constituent 50% des fongicides utilisés en l'agriculture (Azevedo et al. 2015). Sous l'effet de pressions de sélection positives, ces mutations peuvent augmenter rapidement en fréquence dans la population. Ce phénomène a été observé chez plusieurs agents phytopathogènes de cultures, comme *Zymoseptoria tritici* et *Rhynchosporium commune*, responsables de la septoriose du blé et de la maladie de l'orge, respectivement (Omrane et al. 2015; Mohd-Assaad et al. 2016). Selon la théorie de l'interaction « un gène pour un gène », la reconnaissance d'un gène d'avirulence chez le pathogène par le gène de résistance correspondant chez l'hôte conduira à une interaction de type incompatible (i.e. non développement de la maladie). Une mutation ponctuelle sur ce gène d'avirulence pourrait être avantageuse parce qu'aucun gène de résistance correspondant n'existe alors chez l'hôte.

Le taux de mutation est un paramètre complexe, lui-même soumis à l'évolution. Il varie en fonction des organismes mais également d'une région à l'autre du génome. En effet, chez *Leptosphaeria maculans*, agent pathogène de crucifères, on trouve des compartiments possédant un plus grand nombre de mutations ponctuelles répétées (Rouxel 2010). Ce sont aussi dans ces compartiments que l'on trouve le plus grand nombre d'effecteurs, protéines qui modulent l'immunité de l'hôte et permettent l'infection. Chez le pathogène forestier *Phytophthora ramorum*, responsable de l'encre des chênes rouges, plusieurs familles d'effecteurs ont évolué grâce à des mutations ponctuelles qui se sont répandues dans la population sous l'effet d'une pression de sélection positive (Goss 2013). Lorsqu'elles ne sont pas rapidement éliminées par la dérive génétique, les mutations peuvent se répandre rapidement dans les populations grâce à l'effet de la sélection, par propagation de lignées génétiques clonales, comme dans le cas de *P. ramorum* (Dale et al. 2019).

La sélection

La sélection naturelle est un moteur majeur de l'évolution. Qu'elle soit positive (directionnelle ou balancée) ou bien négative (dite aussi purificatrice), la sélection entraîne une

réduction de diversité génétique. Les facteurs biotiques et abiotiques exercent des pressions de sélection sur les agents pathogènes. L'hôte est un facteur biotique crucial façonnant la diversité génétique de l'agent pathogène. Ce dernier doit infecter son hôte pour se reproduire (sauf dans le cas des pathogènes opportunistes), les gènes impliqués dans les processus d'infection sont alors sous une pression de sélection très forte dans le génome de l'agent pathogène. Les signatures moléculaires de la sélection peuvent être observables dans le génome des agents pathogènes et permettent souvent d'identifier des gènes ou des parties du génome impliquées dans l'interaction avec l'hôte. On observe par exemple chez l'agent pathogène du blé, *Mycosphaerella graminicola*, une très forte sélection positive sur un gène codant pour une enzyme capable de dégrader la paroi cellulaire des plantes, permettant d'en extraire les nutriments (Brunner et al. 2009). On peut également détecter la sélection agissant sur des traits liés à la dispersion, comme chez *Seiridium cardinale* induisant le chancre du cyprès (*Cupressus* spp.), chez qui la taille des spores est sous forte pression de sélection (Garbelotto et al. 2015).

Les facteurs abiotiques jouent également un rôle prépondérant dans l'évolution des pathogènes. Chez *Hymenoscyphus fraxineus*, responsable de la maladie du flétrissement du frêne, les températures supérieures à 35° C induisent une réduction du nombre de structure reproductives et de la sévérité de la maladie, limitant ainsi son développement dans les régions plus particulièrement chaudes (Grosdidier et al. 2020). Les conditions environnementales peuvent vraiment façonner les populations d'agents pathogènes, en favorisant par exemple une espèce envahissante par rapport à une espèce indigène, comme dans le cas d'*Heterobasidion*. En effet, il a été montré qu'en Italie, le potentiel reproducteur d'*H. annosum sensu stricto* était réduit lors de la saison sèche par rapport à l'espèce envahissante introduite *H. irregulare*, facilitant ainsi l'invasion de cette dernière (Garbelotto et al. 2009).

Flux génique : migration, hybridation, transfert horizontal de gène

Le flux génique est « l'échange ou le mouvement de gamètes, individus ou population à une échelle géographique » (McDonald 1993). Plusieurs mécanismes peuvent être à l'origine du transfert de variation génétique entre les populations : la migration qui se définit par le déplacement d'individus ou de groupes d'individus, l'hybridation qui résulte de la reproduction sexuée d'individus de deux taxons proches et le transfert horizontal de gène (TGH), qui implique le transfert de gènes ou de parties de génome depuis une espèce vers une autre phylogénétiquement éloignée.

Lorsque des individus de deux taxons différents se croisent et forment une descendance viable, on parle alors d'hybridation fertile. Deux espèces ayant évolué en sympatrie peuvent présenter de fortes barrières reproductives dues à une forte pression de sélection contre les croisements interspécifiques. En revanche, ces barrières peuvent être beaucoup plus faibles entre deux espèces qui sont demeurées longtemps isolées l'une de l'autre, dans ce cas, leur système reproducteur a simplement évolué par mutation et dérive, et on observe alors l'émergence d'individus hybrides (Giraud 2008). L'hybridation joue un rôle très important dans les écosystèmes en augmentant la diversité génétique au sein des espèces, mais aussi en permettant le transfert d'allèles déjà adaptés d'une espèce à une autre. En effet, lorsqu'un individu hybride est fertile, il peut se reproduire avec un individu de l'espèce parentale, une partie du génome hybridé est alors incorporé à la descendance. Ces individus vont également se reproduire et transmettre une partie du génome hybridé à la génération suivante. Ce processus est appelé rétrocroisement (*backcrossing*) et mène à l'introgression, qui se définit par l'incorporation de gènes ou de parties de génome d'une espèce dans le génome d'une autre (Anderson 1949). Si les gènes introgressés fournissent un avantage comme une virulence accrue, ils seront alors sélectionnés positivement et se répandront dans la population. Des analyses de génétique des populations ont par exemple montré l'introgression de traits liés à la virulence chez les champignons pathogènes *Venturia inaequalis* et *Hemileia vastatrix*, responsables de la tavelure du pommier et de la rouille orangée du caféier, respectivement (Feurtey et Stukenbrock, 2018).

L'hybridation peut également aboutir à l'émergence de nouvelles espèces (Mallet 2007). En effet, la plupart hybrides possèdent des phénotypes intermédiaires à ceux de leurs parents et ne sont pas avantageux (gènes avec effets additifs). Cependant, la combinaison des différents traits provenant des phénotypes parentaux peut dans certains cas conférer aux hybrides un plus large éventail de phénotypes différents, leur permettant ainsi d'exploiter une niche écologique plus grande. Le pathogène de crucifères *Verticillium longisporum* regroupe des taxa issus de plusieurs événements d'hybridation tel que *Verticillium dahliae* et d'autres espèces de *Verticillium* non décrites. Cet hybride possède un éventail d'hôte plus important que ses espèces parentales, ce qui lui confère un avantage par rapport à ses parents (Depotter et al. 2016).

Le TGH est l'échange et l'intégration de matériel génétique entre différentes espèces. Il diffère de l'introgression car il n'arrive pas par un mode de transmission dit « vertical », soit par la transmission de matériel génétique des (ou du) parent(s) vers les descendants par reproduction sexuée ou asexuée (Keeling et Palmer 2008). On peut alors observer l'incorporation de gènes

d'une espèce phylogénétiquement très distante dans le génome d'une autre espèce, par exemple l'inclusion de gènes d'organismes procaryotes dans le génome d'eucaryotes. Les avancées technologiques en matière de séquençage ont permis une explosion de données génomiques disponibles pour un nombre d'organismes très variés, favorisant l'identification des TGH et permettant une meilleure compréhension de leur rôle dans l'évolution des champignons (Fitzpatrick 2012). Par exemple, chez *Mycosphaerella populinum*, l'agent pathogène responsable du chancre septorien du peuplier, l'acquisition de gènes impliqués dans l'infection et la colonisation de l'hôte proviendrait d'un transfert de gènes horizontal depuis des espèces de champignons ascomycètes associés avec la dégradation du bois et des procaryotes (Dhillon et al. 2015).

Système de reproduction

Le système de reproduction d'un organisme affecte la distribution de la variation génétique au sein des populations. De nombreuses espèces de champignons sont capables à la fois de reproduction sexuée et asexuée (dite aussi clonale). La reproduction sexuée chez les champignons se fait grâce à l'intervention d'un ou plusieurs locus qui définissent le type reproducteur du champignon (*mating type* en anglais). Les champignons hétérothalliques ont besoin de rencontrer le type reproducteur opposé pour se reproduire, tandis que les champignons homothalliques peuvent le faire de manière autonome. De manière générale, l'hétérothallisme promeut la diversité génétique tandis que l'homothallisme augmente la consanguinité. Les données récoltées grâce à l'étude de plusieurs espèces modèles a permis de montrer que le mode de reproduction affectait plusieurs caractéristiques de l'évolution du génome comme l'évolution des protéines, le taux de mutation et la fréquence des réarrangements chromosomiques entre autres (voir Whittle et al. 2011).

Le maintien de la reproduction sexuée dans les populations naturelles est une question qui fascine les biologistes depuis des décennies. Si ce système est maintenu dans les populations, les bénéfices doivent être supérieurs aux coûts (Nieuwenhuis et James 2016). La conséquence principale de la reproduction sexuée est le brassage génétique des populations, qui grâce à la recombinaison génétique permet la création de nouveaux génotypes à partir de ceux existants. Ces génotypes présentent alors de nouvelles combinaisons d'allèles pouvant posséder un avantage sélectif, ce qui sous l'action de la sélection augmentera la fréquence de ces génotypes dans la population. Les structures résultant de la reproduction sexuée sont elles-mêmes avantageuses : les ascospores produites sont très résistantes et permettent aux champignons de

survivre sous des conditions de stress important ou dans des environnements pauvres en ressources.

Chez les espèces invasives de champignons, les systèmes de reproduction mixtes (mélant reproduction asexuée importante et rares événements de reproduction sexuée) posséderaient donc le plus haut potentiel évolutif (Bazin 2014). Les spores mitotiques, issues de reproduction asexuée, permettent une augmentation de la taille de population et au génotype le plus adapté de se répandre. Plusieurs cycles de reproduction asexuée peuvent être complétés en une seule saison. Les spores méiotiques, issues de la reproduction sexuée, permettent le brassage de la diversité génétique dans la population, résultant en la création de nouveaux génotypes. Ces spores sont également des structures de dispersion et de survie efficaces.

Cependant, un changement de mode de reproduction s'opère chez certaines espèces invasives, et on observe alors une perte totale de la reproduction sexuée dans la population (Gladieux 2015). Des causes proximales peuvent être à l'origine de la modification du système reproducteur : un manque de partenaire possédant le type reproducteur opposé, un événement d'hybridation, ou dans le cas des agents pathogènes possédant un cycle complexe, l'absence de l'hôte alterne. Cependant, cette modification peut également être adaptative. L'absence de reproduction sexuée permet d'éviter la transmission de virus lors de la méiose, de conserver l'hétérozygotie initiale pour les diploïdes, de propager rapidement le génotype le mieux adapté localement si les contraintes maintenant l'avantage de la reproduction sexuée se relâchent (Gladieux 2015). Ce retour à une reproduction strictement clonale a été observé chez plusieurs populations d'espèces d'agents pathogènes comme *Magnaporthe oryzae* infectant le blé (Saleh 2012) ou bien *Cryphonectria parasitica*, le champignon responsable du chancre du châtaignier (Dutech 2012).

Spécificités des espèces invasives

L'être humain a mainte fois introduit des espèces animales ou végétales en dehors de leur aire de répartition, dans des buts divers, comme la chasse ou l'agriculture. Cependant, la dispersion des champignons est rarement intentionnelle et résulte le plus souvent de mouvements ou échanges commerciaux humains. Il a été estimé que 65 à 85% des agents phytopathogènes de plantes étaient des espèces exotiques (Desprez-Loustau et al. 2007). Certaines caractéristiques des champignons favorisent ces dispersions « dissimulées » comme la

production d'un très grand nombre de spores microscopiques ou l'infection d'hôtes asymptomatiques. En effet, de nombreux agents pathogènes ont été introduits via le commerce de leur hôte : à travers du bois de chauffage ou des pousses d'arbre infectés. L'introduction de *Phytophthora cinnamomi* en Australie depuis son aire de répartition présumée en Papouasie Nouvelle-Guinée a par exemple causé énormément de dégâts en agriculture et en horticulture, et a également grandement affecté les écosystèmes naturels (Hardham 2005). Avec une liste d'environ 950 hôtes connus, *P. cinnamomi* est une menace sérieuse pour une grande variété de plantes à travers le monde.

Pour qu'une espèce devienne invasive et soit déplacée en dehors de son aire de répartition naturelle, elle doit surmonter plusieurs barrières. Elle doit d'abord être transportée (intentionnellement ou non) et survivre à ce transport. Une fois déplacée, son nouvel environnement, composé de facteurs abiotiques ainsi que des espèces natives agit comme un filtre de sélection. Si l'espèce parvient à s'établir dans le nouvel environnement, elle doit alors parvenir à se répandre (Sakai et al. 2001). Plusieurs facteurs vont affecter la capacité d'une espèce invasive à s'établir, comme sa plasticité phénotypique, son mode de reproduction, son taux de croissance ou encore la diversité des espèces avec lesquelles elle va entrer en compétition dans le nouvel environnement (Sakai et al. 2001). En théorie, la diversité des plantes dans ce nouvel environnement devrait limiter l'abondance des ravageurs, la quantité et la disponibilité d'hôtes appropriés étant réduite dans une communauté plus diverse. Il a cependant été montré que cette relation n'était pas linéaire. En effet, à une diversité d'arbres faible, la diversité des ravageurs augmenterait avec la diversité des arbres (Guo et al. 2019). Une plus grande diversité spécifique d'arbres fournit alors plus d'environnements à coloniser et donc plus d'opportunités pour les espèces invasives. En revanche, quand la richesse spécifique est haute, on observe un phénomène de dilution. La diversité spécifique des agents pathogènes diminue alors en fonction de l'augmentation de la diversité spécifique des arbres (Guo et al. 2019). Dans ce cas-là, une plus grande diversité d'arbres rend l'hôte approprié plus difficile à trouver et l'espèce invasive a donc plus de mal à s'établir.

L'effet fondateur subit par les espèces déplacées hors de leur environnement aboutit à une réduction de leur diversité génétique, ce qui peut mener à une diminution de leur potentiel adaptatif ou bien à l'échec de leur établissement dans leur nouvel environnement. *C. parasitica* et *Fusarium circinatum* (responsables du chancre du châtaignier et du chancre résineux du pin respectivement) sont deux exemples d'agents pathogènes présentant une variation génétique

réduite dans leur aire de répartition introduite comparé à leur aire de répartition d'origine (Desprez-Loustau et al. 2007). Cependant, les hôtes n'ayant jamais été exposés peuvent être désavantagés lors de l'arrivée d'un nouveau pathogène, car ils n'auront pas coévolué avec celui-ci. En conséquence, le génotype le plus virulent est souvent à l'origine de la fondation de nouvelles populations d'agent pathogènes, qui présentent alors une faible diversité mais qui sont extrêmement destructrices pour les populations d'hôtes (Callaghan et Guest 2015).

Les introductions multiples depuis différentes sources sont un facteur pouvant réduire la sévérité du goulot d'étranglement génétique lors d'événements fondateurs, augmentant ainsi la diversité génétique du pathogène par rapport à une introduction unique. Le flux génique entre des populations introduites différencierées génétiquement joue également un rôle critique dans l'établissement des pathogènes exotiques en favorisant l'émergence de nouveaux génotypes pouvant être mieux adaptés au nouvel habitat (Dlugosch et Parler 2008, Novak 2007). Il est alors possible d'observer des populations invasives possédant une diversité génétique égale ou supérieure à leur population d'origine (Novak 2007).

L'étude de l'adaptation chez les champignons

Niche, fitness et virulence

Le concept de niche est un concept très important en écologie car il permet de comprendre la distribution des espèces dans le temps et l'espace. Ce terme a été défini par Grinnell en 1917 pour la première fois (Grinnell 1917). La niche est alors l'ensemble des facteurs abiotiques qui détermine la distribution d'une espèce particulière. Plus tard, selon Elton, la niche est le rôle qu'une espèce a dans sa communauté, à commencer par son niveau trophique (Elton 1927). Encore plus tard, selon Hutchinson, la niche est alors un « hypervolume à n dimensions », dans lequel chaque axe représente un facteur biotique ou abiotique permettant à l'espèce d'exister (Hutchinson 1957). La définition de ce concept a maintes fois fait débat parmi les écologistes et a parfois pris des significations différentes en fonction de la personne qui l'a employé (Leibold 1995). De nos jours, la paramétrisation mathématique de ce concept est utilisée pour comprendre la distribution et la diversité des espèces, les taux d'extinction et de spéciation, les mécanismes sous-jacents à la spéciation sympatrique et allopatrique, et l'adaptation aux différentes conditions environnementales (Liu et al. 2020). Il a été récemment montré que dans le cas des espèces invasives, les niches étaient similaires dans les milieux indigènes et récemment introduits, supportant l'hypothèse de la conservation de la niche (Liu et al. 2020).

La fitness, ou succès reproducteur, correspond au nombre de descendants fertiles produits par un génotype. Dans une niche donnée, un individu présente alors une fitness plus élevée qu'un autre si, à la génération suivante, on retrouve plus de copies de son génome dans la population. Il est accepté que la virulence serait une conséquence inévitable de la reproduction des agents pathogènes au sein de leur hôte. La virulence serait donc liée à la multiplication intra hôte, un composant majeur de la fitness des pathogènes, et trait pouvant être soumis à sélection. Cependant une virulence trop élevée conduisant à un taux de mortalité trop élevé des hôtes affecterait négativement la transmission inter hôtes, une autre composante majeure de la fitness (Sacristan et al. 2008). La virulence résulterait donc d'un compromis entre la multiplication intra hôte et la transmission inter hôte (Sacristan et al. 2008). De plus, le temps de renouvellement de la forêt naturelle étant assez long, il est attendu que les agents pathogènes soient sélectionnés pour un phénotype qui ne tue pas l'hôte. Bradshaw et ses collaborateurs (2019) ont par exemple observé une réduction de la virulence dans les populations néo-zélandaises de l'agent pathogène *Dothistroma septosporum*, responsable de la maladie en bandes rouges qui affectent plusieurs espèces de conifères. La comparaison d'isolats datant de l'introduction de *D. septosporum* en Nouvelle-Zélande il y a 50 ans et des isolats contemporains a révélé une production d'une toxine fongique, la dothistromine et une biomasse dans les aiguilles de l'hôte bien inférieure dans les échantillons récents (Bradshaw et al. 2019).

La mesure et l'utilisation des concepts de niche et de fitness permettent de comprendre les facteurs et les mécanismes de l'adaptation chez les organismes. Ce genre de résultat peut permettre de mieux prédire et comprendre l'adaptation des espèces aux changements climatiques et aux introductions.

Les méthodes de phénotypage

Mesures de la virulence d'un agent pathogène

Historiquement, les premières mesures de la virulence chez les champignons pathogènes correspondaient à déterminer la quantité d'inoculum nécessaire pour tuer un hôte, similaire au nombre minimal de bactérie à injecter pour tuer une souris susceptible (Grogan et al. 1979). Chez les agents phytopathogènes, des symptômes visibles peuvent être mesurés directement sur la plante hôte (*in planta*). La défoliation est une mesure de la virulence utilisée couramment pour les agents pathogènes des arbres feuillus (Brasier et Webber 1987). Chez les conifères, il est possible d'évaluer le taux de colonisation de l'hôte. Les mesures du nombre de stomates

obstruées par les pseudothèces sur les aiguilles de sapin Douglas, ou encore du nombre de périthèces présents sur une aiguille de pin tordu sont couramment utilisées pour mesurer la virulence de *Phaeocryptopus gaeumannii* causant la rouille suisse du Douglas et de *D. septosporum*, responsable de la maladie en bandes rouges des aiguilles (Winton et al. 2007 et Kabir et al. 2015, respectivement). Chez *Z. tritici*, des méthodes d'analyse d'images automatisée à haut-débit ont été développées pour mesurer quantitativement la virulence en utilisant la taille des nécroses et le nombre de pycnidies présentes sur des feuilles de blé (Stewart et al. 2016).

Des méthodes alternatives à la quantification des dégâts causés chez les hôtes ont parfois été mises au point pour évaluer la virulence de manière simple et peu coûteuse *in vitro*. Des études chez *C. parasitica*, *Chrysoporthe cubensis* et *Ophiostoma ulmi* ont montré que l'inoculation de pommes présentait un moyen rapide d'estimer la virulence chez ces pathogènes (Plourde et al. 2014) (voir partie 3 pour plus de détails sur la méthode chez *O. novo-ulmi*). Chez certains *Phytophtoras*, il est également possible d'inoculer des suspensions de spores sur des poires, et de mesurer la surface des lésions provoquées (Grove et Boal 1991). Bien que n'étant pas une mesure directe de la virulence, ces méthodes présentent un grand intérêt pratique car elles ne nécessitent que peu de temps et de matériel. L'utilisation de cultures cellulaires de tissus de plantes est utilisée depuis plusieurs années dans des programmes d'amélioration génétique des arbres, mais a également trouvé d'autres applications, comme en phytopathologie (Fenning 2019). Cette option est particulièrement intéressante par rapport aux approches traditionnelles quand on considère le long cycle reproducteur des arbres. De nombreuses études ont utilisé ce genre d'approche pour rechercher des variants capables de résister aux attaques des agents pathogènes chez différentes espèces d'arbres, incluant l'orme, le châtaignier, ou encore le peuplier (Fenning 2019). Cette méthode peut également être utilisée de façon inverse, en soumettant la même culture à plusieurs isolats de l'agents pathogènes étudié, permettant d'évaluer les différences de virulences entre eux.

Mesures de croissance

Les champignons pathogènes ont des cycles de vie qui peuvent être complexes et qui varient d'une espèce à une autre. Mesurer leur fitness *in planta* est un véritable défi. Les pathologistes se servent alors d'autres mesures plus simples mais liées à la fitness, comme la croissance, pour pouvoir étudier de nombreux aspects de la biologie de ces organismes. La croissance dans différentes conditions est utilisée dans de nombreuses études pour explorer les différences entre des isolats plus ou moins virulents, la résistance aux fongicides ou pour tester

la corrélation avec des traits impliqués dans la reproduction (Pringle et Taylor 2002). Chez les champignons filamenteux, il est facile de mesurer, au laboratoire, la croissance radiale sur milieux gélosés nutritifs. Ces données de croissance peuvent fournir beaucoup d'informations. Chez *Aspergillus niger*, la surface mycéienne est par exemple un bon prédicteur du nombre de spores (De Visser et al. 1997).

Les méthodes génomiques pour détecter l'adaptation

Un des défis majeurs de la biologie moderne est d'établir le lien entre le génotype et le phénotype des individus. Bien que les processus neutres comme la mutation et la dérive génétique façonnent la diversité génétique des populations, l'environnement local des organismes joue un rôle prépondérant sur l'évolution de l'adaptation locale. Au cours de l'histoire, les naturalistes ont rapidement remarqué que les plantes poussaient mieux dans les endroits où on les retrouvait naturellement. Les premières expériences permettant de d'identifier les bases génétiques de l'adaptation sont les approches dites de « jardin commun ». En plantant des individus de provenances variées dans un même environnement, il est alors possible d'évaluer la différentiation des populations soumises à des traitements différents. Aujourd'hui, l'explosion des techniques de séquençage à haut débit et la multiplication des méthodes de phénotypage ouvre un champ de possibilité incroyable pour détecter les locus impliqués dans certains traits phénotypiques, et ainsi élargir notre compréhension de l'adaptation.

Les techniques de séquençage à haut débit permettent d'obtenir des quantités importantes de données génomiques à faible coût. De plus, les génomes des champignons étant de petite taille comparés aux plantes ou aux animaux (32 millions de paires de base pour *O. novo-ulmi* par exemple), il est possible de séquencer jusqu'à 96 individus en même temps et obtenir une bonne couverture du génome. Après alignement sur un génome de référence, on peut alors obtenir tous les polymorphismes d'un seul nucléotide dans le génome ou SNPs (pour *Single Nucleotide Polymorphism*). L'ensemble des SNPs contenus dans un génome représente la variation génétique des individus séquencés. Ils servent dans des analyses de visant à comprendre la structure génétique des populations ou bien dans des tests d'association entre génotype et phénotype ou génotype et variables environnementales. Il existe deux types d'approches pour étudier l'adaptation. Le premier est l'étude des signatures moléculaire de sélection dans le génome (appelées *bottom-up*). Différents tests statistiques permettent de détecter les gènes sous l'effet de la sélection dans les populations. L'avantage des méthodes de détection de sélection

est qu'aucune mesure de phénotype longue et coûteuse n'est requise, comme on utilise uniquement les données génétiques (voir partie 1 sur la sélection). Un désavantage c'est qu'un variant génétique (SNP) détecté comme étant sous sélection n'est pas toujours directement lié à un phénotype observable, et il est alors difficile de faire le lien avec un phénotype concret. Le second type d'approche est l'étude des différences phénotypiques et l'identification subséquente des mécanismes et gènes associés à ces phénotypes (appelées *top-bottom*). Un test d'association vise à détecter le ou les loci associés avec des variables liés aux individus. Celles-ci peuvent être des facteurs environnementaux (température, précipitations, sécheresse...), on parle alors d'association génotype-environnement ou GEA (pour *Genotype Environment Association*) ou des traits phénotypiques mesurés sur le terrain ou dans le laboratoire, on parle alors d'association génotype-phénotype ou GPA (pour *Genotype Phenotype Association*).

Les analyses de cartographie QTLs (pour *Quantitative Trait Loci*) sont un type de GPA qui se base sur une descendance de deux parents connus, et qui permet d'identifier des régions contenant les loci associés avec le trait considéré. Une condition préalable est donc qu'il soit possible de maintenir et de reproduire par voie sexuée l'espèce considérée au laboratoire. Il a par exemple été possible d'identifier des régions contenant des gènes liés à la virulence chez *Z. tritici* (Stewart et al. 2018) ou encore chez *Heterobasidion annosum* (Lind et al. 2007). Cependant cette analyse présente deux limites. La première est qu'elle ne permet pas d'identifier un gène unique, mais seulement une région associée avec le trait. La seconde est que la puissance du test est déterminée par la diversité génétique et phénotypique entre les deux parents.

Le GWAS (pour *Genome Wide Association Study*) tire parti de la diversité génétique présente dans les populations naturelles et utilise les séquences de nombreux individus. C'est une analyse de haute résolution, statistiquement basée sur un modèle linéaire, testant l'effet de chaque SNP du génome sur le phénotype d'intérêt. Lorsqu'un ou plusieurs SNPs ont un effet significatif sur le phénotype, il est fort probable que le ou les gènes présents dans la région du signal soient directement impliqués dans le développement de ce phénotype. Le nombre d'individus et la qualité des SNPs détectés augmentent la puissance de l'analyse. La résolution du test dépend du taux de recombinaison présent dans la population, et il peut arriver que l'analyse détecte difficilement les variants rares (Korte et Farlow 2013). Dans le cas des champignons pathogènes, la grande majorité des GWAS a été réalisée sur les maladies ayant un impact agronomique, et le plus souvent chez l'hôte, afin de détecter des gènes candidats impliqués dans la résistance et pouvant être intégrés aux programmes d'amélioration génétique des cultures (maïs, soja, blé,

riz...) (Genissel et al. 2017). Cependant, le GWAS peut également être un outil très puissant pour la lutte contre les pathogènes forestier. Stocks et ses collaborateurs (2019) ont par exemple été capables d'identifier des SNPs permettant de prédire la virulence du champignon responsable de la maladie du flétrissement du frêne (ou chalarose) *Hymenoscyphus fraxineus*, sur la santé de l'arbre avec une précision de 90%. Un nombre grandissant d'études s'intéressent maintenant aux agents pathogènes, en essayant par exemple de détecter les gènes impliqués dans la pathogénicité. Chez *Z. tritici* par exemple, la virulence semble être un trait complexe composé de plusieurs régions génomiques différentes, impliquant des gènes associés à la dégradation de la paroi cellulaire, le transport cellulaire et le métabolisme fongique (Hartmann et al. 2017).

Le GEA (pour *Genotype Environment Association*) fonctionne sur le même principe que le GWAS et permet d'identifier les locus associés avec une composante environnementale d'intérêt comme la température ou les précipitations (Sork et al. 2013). Ces analyses aident à comprendre comment les organismes sont distribués dans leur milieu naturel et quels sont les gènes et les mécanismes impliqués dans l'adaptation environnementale. Ils permettent d'étudier l'adaptation des organismes à leur milieu naturel en utilisant comme trait les facteurs abiotiques locaux de l'endroit où l'organisme a été prélevé. Cette approche est appelée génomique du paysage (*landscape genomics* en anglais). Ce domaine a pour but de comprendre les bases génétiques de l'adaptation locale des organismes à leur environnement. Les analyses GEA sont largement utilisées chez les plantes, mais peu se sont penchées sur les champignons pathogènes. Ces analyses devraient prendre de l'importance dans les années à venir car elles permettent de modéliser et ultimement de prédire les changements dans la répartition d'une espèce, ce qui est primordial dans un contexte de changement climatique.

Une approche multi méthodologique combinant la détection de signature moléculaire de sélection, ainsi que la conjugaison des données génétiques, phénotypiques et environnementales dans des tests d'association semble idéale afin de comprendre au mieux l'adaptation chez les organismes vivants. L'association de ces méthodes permet d'identifier des gènes ou SNPs candidats solides, pouvant par la suite être soumis à la validation via des approches de génomique fonctionnelle. Les techniques d'édition de génome comme la populaire méthode CRISPR-Cas9, permettent de confirmer l'impact d'un gène ou d'un SNP candidat sur un phénotype en créant des mutants. L'utilisation de CRISPR-Cas9 était restreinte aux espèces modèles il y a quelques années. Elle est maintenant adaptée à un nombre grandissant d'espèces. Elle permet d'ajouter un gène (*knock-in*), de l'inactiver (*knock-out*), ou d'en moduler l'expression (*knock-up* et *knock-*

down). Cette méthode est utilisée avec succès par un nombre grandissant d'études chez les champignons pathogènes des cultures, cependant l'adaptation de la technique reste limitée chez les pathogènes forestiers (Dort et al. 2020) (voir partie 3 pour l'application chez les champignons pathogènes responsables de la MHO). Cette technique présente un potentiel important pour mieux comprendre les fonctions des gènes des champignons pathogènes et leur impact sur le phénotype.

La maladie hollandaise de l'orme

Historique

La Maladie Hollandaise de l'Orme (MHO) est causée par des champignons Ascomycètes du genre *Ophiostoma*. L'impact dévastateur de la maladie sur les populations d'ormes du monde entier a amené les chercheurs à s'y intéresser depuis plus d'un siècle. Deux vagues successives de pandémies se sont produites durant les cent dernières années. La première vague, causée par *Ophiostoma ulmi* (Buisman) Nannfield, a été observée pour la première fois en Europe du Nord-Est dans les années 1910-1920 (Spierenburg 1921). La maladie s'est rapidement propagée au reste de l'Europe et a ensuite été introduite en Amérique du Nord, vraisemblablement lors via le Canada lors de la Deuxième Guerre Mondiale (Brasier 2000, Pomerleau 1961). Un déclin de la maladie a été constaté en Europe dans les années 1940, rapidement suivi de l'observation d'une deuxième vague se répandant en Europe et en Amérique du Nord simultanément, supposément depuis la Roumanie et la région des Grands Lacs aux États-Unis, respectivement. Cette seconde pandémie, toujours active actuellement, est causée par une espèce proche d'*O. ulmi*, appelée *Ophiostoma novo-ulmi*, et a causé la destruction d'un très grand nombre d'ormes matures en Europe et en Amérique du Nord (Brasier 1991). Les populations européenne et nord-américaine d'*O. novo-ulmi* furent assignées à deux sous-espèces : *O. novo-ulmi* ssp *novo-ulmi* et *O. novo-ulmi* ssp *americana*, respectivement (Brasier & Kirk 2001). La sous-espèce *Ophiostoma novo-ulmi* ssp *americana* a été introduite en Europe dans les années 60, probablement par le transport de bois d'orme infecté depuis le Canada vers le Royaume-Uni (Brasier et Gibbs 1973).

La recherche des origines de la maladie en Asie a conduit à la découverte d'une nouvelle espèce dans les années 90, *O. himal-ulmi* (Brasier et Mehrotra 1995). Cette espèce, endémique de la région de l'Himalaya ne cause pas de symptômes à son hôte local, *Ulmus wallichiana*. En

revanche, l'inoculation de *O. bimacul-ulmi* sur les espèces européenne et nord-américaines d'ormes provoque la MHO.

De nos jours, la MHO semble moins active en Europe de l'Est, ce qui est dû à la faible présence d'ormes matures ayant survécu aux deux vagues pandémiques. Cependant, des populations de l'agent pathogène sont toujours présentes en Europe de l'Est et en Grèce (Pologne : Lakomy et al. 2016, Croatie : Katanic et al. 2020, Grèce : Tziros et al. 2017). La maladie est également active dans l'Est des États-Unis et du Canada, où des arbres infectés sont coupés chaque année par les autorités sanitaires. La MHO semble également continuer son expansion vers l'Ouest de l'Amérique du Nord. Au Canada, le front de migration connu de la maladie se trouve en Saskatchewan. Cependant, des ormes porteurs de la MHO ont très récemment été détectés plus à l'Ouest, en Alberta, où la maladie avait été détectée une seule fois dans les années 80. Aux États-Unis, la présence de la maladie a été reportée jusqu'en Oregon à l'Ouest, et au Texas au Sud. Le fait que cette maladie est toujours active ainsi que la sévérité des effets sur les ormes tant urbains que forestiers démontre l'importance de l'application des mesures de contrôle et d'identification de ces espèces d'agents pathogènes.

Cycle, biologie et virulence

Cycle (hôtes, vecteurs)

Afin de pouvoir infecter leur hôte, les champignons responsables de la MHO ont besoin d'un vecteur, le scolyte. Les scolytes sont des coléoptères de la sous-famille *Scolytinae*. Ils se nourrissent du phloème, la couche de cellules vivantes comprise entre l'écorce des arbres et le xylème. En Amérique du Nord, la dispersion de la MHO est assurée par plusieurs espèces de scolytes : le scolyte de l'orme d'Amérique *Hylurgopinus rufipes*, le scolyte européen *Scolytus multistriatus* ainsi que le scolyte à bandes *Scolytus schevyrewi*, introduit depuis l'Asie et observé au Colorado au début des années 2000 (Jacobi et al. 2007). *Scolytus multistriatus* est le vecteur le plus efficace excepté dans les zones où le climat est plus froid, où le scolyte indigène *H. rufipes* est le vecteur principal. Le nombre de spores observées sur la carapace des scolytes diffère en fonction de la taille de l'insecte, mais également en fonction de son comportement reproducteur (Webber 1990). Lorsque la carapace du scolyte est chargée de spores et qu'il se nourrit sur une jeune branche, la blessure qu'il inflige permet au champignon de passer dans le système vasculaire de

l'arbre. Le champignon se répand alors dans le xylème de l'arbre de manière efficace, tirant probablement avantage de sa capacité à se diffuser sous forme de levure et à coloniser les différents vaisseaux sous forme de mycélium. Les ormes infectés présentent des symptômes externes caractéristiques comme un brunissement et flétrissement des feuilles ainsi qu'une coloration brun-rouge observable sous l'écorce. Les espèces européennes d'ormes (*Ulmus carpinifolia*, *U. glabra*, *U. procera*) présentent une susceptibilité réduite par rapport à l'orme américain, *U. americana*, ce qui serait lié à des différences physiologiques (McNabb et al. 1970). D'autres espèces d'ormes américaines comme l'orme rouge, *U. rubra*, sont également susceptibles (Brunet et al. 2016). Quelques années plus tard, lorsque l'arbre est mourant, il émet des composés chimiques, qui attirent les coléoptères mâles comme femelles (McLeod et al. 2005). Les femelles creusent alors des galeries de pontes sous l'écorce afin d'y déposer leurs œufs. Lors de sa phase de croissance saprophytique, le champignon colonise ces galeries et produit des structures de reproduction asexuées (synnemas) et sexuées (périthèces) surmontées de gouttelettes collantes pleines de spores. Lorsque les jeunes scolytes adultes émergent des galeries au printemps, leur carapace est chargée de spores du champignon qu'ils transportent alors jusqu'à l'arbre où ils iront se nourrir, permettant ainsi au cycle de continuer.

Plusieurs gènes candidats pour la virulence et la pathogénicité ont été identifiés chez *O. novo-ulmi* lors de recherches antérieures. Un premier gène proposé comme impliqué dans la virulence est un gène encodant pour une hydrophobine, la cerato-ulmine. Des observations de mutants naturels ainsi que l'obtention de mutants *knock-out* tout aussi virulents ont rapidement écarté cette hypothèse (Brasier et al. 1995, Temple et al. 2000). Cependant ce gène pourrait avoir un rôle dans la fitness en conférant une meilleure adhérence des spores de champignon à la carapace des scolytes (Temple et al. 2000). L'enzyme Epg1 encodant une endopolygalacturonase était susceptible d'être impliquée dans la colonisation du xylème, cependant des mutants *knock-out* de ce gène ne présentent pas de diminution de virulence (Temple 2009). Le gène Col1 identifié par Pereira et al. (2000) produit des mutants à croissance mycélienne réduite sur milieu nutritif gélosé. Un croisement entre l'isolat peu virulent AST27 et l'isolat très virulent H327 a permis d'identifier le gène candidat le plus prometteur impliqué dans la virulence, appelé Pat1 (Et-Touil et al. 1999). Des données de séquençages de fragments RAPD de Pat1 ont permis d'identifier plusieurs gènes situés sur le chromosome I, dont un transporteur d'azote (Plourde 2011, thèse présentée à la Faculté des études supérieures de l'Université Laval). Des travaux sont actuellement en cours pour confirmer l'implication de gènes encodant des transporteurs d'azote

dans la virulence grâce à l'utilisation de mutants (De Oliveira données non publiées). L'annotation du génome (Khoshraftar et al. 2013, Comeau et al. 2015) et la comparaison de son contenu génique à des bases de données comme PHI-base (Winnenburg et al. 2006) a permis de détecter plusieurs gènes tels que des dioxygénases de type *cox* et des gènes *hex* impliqués dans la formation des corps de Voronine supposément impliqués dans la virulence. Ces différents gènes candidats sont en cours d'étude (Dr. Paul De la Bastide, Biology Department, University of Victoria et Pr. Louis Bernier, Centre d'étude de la forêt (CEF), Faculté de foresterie, de géographie et de géomatique, Université Laval).

Système de reproduction et incompatibilité végétative

Ophiostoma ulmi et *O. novo-ulmi* sont des champignons hétérothalliques, c'est-à-dire qu'ils requièrent la présence du type reproducteur opposé pour réaliser un cycle de reproduction sexuée. Le locus déterminant le type reproducteur se situe sur le chromosome 2. MAT-1 est receveur lors de la reproduction et le type MAT-2 est donneur dans la nature, mais des croisements réciproques sont possibles au laboratoire (Brasier 1991). Lors de la phase saprophytique, deux individus compatibles peuvent produire des structures de reproduction sexuée dans les galeries creusées par les scolytes.

Il existe également un système d'incompatibilité végétative prévenant la fusion des hyphes lors de la rencontre de deux isolats distants génétiquement. Ce système pourrait avoir évolué en réponse à la propagation des virus dans les populations (Milgroom et Brasier 1997). Une vingtaine de loci *vic* différents répartis dans le génome déterminent le type de compatibilité d'un individu (Brasier 1984). L'annotation du génome d'*O. novo-ulmi* a confirmé l'existence et la localisation de ces gènes candidats correspondant à ces loci *vic* (Comeau et al. 2015). Dans certains cas, le locus reproducteur peut prendre le contrôle malgré une incompatibilité végétative des individus et lancer ainsi un cycle de reproduction sexuée.

Phénotypes connus

Ophiostoma ulmi et *O. novo-ulmi* présentent des optimums de croissance différents. La mesure de croissance radiale de colonies mycéliennes de nombreux échantillons à plusieurs températures a permis de déterminer un optimum de croissance à 30°C pour *O. ulmi* et à 20-22°C pour *O. novo-ulmi* (Brasier 1981). C'est cependant à 32°C que la croissance radiale de *O. ulmi* est supérieure à *O. novo-ulmi*, car à 30°C *O. novo-ulmi* présente un taux de croissance quasiment

aussi rapide que *O. ulmi*. *Ophiostoma novo-ulmi* possède donc une gamme de température de croissance plus étendue que *O. ulmi*, élément essentiel à l'invasion d'un agent pathogène dans un nouvel environnement.

Autrefois respectivement appelée la souche non-agressive et la souche agressive, il a été montré que *O. ulmi* était moins virulente que *O. novo-ulmi* (Gibbs et Brasier 1973). Il est possible d'évaluer la virulence des champignons causant la MHO en utilisant directement des ormes (Solla et Gil (2002) utilisent par exemple *Ulmus minor* dans leurs expériences). Les arbres ou les pousses peuvent être inoculés sur le terrain ou bien dans des conditions stables et contrôlées, en les plaçant en serre ou en chambre de croissance. Le pourcentage de défoliation est alors mesuré de manière régulière pendant les trois mois qui suivent l'inoculation (Solla et Gil 2002). Le point fort de cette démarche est que l'effet du champignon peut être mesuré sur son hôte naturel. Cependant ces méthodes restent extrêmement difficiles à mettre en place à grande échelle en raison des coûts financiers engendrés et de l'espace nécessaire pour mener à bien l'expérience. Une méthode alternative a été proposée par Plourde et Bernier (2014), permettant d'évaluer la virulence des champignons provoquant la MHO. Une pastille de milieu nutritif gélosé portant du mycélium fongique est inoculée sur des pommes de la variété 'Golden Delicious'. Le champignon provoque alors le développement d'une nécrose brune, observable autour de l'insertion de la pastille. La taille de cette nécrose est mesurée 14 et 28 jours après l'inoculation. Celle-ci est positivement corrélée avec le pourcentage de défoliation que le même isolat provoquerait sur un arbre, et n'est pas corrélée au taux de croissance radiale sur gélose, ce qui n'en fait donc pas une mesure de croissance (Plourde et Bernier 2014). Bien qu'évaluant la virulence de manière indirecte, cette méthode rapide et peu coûteuse présente une bonne alternative pour évaluer la virulence chez les champignons causant la MHO.

Structure des populations et diversité

Génétique des populations

La structure des populations d'*O. ulmi* diffère en Europe et en Amérique du Nord. Les populations européennes présentent un plus grand nombre de types d'incompatibilité végétative, ainsi qu'un ratio égal des deux types reproducteurs, suggérant la possibilité de faire de la reproduction sexuée régulièrement (Mitchell et Brasier 1994). En Amérique du Nord, le type d'incompatibilité végétative dominant est AMSG, et celui-ci est largement associé au type reproducteur MAT-2. Ces éléments suggèrent une reproduction sexuée limitée en Amérique du

Nord, ainsi qu'une diversité génétique réduite par rapport aux populations européennes. Ces observations sont cohérentes avec un scénario d'effet fondateur lors de l'introduction de *O. ulmi* en Amérique du Nord depuis l'Europe. Il est également possible que le changement d'hôte ait imposé une pression sélective ayant façonné la diversité génétique d'*O. ulmi* en Amérique du Nord.

Les observations de *O. novo-ulmi* ont remplacé celles de *O. ulmi* lors de la deuxième vague pandémique qui a débuté dans les années 1940 et qui est toujours active aujourd'hui. Les populations européennes et nord-américaines de cette espèce plus virulente (autrefois races EAN et NAN) ont été désignées comme deux sous espèces en 2000 (Brasier et Kirk 2001). Cette distinction s'est faite sur la base de critères phénotypiques et moléculaires (voir ci-dessus) dans une volonté de formaliser la désignation taxonomique de ces deux populations. Une étude de la structure des populations d'*O. novo-ulmi* ssp *americana* en Amérique du Nord a été réalisée à la fin des années 1990 et a révélé la présence de trois principaux groupes d'incompatibilité végétative AMSG, AM2SG et EUSG et de quelques types minoritaires (Brasier 1996). Le nombre de types d'incompatibilité végétative était plus élevé dans la région des Grands Lacs (Indiana et Michigan, États-Unis) et diminuait progressivement en s'éloignant de ceux-ci, ce qui semble supporter l'hypothèse d'une expansion rapide et clonale depuis une émergence de la deuxième vague de pandémie dans cette région (Brasier et Kirk 2000). Cependant aucune autre étude n'a creusé cette hypothèse depuis. Lors de l'étude de la structure de la population espagnole d'*O. novo-ulmi* ssp *americana*, deux sous-groupes génétiques ont été mis à jour. Il a été proposé qu'un de ces groupes correspondrait à la sous espèce américaine d'*O. novo-ulmi* et que l'autre serait composé d'hybrides entre les deux sous espèces (Solla et al. 2008). Cependant ces groupes génétiques pourraient possiblement correspondre aux deux types principaux d'incompatibilité végétative présents en Amérique du Nord, nommés AMSG et EUSG (Brasier 1996).

Hybridation

Les différences phénotypiques observées entre *O. ulmi* et *O. novo-ulmi* laissent à penser que *O. novo-ulmi*, en tuant les hôtes plus rapidement, aurait éliminé *O. ulmi* dans une compétition pour la ressource limitante, les hôtes. L'observation de deux vagues épidémiques successives semble aller dans ce sens. Cependant, les populations de *O. ulmi* et *O. novo-ulmi* ont coexisté en Europe assez longtemps pour que des hybrides entre les deux espèces soient observés (Brasier

et al. 1998). Bien que rares et semblant avoir une fitness moins élevée que leurs parents (Brasier et al. 1998), ces hybrides fertiles ont pu agir comme pont génétique entre les espèces parentales, menant à l'introgression de parties du génome de *O. ulmi* dans *O. novo-ulmi* (Et-Touil et al. 1999, Paoletti et al. 2006). Ces événements d'introgression ont permis l'acquisition du type reproducteur MAT-1 chez *O. novo ulmi*, alors que le type reproducteur MAT-2 seulement était présent dans la population (Paoletti et al. 2006). La présence des deux types reproducteurs chez *O. novo-ulmi* confère un avantage évolutif en débloquant la possibilité de faire de la reproduction sexuée et ainsi augmenter la recombinaison génétique et le brassage des allèles dans les populations.

L'introduction et la propagation d'*O. novo-ulmi* ssp *americana* en Europe dans l'aire de répartition d'*O. novo ulmi* ssp *novo-ulmi* a conduit à la formation de nombreux hybrides, permise par une barrière reproductive réduite entre ces deux taxons (Konrad et al. 2002). Ces hybrides présentent le même degré de virulence que le taxon américain parental, et peuvent présenter de nouvelles combinaisons de types végétatifs, pouvant procurer un avantage contre les mycovirus (Webber 1993). Les mycovirus sont transmis de manière passive lors de la fusion des hyphes, ils ralentissent la croissance mycéienne et réduisent la sporulation (Brasier 1997). Ces mycovirus sont souvent associés à des segments d'ARN double brin qui perturbent le fonctionnement normal des mitochondries (Brasier 1997). Ces éléments ont permis d'envisager l'utilisation de mycovirus comme moyen de contrôle de la MHO, cependant cette stratégie pourrait se révéler inefficace dans les zones où la diversité de types végétatifs est forte (Martín et al. 2018). Les conséquences écologiques d'un déploiement de scolytes portant des spores infectées de mycovirus restent également à évaluer.

Génomique

Les premiers assemblages de génome de l'isolat de *O. ulmi* W9 et l'isolat de *O. novo-ulmi* H327 paraissent en 2013 (Koshraftar et al. 2013, Forgetta et al. 2013, respectivement). Bien que la qualité des données n'aie pas permis un assemblage au niveau chromosomique pour *O. ulmi*, la conjugaison des données de séquençage d'ARN et de marqueurs de séquences exprimés (en anglais *expressed sequence tag*) a permis une première annotation du génome, avec 8639 modèles de gènes prédicts (Koshraftar et al. 2013). Les données obtenues lors du séquençage de l'isolat H327 d'*O. novo-ulmi* ont permis d'obtenir un assemblage au niveau chromosomique, permettant une meilleure compréhension de l'architecture du génome (Forgetta et al. 2013). Par la suite,

L'annotation de ce génome a mis en lumière des gènes impliqués dans des fonctions très importantes comme la croissance, la pathogénicité et le métabolisme, avec 78% des gènes assignés à une fonction parmi les 8640 modèles prédits (Comeau et al. 2015). L'analyse a également révélé que les régions répétées comptaient 3,4% du génome, soit une portion relativement faible (Comeau et al. 2015). Cependant, les mécanismes de mutation ponctuelles causées par des séquences répétées sont actifs dans ce génome (Comeau et al. 2015).

Des outils moléculaires ont été développés chez les champignons responsables de la MHO afin de transformer des isolats. Royer et ses collaborateurs (1991) sont les premiers à développer une méthode de transfert de gène permettant d'ajouter des copies ou de remplacer des gènes. Cette technique générant de nombreux mutants ectopiques et au taux de réussite bas (Plourde 2008) a récemment été améliorée grâce à l'utilisation d'un plasmide OSCAR, et permet maintenant d'obtenir des mutants avec un taux de réussite de 88% (Villamil et al. 2020). La technique d'édition de génome CRISPR-Cas9 a tout aussi récemment été adaptée à *O. novo-ulmi* (Tanguay 2019). Ces méthodes ouvrent un champ très vaste de possibilités pour l'étude de la génomique fonctionnelle des champignons responsables de la MHO. En effet, la détection de gène candidats étant facilitée par l'accessibilité des techniques de séquençage à haut-débit, la validation fonctionnelle de ces candidats reste le facteur limitant dans de nombreuses études de phytopathologie. La disponibilité de ces méthodes permet d'envisager la création de nombreux mutants, qui une fois phénotypés, aident à mieux comprendre l'effet de certains gènes impliqués dans la croissance ou la virulence de ces champignons.

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Objectifs de la thèse

La MHO a suscité énormément d'intérêt dans la communauté scientifique depuis sa découverte au début du siècle dernier, et plusieurs équipes de recherches ont permis d'avoir une bonne compréhension de la taxonomie et de la biologie des champignons provoquant cette maladie. Les dernières décennies ont cependant vu passer un déclin du nombre d'études sur cette maladie, probablement dû à la quasi-destruction des ormes en Europe et au peu de succès connu par les traitements tentés. La MHO est cependant toujours active et son avancée continue de faire les actualités en Amérique du Nord. Le premier objectif de cette thèse est de comparer et contraster la génomique des populations et la génomique évolutive des agents pathogènes forestiers et agricoles en réalisant une revue de littérature. Le second objectif de cette thèse est de se servir des dernières innovations technologiques et méthodologiques en matière de génomique (séquençage à haut débit de génomes complets et méthodes computationnelles d'analyses) pour mettre à jour les connaissances sur la maladie. L'utilisation de ces outils génomiques permet d'étudier la structure des populations des agents pathogènes responsables de la MHO au niveau mondial, ainsi que de comprendre les mécanismes évolutifs moteurs de l'adaptation et de la diversité. Nous nous intéresserons également aux impacts de l'hybridation et de l'introgression sur la diversité et les traits adaptatifs de ces populations. La seconde vague de pandémie continue de faire l'actualité en Amérique du Nord, avec une observation très récente de l'arrivée de la MHO en Alberta au Canada. Les champignons de la MHO ont été capables de coloniser une grande variété de régions, avec des climats parfois très différents, du sud du Texas aux États-Unis au nord du Québec au Canada. Les gènes impliqués dans l'adaptation à tant de milieux différents restent inconnus. Enfin, le dernier objectif de travail a pour but d'aider à mieux comprendre les marqueurs sous-jacents à l'adaptation et à la virulence chez ces agents pathogènes, en utilisant des données de phénotypage. L'ensemble des connaissances produit grâce à ce travail fournit des fondations pour le développement d'outils de caractérisation génomique des pathogènes responsables de la maladie hollandaise de l'orme. En effet, cette thèse s'inscrit dans le cadre du projet bioSAFE (www.biosafegenomics.com), ayant pour but de développer des outils de détection et de surveillance des espèces invasives. Ces outils permettent une identification rapide et précise des organismes pathogènes ainsi que des risques qu'ils posent pour les écosystèmes forestiers.

Chapitre 1 Evolution and adaptation of forest and crop pathogens in the Anthropocene

1.1 Résumé

L'Anthropocène est l'ère pendant laquelle l'espèce humaine a l'impact le plus marqué sur les systèmes écologiques et biogéographiques de la planète. Certains agents pathogènes peuvent tirer avantage des activités humaines et évoluent en réponse à l'expansion des systèmes agronomiques et forestiers, menant souvent à l'éclatement d'épidémies. La disponibilité d'assemblage de génomes de qualité et de données de génomique des populations joue un rôle clef dans la compréhension de l'adaptation et de l'évolution de ces agents pathogènes. D'autre part, les cultures et les arbres ont des caractéristiques biologiques et des degrés de domestication différents. Malgré ces différences, certains mécanismes évolutifs sont communs aux pathogènes des cultures et des arbres, comme l'hybridation, le changement d'hôte, la sélection, la spécialisation ou encore l'expansion clonale. Des solutions comme l'intégration de diversité spécifique et génétique ou l'édition génomique dans les cultures pourraient aider à maintenir la production alimentaire ainsi que les services écosystémiques dans le futur.

1.2 Abstract

Anthropocene marks the era when human activity is making a significant impact on earth, its ecological and biogeographical systems. The domestication and intensification of agricultural and forest production systems have had a large impact on plant and tree health. Some pathogens benefitted from these human activities and have evolved and adapted in response to the expansion of crop and forest systems, resulting in global outbreaks. Global pathogen genomics data including population genomics and high-quality reference assemblies are crucial for understanding the evolution and adaptation of pathogens. Crops and forest trees have remarkably different characteristics, such as reproductive time and the level of domestication. They also have different production systems for disease management with more intensive management in crops than forest trees. By comparing and contrasting results from pathogen population genomic studies done on widely different agricultural and forest production systems, we can improve our understanding of pathogen evolution and adaptation to different selection pressures. We find that in spite of these differences, similar processes such as hybridization, host jumps, selection, specialization, and clonal expansion are shaping the pathogen populations in

both crops and forest trees. We propose some solutions to reduce these impacts and lower the probability of global pathogen outbreaks so that we can envision better management strategies to sustain global food production as well as ecosystem services.

1.3 Context

In the age of the Anthropocene, human activities have caused deep global changes (Zalasiewicz et al. 2008). The transformation of the world ecosystems during this period has had a serious impact on biodiversity and important consequences on natural resources (Hobbs et al. 2006). The domestication of plants and animals, the intensification of agriculture, and deforestation are among the most important anthropogenic drivers of these changes.

1.3.1 Domestication of annual plants

Plant domestication (see Glossary), the accelerated evolutionary process operating under human selection (Harlan 1992), has transformed the course of human history. Nearly 70% of the calories consumed by humans are supplied by only 15 domesticated crops (Ross-Ibarra et al. 2007). Agriculture began more than 50,000 years ago although the domestication of today's major crops (wheat, barley, oat, rice, maize, etc.) occurred within the last 10,000 years (Brown et al. 2009; Brown 2018). Plants with the favourable traits have been preferentially selected and multiplied for thousands of generations, gradually shaping genetic variation in local populations and isolating domesticated from wild populations. Over time, artificial selection and the restriction of gene flow caused domesticated populations to diverge genetically and phenotypically from the wild ancestral populations (Olsen and Wendel 2013; Zeder et al. 2006). The outcome of this process differs according to the degree of domestication, resulting in a continuum of plant populations that ranges from exploited wild plants to cultivated human-dependent crops.

A crop is a plant that usually resulted from a long process of domestication and genetic improvement. Crops are grown and harvested for commercial or subsistence purposes. Their yields have increased dramatically, particularly during the 20th century, by exploiting their genetic potential and improving their management by capitalizing on the use of fertilizers in monoculture systems and minimizing competition from weeds, insect-pests, and pathogens with the intensive use of agrochemicals (Miflin, 2000).

Glossary

Agroforestry: A land-using system where long-lived perennials are planted in combination with herbaceous plants, taking advantage of the ecological interactions existing between species with different life history traits.

Cost of domestication: The evolutionary burden caused by domesticating a wild species and subsequent breeding; these effects include small effective population size, inbreeding and strong selection on domestication genes that will result in an increase of deleterious mutations, imposing a genetic load to the domesticated population.

Cost of pestification: By analogy to the cost of domestication, adaptation of a new pathogen lineage to a domesticated plant species impose some evolutionary effects such as reduction in population effective size, strong selection on “pestification” genes and sometimes absence of sexual recombination that will lead to the accumulation of deleterious mutations.

Crop: A plant that is grown in managed plantations, often originating following a process of domestication and improvement and is harvested for commercial or subsistence purposes.
Domestication: An accelerated evolutionary process operating under human selection. In its most simple form, plants with the most interesting traits have been selected and multiplied via seeds or propagules over multiple generations, gradually shaping genetic variation in local populations and isolating the domesticated population from external gene flow.

Domesticated species: A species is domesticated when it has been selected and reproductively isolated for a period of time long enough to cause genetic divergence from its wild relative.

Domestication syndrome: The set of traits selected by humans during domestication that differentiates modern crops from their wild progenitors. **Domesticated pathogen:** A pathogen is domesticated when it coevolves with its main host which is under “domestication” process.

Natural forest, seminatural forest and plantation: Terms describing a gradient of management practices where trees grow and disperse naturally with limited to nonexistent human intervention (natural forest) to highly managed systems where tree species and diversity is controlled and trees are planted artificially (plantation).

Plant breeding: Human-associated/preferential selection and propagation of genotypes with desirable traits.

1.3.2 Domestication of long-lived perennials

In contrast, the domestication of long-living woody perennials, including fruit trees, started only during the last few centuries (Campbell et al. 2003; Gaut et al. 2015; Harfouche et al. 2012; Miller and Gross 2011; Sederoff et al. 2009; Zohary and Hopf 2000). While some fruit trees cultivars are almost undomesticated and indistinguishable from their wild relatives (e.g. cacao trees; Efombagn et al. 2009), others have been bred and selected for hundreds of years, producing many different cultivars, such as apple trees (Cornille et al. 2014). Horticultural traits in these trees have been selected and then fixed by human-mediated methods such as vegetative propagation with cuttings and grafting, leading to a gradual genetic isolation of selected cultivars from the original populations.

Domestication is also recent for forest trees used for lumber, pulp, and wood products in part because of their long reproductive cycle and the easily accessible timber in primary natural forests [Food and Agriculture Organization (FAO) 2020]. As a result, most planted forests are composed of non-domesticated trees that are characterized by high genetic diversity, age, and species heterogeneity, and long time scales. There is a wide diversity of forested landscapes, reflecting different degrees of anthropogenization. In the past half century, the increasing demand for fiber and wood products has led to a strong expansion of tree plantations, representing now three percent of forest area worldwide (FAO 2020). In the southern-hemisphere highly-productive monoculture plantations of eucalyptus, acacia, and pine account for most of the wood supply (Paquette and Messier 2010). Tree planting as a means to fix carbon and reduce greenhouse gases is creating further demand to increase forest acreage, with countries such as China, India, the USA, and Canada proposing plans to plant trillions of trees.

Therefore, long-lived perennials comprise a mixture of highly domesticated and intensively managed plantations and forest stands as well as highly heterogeneous undomesticated forests. This provides a striking contrast with the genetically homogeneous, annual, and highly managed crops.

1.3.2.1 Exploring pathogen evolution along the domestication gradient

Crops and forest trees are affected by diseases caused by pathogens that are sometimes phylogenetically closely related. Because of the contrasts highlighted in the previous sections, we expect different impacts on pathogen populations. However, there is a gradient along the

domestication and management continuum (Figure 1). Forest trees occurring in natural forests such as oaks and chestnuts are less domesticated and managed than the pines and poplars grown in intensive plantations. Fruit trees are woody long-lived perennials and their genetic domestication is mostly recent; yet they are intensely managed, in a way very similar to crops, with frequent human interventions to increase pest and disease protection and increase yield.

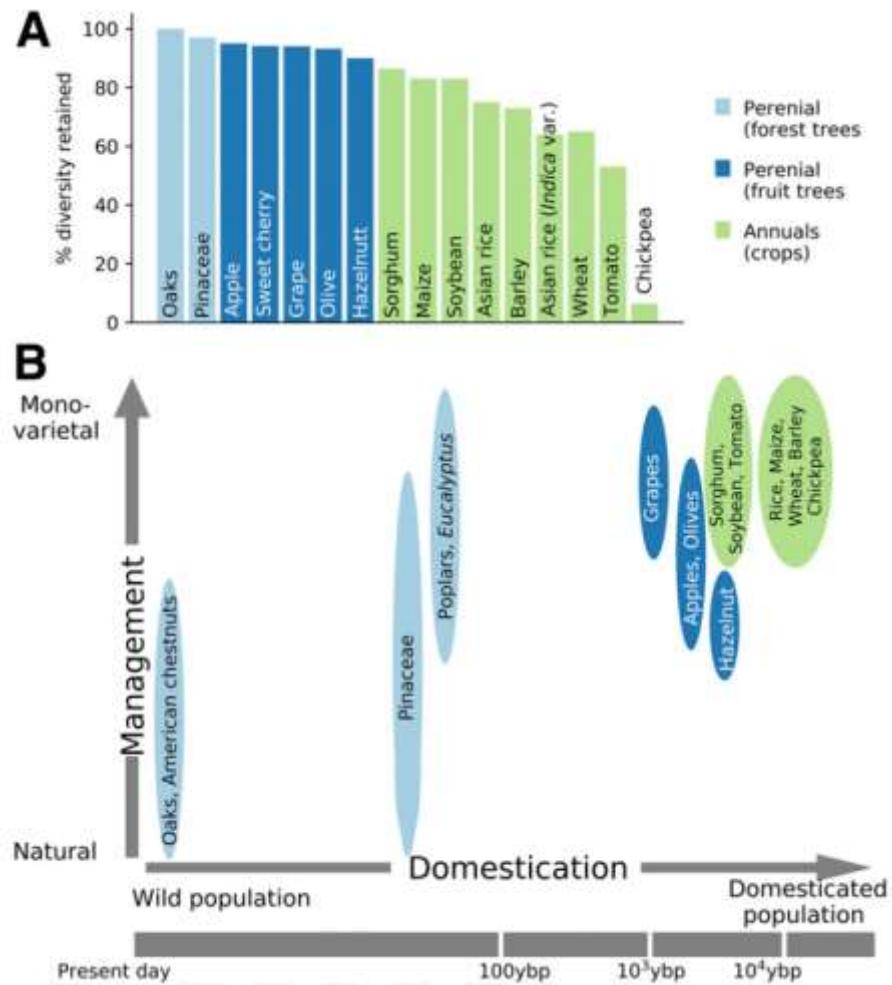


Figure 1. Influence of management and domestication on aspects of agricultural and forest plant species. **A**, Proportion of the diversity retained (relative to the total diversity found in the wild counterpart) in long-lived perennials (forest and fruit trees) and in annual plants (crops) following domestication. **B**, Position of long-lived perennials and annual plants on the domestication gradient; the grayscale in the bottom represents the time in years before present (ybp) at which the domestication process started. Color codes are the same as shown for A.

Exploring how these different production systems impact pathogen population evolution and adaptation might help the community of crop and forest pathologists, geneticists, breeders, and conservationists better understand and manage diseases. In this review, we synthesize the current knowledge on the impact of domestication and management of crop and forest production systems on evolving pathosystems. In particular, we focus on the development of global genomics data such as worldwide populations genomes and the accessibility to high quality reference assemblies for understanding the evolution and adaptation of pathogens and to answer hypothesis-driven questions. A summary of the main pathogens of annual and perennial plants pathogens discussed/mentioned in this review is presented in Table 1.

1.4 Agriculture and forest ecosystems in the age of the anthropocene

1.4.1 Impact of domestication on agriculture and forest ecosystems

Ecosystem design and subsequent plant domestication have both, to some extent, been carried out in very linear ways. Wild and genetically diverse plant resources were selected into phenotypically and genetically homogeneous cultivars while complex and diversified ecosystems were simplified into monocultural and/or uniform plantations. The term “domestication syndrome” was described by Karl Hammer in 1984 as the set of traits that humans selected during domestication and differentiates modern crops from their wild progenitors reviewed in (Box 1; Olsen and Wendel 2013). The domestication syndrome may evolve over a period ranging from few to thousands of generations, as desirable traits are selected and become fixed within the crop population (Meyer et al. 2012).

1.4.1.1 Comparative features of wild vs domesticated annual plants

Annual crop plants were the first to be domesticated from their wild ancestors. In annual crop plants, the domestication syndrome traits include loss of seed shattering, decreased seed dormancy, seed size, and seed number (Zohary and Hopf 2000). Originally, small numbers of progenitor genotypes were selected by early farmers for traits usually related to overall yield, harvesting, and edibility. Some of those annual crops are self-pollinated such as annual herbaceous crops. The strong human-made selection process, often combined with inbreeding and sometimes selfing (e.g. rice; Kovach et al. 2007), causes genetic bottlenecks of varying degrees that result in reduction of the level of genetic variation (Olsen and Gross 2008),

generating in some cases a “cost of domestication” (Glossary) due to the accumulation of deleterious mutation in the domesticated population (Moyers et al. 2018; Lu et al. 2006). Domesticated *Poaceae* such as maize, wheat, oats, and barley lost one third of the nucleotide diversity relative to their wild progenitors (Buckler et al. 2001). Genome re-sequencing of cultivated and wild rice indicated that the average nucleotide diversity in cultivated rice varieties was 25% lower than in their wild counterparts, suggesting reduction in effective population sizes during domestication bottlenecks (Xu et al. 2011). The reduction of genetic diversity has been even more drastic in wheat which is dominated by intensive selection of a handful of loci (Hao et al. 2017; Liu et al. 2019). In wheat, selective sweeps around the genes controlling flowering time and phenology are evident (Cavanagh et al. 2013). Similarly, intensive selection of reduced height and photoperiod insensitivity genes resulted in semi-dwarf ‘Green Revolution’ cultivars which replaced all old cultivars in south Asia (Borlaug 2007; Cavanagh et al. 2013). In addition to this reduction in genetic diversity, there is an intrinsic genetic homogenization since fields are often planted to a single variety.

Table 1. Passport information on crop and forest pathogen species and effect of host domestication on the population of these pathogens

Species (taxonomy)	Disease	Host(s)	Lifestyle	Origin	Actual distribution	Genomic characteristics of host domestication and management	Impact	Reference(s)
Pathogens of annual plants								
<i>Verticillium longisporum</i> , ascomycete, Sordariomycetes	Verticillium stem striping of winter oilseed rape	Brassicaceous hosts (including winter oilseed rape and cauliflower)	Necrotroph, allodiploid hybrid	nd	Global in winter oilseed rape areas (Europe)	Inter-specific crosses (by hyphal fusion) between <i>Verticillium</i> spp. gave rise to the new species <i>V. longisporum</i> , expanding the host range of these pathogens in the Brassicaceae family	WGS: Depoter et al. 2017; Fogelqvist et al. 2018	
<i>Magnaporthe oryzae</i> (=Pyricularia oryza), ascomycete, Sordariomycetes	Rice and wheat blast	Poaceae	Necrotroph, haploid	South America	Global in wheat and rice growing areas	Emergence of a specialized <i>M. oryzae</i> lineage on rice following domestication. Occasional gene flow between <i>M. oryzae</i> lineages when co-infecting wild grasses and weeds	SNPs-WGS1: Gladieux et al. 2018a,b; Latorre et al. 2020	
<i>B. graminis</i> f. sp. <i>tritice</i> , ascomycete, Leotiomycetes	Grass powdery mildew	Specialized on triticale, and intergeneric hybrid between wheat (<i>Triticum</i>) and rye (<i>Secale</i>)	Obligate biotroph, haploid	nd	Global in triticale (Europe)	Hybridization between <i>B. graminis</i> f. sp. <i>tritici</i> and <i>B. graminis</i> f. sp. <i>secalis</i> resulted in the new subspecies <i>B. graminis</i> f. sp. <i>tritcale</i> specialized on the artificially made polyploid crop triticale	SNPs-WGS: Menardo et al. 2016	

<i>Z. tritici</i> (=Mycosphaerella graminicola) ascomycete, Dothideomycetes	Septoria tritici blotch	Wheat (<i>Triticum</i> sp.)	Hemibiotroph, haploid	Fertile crescent (Middle East)	Global in wheat growing areas	<i>Z. tritici</i> was differentiated from wild grass infecting species <i>Z. pseudotritici</i> and <i>Z. ardabilliae</i> during wheat domestication. Recombination and transposable elements mobility increase nucleotide diversity and structural variation in <i>Z. tritici</i> .	SNPs-WGS: Stukenbrock et al. 2011; 2012b; Oggenfuss et al. 2020; LRA: Plissonneau, et al. 2018; Badet et al. 2020
<i>P. striiformis</i> f. sp. <i>tritici</i> , basidiomycete, Pucciniomycetes	Wheat yellow (stripe) rust	Wheat, cultivated barley and rye (uredinial/telial) Barberry and Oregon grape [pycnial/aecial (alternative hosts)]	Obligate biotroph, dikaryotic	Himalayan region	Global in wheat growing areas	Eradication programs/absence of the alternative hosts triggered the emergence of asexual lineages. Diversity of asexual lineages and host adaptation on wheat promoted by transposable element activity, increased heterozygosity and effectors	Comparison of WGS: of isolates from sexual and asexual lineages: Schwessinger et al. 2020
<i>P. graminis</i> f. sp. <i>tritici</i> , basidiomycete, Pucciniomycetes	Wheat stem/black rust	Wheat (uredinial/telial) Barberry [pycnial/aecial (alternative hosts)]	Obligate biotroph, dikaryotic	Fertile crescent (Middle East)	Global in wheat growing areas	Eradication programs/absence of the alternative hosts triggered the emergence of asexual lineages. The Ug99 lineage that spread clonally through Africa and the Middle East following cultivation of wheat cultivars with a single R-gene emerged following somatic hybridization with another lineage.	Comparison of WGS of isolates from different lineages: Li et al. 2019

Pathogens of perennial fruit trees

<i>Venturia inaequalis</i> , ascomycete, Dothideomycetes	Apple scab	<i>Malus</i> spp.	Hemibiotroph, haploid	Central Asia	Global, followed the dispersal of domesticated apple	Differentiation of an agricultural-type population of <i>V. inaequalis</i> by host-tracking of cultivated apple trees after domestication. Introgression of the wild-type population by the agricultural-type increased virulence towards the wild apple <i>M. sieversii</i>	SNPs-WGS: Fleurtey et al. 2020
<i>Hemileia vastatrix</i> , basidiomycete, Pucciniomycetes	Coffee rust	<i>Coffea</i> spp.	Obligate biotroph, dikaryotic	Yemen	Asia, America and Africa, followed dispersal of <i>C. arabica</i>	Emergence of a domesticated lineage during the domestication process of <i>C. arabica</i> via host shift from parental coffee hosts (<i>C. canephora</i> and <i>C. eugenioides</i>)	SNPs-WGS: Talhinas et al. 2017

Pathogens of perennial forest trees

<i>Cryphonectria parasitica</i> , ascomycete, Sordariomycetes	Chestnut blight	Asian, American and European chestnuts	Necrotroph, haploid	Eastern Asia	America and Europe	Dominance of a single asexual lineage in southern and south-eastern Europe. Limited sexual recombination, transposon activity and epigenetic modifications increase diversity of this lineage	SNPs-WGS: Stauber et al. 2020; Vuković et al. 2019
<i>Ophiostoma novo-ulmi</i> , ascomycete, Sordariomycetes	Dutch elm disease	Elm trees	Saprotoph, haploid	nd	Eastern North America, almost inactive in Europe	Introduction of American lineages to Europe through wood transport facilitated admixture between lineages, impacting adaptation	SNPs-WGS: Hessenauer et al. 2020

<i>Dothistroma septosporum</i>, ascomycete, Dothideomycetes	Dothistroma needle blight	Pines of subgenus <i>Pinus</i>	Hemibiotroph, haploid	North America	North America, Europe, and Europe	The intensive plantation of radiata pine (<i>Pinus radiata</i>) resulted in the introduction and emergence of clonal lineages of <i>D. septosporum</i> in the Southern Hemisphere; aneuploidy and increase in the copy number of virulence factor	SNPs-WGS: Bradshaw et al. 2019
<i>Sphaerulina musiva</i> (=Mycosphaerela populinum), ascomycete, Dothideomycetes	Septoria leaf spot and canker	Native on <i>P. deltoides</i> . Epidemic on hybrid poplars of the <i>Populus</i> and <i>Populorum</i> , Tacamahaca botanical sections	Hemibiotroph, haploid	North America	North America; introduced in South America	Intensification of poplar cultivation in North America resulted in the change of an innocuous foliar pathogen into a destructive and epidemic canker pathogen. Acquisition of a secondary metabolite gene cluster by horizontal transfer is responsible for the adaptation of <i>S. musiva</i> to colonize wood and cause a lethal canker on poplars. Hybrid poplar cultivation promoted the introduction of the pathogen in new areas	WGS: Dhillon et al. 2015; SNPs-WGS: Tabima et al. 2020
<i>Armillaria ostoyae</i> basidiomycete, Agaricomycetes	Armillaria root disease	Woody perennials	Saprotroph, facultative necrotroph, diploid	nd	North Hemisphere	Intensive plantation of <i>Pinus pinaster</i> in southwest France accelerated the spread and success of <i>A. ostoyae</i> in this area	SNPs: Labbé et al. 2017
<i>Melampsora larici-populina</i>, basidiomycete, Pucciniomycetes	European poplar leaf rust	Native on <i>P. nigra</i> ; Infects hybrid poplars of the <i>Populus</i> and Tacamahaca botanical sections	Obligate biotroph, dikaryotic	Europe	Europe, introduced in eastern North America, Australia,	Intensive plantation of hybrid poplars carrying an R-gene triggered the emergence of a new genetic group that	SNPs: Persoons et al. 2017

				South Africa, New Zealand	replaced the initial rust population in the North of France	
<i>Phytophthora</i>	Sudden oak <i>ramorum</i> , oomycete,	>150 woody perennials, including forest trees and sudden sudden	Hemibiotroph, diploid	Introduced in North America and Europe	Plant trade promoted spread of the pathogen; asexual lineages rapidly evolve by using non-meiotic mechanisms such as mitotic recombination (associated with transposon activity)	SNPs-WGS: Dale et al. 2019; Yuzon et al. 2020
Peronosporales	larch death and ramorum blight					

1.4.1.2 Comparative features of wild vs domesticated long lived perennials

Natural forests have built-in diversity with different tree and plant species and different age classes. Because of their long-lived nature, forest trees maintain genetic variation for decades which slows the erosion of diversity by drift. Trees often carry a heavy genetic load of deleterious recessive alleles (Boshier and Billingham 2000) despite rather large effective population sizes covering extensive geographic areas and largely outcrossing mating systems. In most plant species, there is a positive relationship between population size, genetic diversity, and fitness (Leimu et al. 2006). Taken together, these life-history traits promote the maintenance of abundant levels of genetic variation in most forest tree populations compared to annual plants (Hamrick and Godt 1996), which in theory should help adaptation to changing environmental conditions but also pathogen resistance.

Tree life-history traits and biological features, multiple geographical origins, and ongoing crop-wild gene flow, contribute to mild domestication bottlenecks. For example, perennial fruit crops such as apple and olive trees have on average retained 90% to 95% of the neutral genetic variation found in wild populations (Fig. 1A), even for those supposedly under domestication since a long time (e.g. 10,000 to 5,000 years ago for the European hazelnut, *Corylus avellana*; Helmstetter et al. 2020). For some recently domesticated perennial fruit crops, this slight reduction in genetic diversity was more likely due to selective propagation in a cultivated setting, rather than to many generations of selective breeding (Miller and Gross 2011). Impact of domestication on forest trees is usually mild, too. Only a slight decrease in genetic diversity was observed following the first steps of the domestication process (i.e. phenotypic selection and tree improvement cycles) of western hemlock (Wellman et al. 2003), interior hybrid spruce (Stoehr and El-Kassaby 1997), and Douglas fir (El-Kassaby and Ritland 1996) in British Columbia, Canada. Likewise, no difference in gene diversity was found between natural and managed populations of *Picea glauca* and *Pinus banksiana* (Godt et al. 2001). Lessons have been learned from the domestication experiences with annual crops and subsequent loss in genetic variation, and most forest tree breeding programs have ensured that selected populations were large enough to capture a maximum of genetic diversity and allelic richness (Harfouche et al. 2012; Ingvarsson and Dahlberg 2019).

In forestry, genetic and genomic techniques combined with conventional breeding is accelerating tree domestication (Boerjan 2005; Campbell et al. 2003; Sederoff et al. 2009). It is

still unclear which genetic and genomic changes might become associated with tree domestication (Boerjan 2005; Sederoff et al. 2009). Forest tree species are in various stages of domestication depending on the degree of selection exerted (Boerjan 2005; Neale 2007). For example, cottonwoods (poplars of the botanical section *Populus*) are among the most advanced most domesticated forest trees. They have emerged as extremely versatile trees with natural attributes favorable to their domestication: ease of vegetative propagation, rapid growth, relatively short juvenile phase, and interspecific compatibility that allows breeding hybrids with broad adaptability, improved growth, and acceptable levels of disease resistance. This has contributed to the recognition of poplars as a model for tree domestication (Boerjan 2005; Bradshaw et al. 2000; Taylor 2002). In contrast, most deciduous and conifer tree species are long-lived perennials with lengthy juvenile phases, high levels of genetic diversity, extensive outcrossing, widespread natural hybridization, and weak population structure. Despite limited among-population structure, natural tree populations are usually locally adapted.

1.4.1.3 The domestication gradient of annuals and long lived perennials

“Plant domestication” (Glossary) can be seen as a gradient correlated with different features such as the time (reported as a number of years the breeding process started or a number of breeding generations), the level of genetic isolation to the wild population of origin or the proportion of natural diversity retained in the end of the domestication process. Due to differences in the time the domestication process started and in their life traits and biological features, annual plants and long-lived perennials are positioned differently along this gradient (Fig. 1B). At one end of the gradient are annual plants that have received intensive human-selection pressures starting thousand of years ago while at the other end of the spectrum are the wild counterparts of these crops, conifers, and deciduous forest trees that remain “untouched” (with the exception of management with the plantation of particular genotypes or provenances of interest; see ‘Management of crops and forests’ section). Between these two extreme points, long lived perennials are scattered. Although perennial fruit crops don’t have the biological features of annual plants to facilitate the domestication process (see above) they have received much more attention than forest trees in the history of plant domestication. Therefore they can be placed at an intermediate level on the domestication gradient. Then, forest tree species tend to be rather close to the second end of the gradient, in various stages of domestication depending on the degree of selection exertable and exerted (Boerjan 2005; Neale 2007). For example,

cottonwoods (poplars of the botanical section *Populus*) are among the most advanced species in the domestication process of forest trees. They have emerged as extremely versatile trees with natural attributes favorable to their domestication: ease of vegetative propagation, rapid growth, relatively short juvenile phase, and interspecific compatibility that allows breeding hybrids with broad adaptability, improved growth, and acceptable levels of disease resistance. This has contributed to the recognition of poplars as a model for tree domestication (Boerjan 2005; Bradshaw et al. 2000; Taylor 2002). Eucalyptus trees have similar features, making them of particular interest for breeding and domestication, too. In contrast, most conifer and deciduous tree species are long-lived perennials with lengthy juvenile phases, high levels of genetic diversity, extensive outcrossing, widespread natural hybridization, and weak population structure, which are hardly compatible with the requirement of the domestication process.

1.4.2 Management of crops and forests

In addition to the reduction in genetic diversity associated with domestication, there has been a drastic reduction in plant diversity as only a handful of cultivated crop species dominate the arable land globally; for example, maize, wheat, soybean, and cotton are grown over two third of the total arable land in the United States (Margosian et al. 2009). This combination of low genetic and species diversity has invariably been accompanied by increases in pest and disease problems. Hence, human management has become an essential part of production of crops and trees in monoculture conditions (Fuller et al. 2010). In these conditions pathogens are mostly controlled by crop and land management and genetic selection for certain disease resistance traits. Similarly, as a part of management, pesticides and fungicides have increased crop productivity and are required for maintaining the high quality of the produce; they have become an integral part of modern agricultural ecosystems (Ishii 2015). These intensive management approaches are not sustainable in the long-term, in part because of the intense selection pressure on the pathogen population to become resistant to fungicides or evolve to overcome resistance (Storkey et al. 2019).

Most modern crop cultivars carry one or few major resistance genes for each of the locally important diseases. Relying on single genes for resistance exposes the crop to a major risk as pathogens can easily evolve to overcome a single resistance gene (Brar et al. 2019). Reduction in the selection pressure can be achieved by breeders sequential release of resistance genes or gene pyramiding which is the combination of multiple resistance genes in the same crop cultivars.

Overall crop management practices combined with human selection has led to a dramatic reduction in crop diversity at multiple levels.

By contrast, forests are managed quite differently from most crops and vary dramatically with the type of forest. Overall, use of pesticides is rare and usually only applied to combat invasive species. Human intervention in forest management usually involves silvicultural approaches such as species and provenance selection for planting, spacing, thinning or pruning. These management approaches can be conducive to disease outbreaks. For example, root pathogens such as *Heterobasidion spp.* and *Armillaria spp.* take advantage of the logging or thinning practices to invade the root systems and spread from tree to tree. Genetic resistance is often present in natural populations, but its exploitation, via breeding, is rare because of the long breeding cycle unlike agricultural crops (Goheen and Goheen 1990). Even breeding horticultural woody perennial crops is faster and easier than breeding forest trees. Some of the best-documented cases of resistance breeding in trees are against rusts [poplar rust (caused by *Melampsora spp.*), fusiform rust (caused by *Cronartium quercuum f. sp. fusiforme*), white pine blister rust (caused by *Cronartium ribicola*); Jorge et al. 2005; Snieszko et al. 2014]. Deployment of resistance using a single gene approach similar to crop breeding has also resulted in selection of pathogen populations capable of overcoming resistance.

There are many different ways in which associated activities throughout tree management practices may influence genetic diversity of trees [reviewed in Ratnam et al. 2014]. Stand- or landscape-genetic variation can be altered by planting seedlings, thinning, selective harvesting, or through deployment of material from tree improvement programs. Forest fragmentation, which inevitably follows clearcut harvesting, is expected to have an impact on genetic diversity and population structure of trees by affecting a magnitude of evolutionary processes, such as genetic drift, gene flow, and selection. However, studies investigating the effect of harvesting and regeneration on genetic diversity reported mixed results (Ratnam et al. 2014). The extent of genetic erosion depends on the management system applied, stand structure, as well as species' distribution and demography, biological attributes and ecology.

Forest plantations have been seen as a way to reduce pressure on natural forests while answering the increasing demand for wood products and providing multiple ecosystem services (Paquette and Messier 2010). While the implementation of industrial large-scale and

monocultures of fast growing and exotic species has been the gold standard, there is a growing interest in accelerating development of mixed-species plantations and agroforestry systems (Liu et al. 2018; Payn et al. 2015). Agroforestry is the integration of some tree species with crop plants on the same piece of land. Agroforestry then bridges the gap between crop ecosystems and forest ecosystems and increases resilience of crop plant species to environmental changes by increasing ecological services (Dainese et al. 2019; Hajjar et al. 2008).

1.5 Plants versus pathogens: the consequences of crop domestication and management

In natural ecosystems, the antagonistic interaction between host plants and their pathogens drives a co-evolutionary arms race of reciprocal adaptations. This co-evolutionary process shapes genetic variation in both plants and pathogens (Brown and Tellier 2011), sometimes resulting in mirrored genetic structures (Barrett et al. 2008; Feurtey et al. 2016; Hartmann et al. 2020). One of the main consequences of domestication is the “artificial” evolution (through breeding) of the plant, and the absence of “natural” response to pathogen pressure. In turn, plant domestication and management have increased the selection pressure on pathogens. Domestication, combined with globalization, facilitates the rapid dispersal of pathogens and provides new ecological niches leading to the emergence of a succession of specialized pathogens. This process is a major shift in the co-evolutionary dynamics found in natural ecosystems between hosts and pathogens, resulting in significant changes in the structure of pathogen populations and in some instances in the genome architecture (e.g. Giraud et al. 2010; Lebarbenchon et al. 2008; Möller and Stukenbrock 2017; Stukenbrock and McDonald 2008).

An implicit hypothesis is that the accelerated genetic selection in crops results in strong directional selection in the pathogen population leading to the rapid emergence of specialized populations that can exploit the niche more effectively. The hypothesis, originally proposed for human pathogens (Pearce-Duvet 2006), has now been widely tested for crop pathogens. In the last 20 years, the comparison of several natural and agricultural systems on the basis of host specificities, phylogenetics, and estimates of divergence time supported the hypothesis that the divergence of new crop pathogens and specialized lineages from their wild ancestors was coincident with the development of domesticated crops (Couch et al. 2005; Stukenbrock and

McDonald 2008; Stukenbrock et al. 2007; Zaffarano et al. 2008; however, see also Munkacsi et al. 2007).

There is little evidence that pathogens of domesticated crops often exhibit rates of adaptive substitutions different from their close wild relatives or populations on wild host plants [e.g. *Zymoseptoria tritici* (syn. *Mycosphaerella graminicola*) (Brunner et al. 2009; Stukenbrock et al. 2011; Grandaubert et al. 2019)]. Yet, specific adaptation signatures vary and depend on several factors, including level of specialization and pathogen genetics and biology. These signatures are often linked to intrinsic genomic, reproduction, and infection features present before the domestication event. Co-evolutionary host-tracking, in which the pathogens follow their host along the domestication course, and the emergence of new pathogen lineages, sometimes clonal, through host jump, hybridization and positive directional selection constitute the main mechanisms underlying the rapid emergence of specialized populations. However, the combination of some of these features may have a reverse effect. Small effective population sizes, strong directional selection on few candidate genes and absence of sexual recombination in pathogen populations specializing on domesticated plants may induce a genetic load (i.e. a cost of pestification by analogy to the cost of domestication; see Box 1) resulting from the accumulation of deleterious mutations (e.g. Gladieux et al. 2018a and Fleurtey et al. 2020a; see Stukenbrock et al. 2011 for a counter-example in a sexual lineage).

1.5.1 Where the grass is greener: emergence of new species following host jump, selection and specialization

The emergence of plant pathogens in agro-ecosystems has been extensively described using genetic markers (see Stukenbrock and McDonald 2008 for a review). Genome sequencing and population genomics are now shedding new lights on this process. Evolutionary processes related to the emergence of novel pathogen species via host-range expansion and host jumps are becoming easier to track by analyzing whole genomes. Such events have shaped the diversification of powdery mildew pathogens. Although there is only one species of powdery mildew on grass, *Blumeria graminis*, different formae speciales are defined by the host they infect. Genome sequencing of *Blumeria graminis f. sp. hordei* (Spanu et al. 2010) revealed that *B. graminis f. sp. hordei* and *B. graminis f. sp. tritici* diverged about 6 million years ago (MYA) from *B. graminis f. sp. tritici*, after the divergence of their hosts (barley and wheat, respectively) (Middleton et al. 2014; Wicker et al. 2013). The *formae speciales secalis* and *tritici* diverged more recently between 0.25

and 0.16MYA, while the divergence between their hosts (rye and wheat, respectively) was estimated at 4MYA. Host jump events and recent horizontal gene flow were also identified as processes that shaped current diverged lineages (Menardo et al. 2017).

Evolutionary processes related to domestication of the host can be tracked in a similar way. The evolution of the wheat leaf blotch pathogen *Zymoseptoria tritici* follows a pattern of host-tracking in response to wheat domestication. The pathogen invaded the wheat-growing regions of the world together with its host *T. aestivum* and *T. durum* (Stukenbrock et al. 2007; Stukenbrock et al. 2012a). *Zymoseptoria tritici* differentiated from its sister species *Z. pseudotriticis* (S1) and *Z. ardabiliae* (S2) in the fertile crescent likely during wheat domestication and is currently a major invasive pathogen of wheat globally (Stukenbrock et al. 2011; Stukenbrock and Dutheil 2018). Genome sequencing and comparison revealed collinearity and high conservation of genomic, transcriptomic and epigenomic signatures of genome architecture (Feurtey et al. 2020). High gene content variability both within and between species, mainly limited to the accessory chromosomes and compartments indicates compartmentalization that allows purifying selection to retain a functional core genome and relaxed selection on the accessory genome.

1.5.2 Fast and furious: shifting gears and speeding up evolution

Some pathogens of domesticated crops have undergone a shift to speed up evolution. The increased evolutionary capacity of *Z. tritici* compared to its sister species is driven by recombination, and transposable elements (TE) variation, high nucleotide diversity, extensive structural variation (Badet et al. 2020; Oggendorf et al. 2020; Plissonneau et al. 2018). In comparison, *Z. pseudotriticis*, which infects naturally-occurring grass species and is mostly found in Iran (Stukenbrock et al. 2012a,b), shows significantly less variation along its genome with only a limited number of haplotype groups. Alternatively, the difference in population effective size with *Z. pseudotriticis* could explain the higher evolutionary potential for *Z. tritici*.

The high level of diversity in *Z. tritici* is driven by intrinsic factors such as high recombination rates and loss of part of its DNA methylation machinery which leads to relaxation of TE expression and hence likely enhanced TE mobility that might lead to higher mutation rates due to transpositions (Grandaubert et al. 2019; Möller et al. 2020). Differentiation of *Z. tritici* likely is driven by positive selection on secreted proteins and changes in mating type genes. Indeed, effector candidates display twice the rate of adaptive substitution when compared to

other genes and *Z. tritici* effectors have high levels of presence-absence polymorphism. Phenotypic characterization of some of the effector candidates with the largest signal for positive selection confirmed that these genes contribute to wheat infection by *Z. tritici*. The elevated rates of positive selection in *Z. tritici* at the genome level can be interpreted as increased adaptive evolution (Poppe et al. 2015). It will be important to discover the biochemical mechanisms that enable this increased adaptive evolution in *Z. tritici* for which loss of de novo DNA methylation might represent one example. Adaptive substitutions in DNA repair machinery genes are another set of prime candidates causing these phenotypes (Hartmann et al. 2020).

1.5.3 Evolutionary fast-track and host-range expansion

Interspecific hybridization plays a major role in natural ecosystems and can provide an evolutionary fast-track in filamentous pathogens (Brasier 2001; Milgroom et al. 2014; Park and Wellings 2012; Stukenbrock 2016). From a theoretical point of view, allopatric species are more likely to have a permeable reproductive barrier, thus allowing the emergence of hybrid individuals (Giraud et al. 2008; Le Gac and Giraud 2008). Pure F1 hybrids are rarely found in the wild because they often exhibit intermediate phenotypes that have a reduced fitness compared to their parents, or they can suffer from incompatibilities between parental alleles and repeated backcrossing with the parental species can lead to the transfer of adaptive traits between species and thereby be a mechanism that speeds up adaptive evolution (Depotter et al. 2016; Feurtey and Stukenbrock 2018; Gladieux et al. 2011; Stukenbrock 2016).

Hybridization played a role in the emergence of *B. graminis f. sp. tritice* on triticale (\times *Triticosecale*), an artificial human-made hexaploid crop species from a cross between wheat (*Triticum*) and rye (*Secale*) (Menardo et al. 2016). Triticale was resistant to *B. graminis* when first introduced commercially in the 1960s, until it was attacked by *B. graminis f. sp. tritice* in 2001. The whole-genome sequencing of isolates of *B. graminis* from four *formae speciales* revealed that *B. graminis f. sp. tritice* is a hybrid between *B. graminis f. sp. tritici* and *B. graminis f. sp. secalis* (Menardo et al. 2017) that is estimated to have originated from at least two independent hybridization events after the introduction of triticale as a commercial crop in the 1960s (Menardo et al. 2016). A portion of the *Z. tritici* genome has also been recently introgressed from its sister species and interspecies hybridization appears to be common between *Zymoseptoria spp.* (Feurtey et al. 2019, 2020b).

Interspecific hybridization can also expand host range (Vlaardingerbroek et al. 2016). The wilt disease of crucifer crops caused by *Verticillium longisporum*, regroups taxa originating from at least three hybridization events between *V. dahliae* and un-described *Verticillium* parental species (Depotter et al. 2017; Inderbitzin et al. 2011). The parental species *V. dahliae* is the causal agent of a vascular wilt disease on more than 200 host species, including many economically important crops. This fungus is a notorious example of an asexual plant pathogen with a high genome plasticity mediated by mechanisms different from meiotic recombination, such as horizontal gene transfer (Shi-Khunne et al. 2019), transposon activity and large-scale genomic rearrangements (de Jonge et al. 2013; Faino et al. 2016). In contrast to *V. dahliae* and other *Verticillium* species, *V. longisporum* is not a haploid organism but rather an allotetraploid hybrid (Depotter et al. 2017; Inderbitzin et al. 2011). Fixation of the MAT1-1 idiomorph in *V. longisporum* suggested that sexual recombination without separation of homologous chromosomes was not the mechanism at the origin of this new species; instead, hyphal fusion followed by karyogamy was a more likely scenario to explain the creation of a stable, diploid nucleus (Inderbitzin et al. 2011). Allotetraploid hybridization in *V. longisporum* expanded the host range of the pathogen in the *Brassicaceae* family, compared to the parental species *V. dahliae* that does not colonize these plants (Novakazi et al. 2015). In addition, the three separate hybridization events have affected the pathogenicity of the different *V. longisporum* clonal lineages. The most widespread lineage and main causal agent of *Verticillium* stem striping on oilseed rape, A1/D1 is often found on multiple brassicaceous hosts, whereas lineage A1/D2 is specialized to horseradish (Depotter et al. 2017; Inderbitzin et al. 2011). Analysis of the genome structure and content of *V. longisporum* supported the allotetraploid hybrid origin of this pathogen and identified a higher gene content than in *V. dahliae*, including many secreted proteins and carbohydrate active enzyme (CAZy) encoding genes of the glycoside hydrolase group (Fogelqvist et al. 2018). In a context of accentuated global exchange and climate change, as more species distribution overlap, such events of interspecific hybridization leading to an increased pathogenicity could become a major challenge in crops disease management.

1.5.4 The battle of the clones

Some crop pathogens appear to consist almost entirely of globally distributed asexual lineages. Monocultures, i.e. cultivation of genetically homogeneous crop variety, has added an additional layer of selection pressure favoring the spread of lineages with high fitness that can

cause devastating epidemics. The most notorious example are the wheat rusts, with their ability to track their host by evolving rapidly new lineages. These rusts are heteroecious, requiring infection of an alternate ‘sexual’ host to complete their life-cycle; sexual recombination can lead to high diversity in the wheat yellow (stripe) rust pathogen *P. striiformis* f. sp. *tritici* in the Himalayan region, where the alternate host occurs, and provides the advantage of a quick turnaround of races (Ali et al. 2014; Hovmöller et al. 2016). However, where the alternate host is absent or scarce due to its natural distribution or eradication programs, the fungus is restricted to asexual propagation with lineage diversification usually arising by mutation.

Global populations of *P. triticiina* and *P. striiformis* f. sp. *tritici* are composed of clonal lineages that maintain high levels of heterozygosity, due to their dikaryotic nature (Ali et al. 2014; Brar et al. 2018; Fellers et al. 2020; Hovmöller et al. 2016; Kolmer et al. 2019; Lei et al. 2017). Population shifts can be attributed to mutations and selection within clonal lineages, and/or to the emergence of new exotic lineages (Hubbard et al. 2015). Some clonal lineages have different adaptive traits such as warm-temperature growth or the production of telia (Brar et al. 2018a). An emergent population of *P. striiformis* f. sp. *tritici* virulent on previously resistant wheat varieties suggests a rapid lineage turnover following host genotype replacement facilitated by long distance migration (Hubbard et al. 2015).

Even in the absence of sexual reproduction, there are mechanisms that ensure diversity in asexual lineages. Long-term asexual evolution appears to drive genome expansion via TE activity and leads to increased heterozygosity. The genomes of recent sexual isolates of *P. striiformis* f. sp. *tritici* are smaller and less heterozygous compared to long-term asexual lineages (Schwessinger et al. 2020). Presence/absence polymorphism in *P. striiformis* f. sp. *tritici* gene content is not driven by effector candidates in general. Instead, other genes of unknown functions appear to be specific to sexual isolates and these genes might be linked to infection of barberry during the sexual cycle as it has been shown that long-term asexual lineages are compromised in entering the sexual cycle with potential specific selection against it (Schwessinger et al. 2020).

In asexual lineages, the exchange of genetic material via hyphal fusion constitutes an adaptive mechanism that can rapidly generate new traits (Furtey and Stukenbrock 2018). Somatic genetic exchange between individuals co-infecting the same host has been experimentally demonstrated (Lei et al. 2017; Park and Wellings 2012). The most important

example of somatic hybridization was provided by the Ug99 strain of *P. graminis f. sp. tritici* that emerged following cultivation of wheat cultivars carrying resistance gene Sr31 (Basnet et al. 2015). The haplotype-phased genomes of races Ug99 and Pgt21 collected in Uganda and Australia share one haploid nucleus with no trace of recombination or chromosome reassortment, a clear indication of somatic hybridization (Li et al. 2019). Ug99 has spread clonally through Africa and the Middle East causing devastating epidemics and is a significant threat to global wheat production (Basnet et al. 2015).

1.5.5 Contacts between pathogens species from wild and domesticated crop plants

Genetic exchanges between domesticated and wild species can impact disease outbreaks or result in the emergence of new pathogens (e.g. Gladieux et al. 2018a; Stuckenbrock et al. 2012a). In agro-ecosystems, wild crop relatives or related wild/cultivated species can serve as sources of new pathogens. The apple tree pathogen *Venturia inaequalis* from the agricultural population evolved a higher virulence on both wild and domesticated trees. Secondary contact between this pathogen and wild apple trees would result in an invasion of this pathogen and potential introgression in the wild type pathogen (Feurtey et al. 2020a).

One of the recent examples of host jump is of *Magnaporthe oryzae* (*Pyricularia oryzae*), the wheat blast pathogen that was reported in South America (Cruz and Valent 2017) and then in Bangladesh, causing substantial losses (Islam et al. 2016). Phylogenomic analyses revealed that this outbreak was most likely caused by a wheat-infecting strain from South America (Islam et al. 2016). The wheat-infecting lineage of *M. oryzae* is believed to have emerged from '*Lolium*' and '*Arena*' pathotypes (Couch et al. 2005; Farman et al. 2017; Inoue et al. 2017) and coexists with multiple host-specialized and genetically divergent lineages that infect other cereals and grasses (Chiapello et al. 2015; Islam et al. 2016; Yoshida et al. 2016). The rice infecting lineage of *M. oryzae* is highly differentiated, a likely consequence of the host jump from *Setaria* 2,500 to 7,500 years ago after rice domestication (Gladieux et al. 2018a; Latorre et al. 2020). Host selection could have been important in the emergence of the wheat blast pathogen. Two avirulence genes of *M. oryzae*, PWT3 and PWT4, were implicated in host specificity and avirulence on wheat cultivars carrying Rwt3 and Rwt4 genes (Inoue et al. 2017). Widespread cultivation of Rwt3 wheat cultivars in Brazil could have allowed the *Lolium* pathotypes of *M. oryzae* containing PWT3 to establish in that country (Cruz and Valent 2017). However, recent population genomic

analyses could not determine whether the wheat blast pathogen in Brazil had single or multiple origins (Ceresini et al. 2018). Genome-wide analysis of an extensive collection of the blast fungus, confirmed that *M. oryzae* consists of an assemblage of differentiated lineages associated with particular host genera (Gladieux et al. 2018b). This pattern of genetic isolation could be neutralized by the capacity of distinct lineages to colonize common cereals and wild grasses and weeds, increasing the chance for occasional gene flow between isolates from different lineages with overlapping host ranges (Gladieux et al. 2018b).

1.6 Not so slow, after all: longer generation time in trees doesn't rhyme with slower forest pathogen evolution

Trees have a longer reproductive cycle, are longer-lived and are less advanced on the domestication gradient than crop pathogens. Intuitively, we could expect the selective pressure imposed by tree domestication to be milder compared to crops with longer histories of domestication, with resulting differences in pathogen population structure and genome architecture. The consequence of tree domestication on populations of forest pathogens is much less documented largely due to the fact that most of the forest tree species are still in domestication infancy. Some woody trees such as coffee and apple have wild relatives but have been domesticated for thousands of years. Others, such as conifers from the northern hemisphere, are essentially undomesticated. Yet, similar evolutionary mechanisms appear to shape populations of tree and crop pathogens. The expansion of clonal lineages and the emergence of novel lineages following hybridization and host jump are also clearly important in the evolution of forest pathogens.

1.6.1 The return of the clones: when forest trees are threatened too

Similarly to crop pathogens, perennial trees can be threatened by clonal populations and lineages of pathogens. Trade of germplasm, genetic material and plant and forest products has resulted in some of the most dramatic introduction of exotic pathogens in naive environments. The introductions of the chestnut blight in North America and Europe and the white pine blister rust in North America are among the most dramatic examples of entire ecosystem and landscape level changes induced by invasive pathogens. Despite the effect of genetic drift and inbreeding caused by severe genetic bottleneck and clonality, invasive pathogens are still successful at occupying their new environment. This intriguing aspect often described as “the genetic paradox

of invasiveness” has been discussed in multiple publications (Allendorf and Lundquist 2003; Gladieux et al. 2015; Sax and Brown 2000; Tsutsui et al. 2000). The phenotypic plasticity and the adaptability required for these clonal populations to be competitive and succeed on a new host and a new environment can be explained by mechanisms similar to those described in the previous section for crop pathogens (The battle of the clones).

The causal agent of chestnut blight, *Cryphonectria parasitica*, is native to eastern Asia and was introduced in North America where it devastated the native tree species *Castanea dentata* and later in Europe where it attacks the European sweet chestnut *C. sativa* (Dutech et al. 2012). In southern and southeastern Europe, the invasive population of this fungal pathogen is dominated by one single asexual lineage (identified as S12) consisting nearly exclusively of a single mating type. Genome sequence analyses showed that this lineage was already preadapted to success across the European niches as it likely arose from a bridgehead (sensu Bertelsmeier and Keller 2018; Lombaert et al. 2010) pool of genotypes that were previously established in Europe through multiple introductions from North America (Stauber et al. 2020). Additional evolutionary forces such as cryptic sex purging deleterious mutations, transposon activity (Stauber et al. 2020) and epigenetic modifications (Vuković et al. 2019) keep diversifying this clonal lineage, underpinning the hypothesis of adaptation to his niche and rapid success of its expansion on sweet chestnut in Europe.

The oomycete *Phytophthora ramorum*, responsible for sudden oak death in the United States, sudden larch death in Europe and ramorum blight of trees and ornamental shrubs in North America and Europe, is probably one of the most successful invasive forest pathogens, with the ability to infect more than 150 different woody perennial host species (Grünwald et al. 2019). To date, four reproductively isolated lineages, presumably asexual and comprised of one single mating type are spreading in Europe and North America. Despite their supposed clonality the four lineages exhibit substantial amounts of intralineage phenotypic and genotypic variation. At a small geographical scale (e.g. in the western US), local environmental conditions and the presence of sporulating hosts seem to be the driver of outbreak development and expansion of new clonal genotypes (Peterson et al. 2015; Yuzon et al. 2020); a similar scenario has been proposed to explain the local dispersal of clonal lineages of the needle pathogen of Douglas fir *Phytophthora pluvialis* in the western US and New Zealand (Brar et al. 2018b; Tabima et al. 2021). Non-sexual mechanisms of genotypic diversification can explain the intralineage variability and

the resulting potential of generating new genotypes adapted to local conditions, even in the absence of sexual reproduction. Structural variation such as aneuploidy and copy number variation were observed within each *P. ramorum* lineage (Dale et al., 2019; Kasuga et al. 2016). In particular, by sequencing multiple genomes of each *P. ramorum* lineage, Dale et al. (2019) showed that mitotic recombination likely associated with transposon activity generated runs of homozygosity (ROH) affecting hundreds of genes simultaneously and generating nonsynonymous changes in about 17.0% of the *P. ramorum* proteome. Although some slight differences in fitness were observed between the *P. ramorum* isolates with and without ROH, additional testing of different hosts under different conditions is required to obtain a comprehensive picture of the effect of these changes on the pathogen phenotype (Dale et al. 2019; Grünwald et al. 2019). Similarly, a high ROH incidence was observed in the triploid hybrid *P. xalni*, the causal agent of alder decline in Europe. High ROH incidence in this triploid hybrid has been interpreted as a mechanism promoting genome stabilization (by reducing) and clonal diversification and response to stress such as winter temperature and soil acidity (Mizeriene et al. 2020).

1.6.2 The rise of the hybrids

Interspecific hybridization and host jumps have been widely described in forest pathogens and is thought to provide a fast-track to evolution (Brasier 2000). In Dutch elm disease pathogens, interspecific hybridization with *Ophiostoma ulmi* has allowed the acquisition of a missing mating type in *Ophiostoma novo-ulmi*, the more virulent species (Paoletti, Buck, and Brasier 2006). The introgression of this gene in *O. novo-ulmi* facilitated adaptation of this invasive species by enabling sexual reproduction in the population, thus allowing recombination and genetic shuffling. In addition, adaptive genes involved in growth at high temperature and in virulence were introgressed from *O. ulmi* to *O. novo-ulmi* (Hessenauer et al. 2020). In the basidiomycete species complex *Heterobasidion annosum*, the introduction of the North American (NA) taxon in Italy was linked to US military activity during World War II (Gonthier et al. 2004). This exotic taxon hybridized with the local infertile European (EU) taxon, impacting the evolutionary trajectory of this organism in Europe (Gonthier et al. 2007). It resulted in massive introgression from the native to the introduced species, representing 5 to 45% of the genome content in admixed individuals (Gonthier and Garbelotto 2011). While these introgressed regions seem to

occur randomly and not driven by selection, such an increase in genetic diversity could be a key factor for evolving adaptive alleles (Gonthier and Garbelotto 2011).

The coffee tree, *C. arabica*, is a domesticated tree species that resulted from the hybridization of the diploid species *C. canephora* and *C. eugenioides* about 10,000 years ago (Cenci et al. 2012). It accounts for about 60% of the world's coffee production. Domestication of arabica coffee in Yemen, followed by intensive cultivation and spread to Asia, America, and Africa resulted in several severe genetic bottleneck narrowing drastically the genetic diversity of this tree crop. It is highly susceptible to the coffee leaf rust caused by the obligate biotrophic fungus *Hemileia vastatrix* (Talhinhas et al. 2017). The global coffee rust population comprises three distinct lineages, including the ‘domesticated’ *H. vastatrix* lineage (“C3” group) that emerged and specialized on tetraploid hosts during *C. arabica* domestication process via an host jump from diploid coffee hosts (Silva et al. 2018). Episodes of introgression between this lineage and those specialized on diploid hosts raise the possibility that virulence factors may be quickly exchanged between groups and increase the risk of emergence of new hypervirulent strains.

1.6.3 Rapid tracking of slow moving trees

There is a seemingly unbalanced advantage in the evolutionary arms-race of trees and their pathogens. Trees have long generation times and are expected to adapt slowly to biotic and abiotic changes. By comparison, their pathogens have a shorter generation time, providing a systematic advantage in the evolutionary race with their host. This has been particularly well documented for the domestication of the cultivated apple, *Malus domestica* in central Asia 4,500 years ago from the wild apple *M. sieversii* and the apple scab pathogen, *Venturia inaequalis* (Gladieux et al. 2010). *Venturia inaequalis* is subdivided in a wild population representing a relic of the ancestral population and a current derived agricultural-type population that was disseminated westward by host-tracking of cultivated apple trees and introgressed the wild-type populations after having diverged in strict isolation during apple tree domestication (Feurtey et al. 2020a). This secondary contact caused by the introduction of domesticated apple trees in Central Asia about 100 years ago has led to the recent invasion of the agricultural-type population in the wild *M. sieversii* forest and to the emergence of hybrids. The agricultural *V. inaequalis* population and the hybrids display greater virulence on wild apple trees than its own endemic wild-type population, facilitating invasion of *M. sieversii* trees.

Increased virulence on the wild host was likely inherited during tracking of apple tree domestication. The agricultural *V. inaequalis* population has probably accumulated multiple alleles such as a truncated small secreted protein recognized by domesticated apple trees selected for resistance to apple scab (Cornille et al. 2012). This pattern is consistent with the concept known as “pestification” (Feurtey et al. 2020a; Saleh et al. 2014), under which selection by humans of more resistant plants unwittingly leads pathogens to accumulate virulence traits and to cause more severe symptoms. In spite of the long reproductive cycle, the domestication and deployment of homogeneously resistant cultivars is imposing selection pressures that are comparable to those described in annual crops.

Eucalyptus, poplars, and pines are among the most advanced trees on the domestication gradient in woody perennials. Yet, these trees grown for fiber and wood have been domesticated for less than a century, compared to thousands of years for most crops. However, the global deployment of fast-growing clones and interspecific hybrids carrying resistance genes in quasi-monoclonal stands over several decades has imposed a strong selection pressure on pathogen populations. One of the best examples is the European poplar leaf rust pathogen, *M. larici-populina*, that undergoes demographic fluctuations consistent with a history of rapid adaptation of the pathogen in response to the deployment of resistance genes in the cultivated poplars. Rapid fixation of the *M. larici-populina* virulence factor corresponding to a widely deployed resistance gene (R7) in poplar cultivars has resulted in the emergence of a unique, homogeneous and virulent rust genetic group that replaced the initial population in the North of France where the sexual host is absent (Persoons et al. 2017). By contrast, the more diverse *M. larici-populina* population in Southern France, where the sexual host, larch, is present, was at equilibrium on the wild poplar *Populus nigra* (Xhaard et al. 2011; 2012).

Perhaps the proximity of natural populations of both pathogens and hosts in a forestry context can serve as a buffer to restore diversity. The bottleneck signature in the *M. larici-populina* populations on cultivated resistant poplars was erased rapidly and genetic diversity was gradually increased over time. This contrasts with the usual pattern of host tracking associated with a reduction in diversity away from the centre of domestication of the host plant (e.g. wheat yellow rust and apple scab; Ali et al. 2014; Gladieux et al. 2008). The presence of naturally occurring sexual populations in relatively close proximity could explain the rapid increase in genetic diversity (Persoons et al. 2017).

1.6.4 Increasing forest productivity: a tree pathogen paradise

The rapid intensification of forestry is likewise having an impact on the evolutionary and demographic history of pathogens that have a long co-evolution history with their host trees. The combination long-lived host with low diversity often deployed in clonal plantations could create ideal conditions for disease outbreaks. However, while the impact of forest management practices on genetic diversity and structure of forest trees is particularly well documented (e.g. Ratnam et al. 2014), their consequences on tree-pathogen populations remain poorly documented. Most significant examples available are those related to the impact of industrial large-scale plantations and monocultures (e.g. european poplar leaf rust *M. larici-populina* - see above). Indeed, the plantation of monospecific (or in some cases monoclonal) forests represents an extreme case on the spectrum of forest management practices (Fig. 1B). The increase of such a forest system in the last 50 years has provided favorable conditions for the build-up and spread of local pathogen populations (Labbé et al. 2015; Sakalidis et al. 2016; Tabima et al. 2020; Xhaard et al. 2011).

Two striking examples are *Dothistroma septosporum* and *Armillaria ostoyae* with the domestication and expansion of pine (*Pinus spp.*) cultivation. At least three isolated genetic lineages of the Dothistroma needle blight (DNB) pathogen *D. septosporum* emerged following the introduction and establishment of commercial plantations of the Monterey pine, *P. radiata*, in the Southern Hemisphere (Barnes et al. 2014; Bradshaw et al. 2019). Each of these genetically isolated DNB lineage arose from a highly diverse genetic pool in the endemic area of DNB in the Northern Hemisphere and have a clonal structure consistent with a scenario of recent introduction of a few founder individuals and high adaptation potential to their host (Bradshaw et al. 2019). In Southwestern France, the large monospecific plantation of maritime pines (*Pinus pinaster*) in the Landes de Gascogne during the nineteenth and twentieth centuries favoured the expansion of the native root and butt-rot pathogen *Armillaria ostoyae*. The best fitting models using genome-wide SNP and microsatellite markers are consistent with the recent and intensive pine plantation accelerating the rapid spread and success of the root pathogen (Dutech et al. 2016; Labbé et al. 2017).

Tree genetic improvement programs are still at the stage where they are capitalizing on the range-wide capture of trait variation of valuable tree species by establishing tree provenances and progeny trials (Koskela et al. 2014). However, transfer of germplasm and genetic material

have increased the risk of emergence and spread of diseases (Koskela et al. 2014). There have been a significant number of documented examples of introduction of exotic pathogens of trees while transferring reproductive material (Liebhold et al. 2012). Introduction and intensive plantation of new host species may also provide an opportunity for the rapid emergence of modified pathogen populations and new diseases. The intensification of poplar cultivation in North-America resulted in the emergence of a new disease with the rapid change of an innocuous foliar pathogen into a destructive and epidemic canker pathogen (Bier 1939; Thompson et al. 1941). The ascomycete fungus *Sphaerulina musiva*, previously known as a necrotic leaf-spot pathogen on the native eastern cottonwood *Populus deltoides* (Bier 1939; Thompson et al. 1941), adapted to infect and colonize poplar woody tissues as a result of horizontal transfer of genes from prokaryotes and fungi associated with wood decay (Dhillon et al. 2015). The pathogen population structure shows a strong association between multiple pathogen introductions and dissemination, creating genetic admixture, and poplar culture (Sakalidis et al. 2016; Tabima et al. 2020).

1.7 Brave new world: new solutions to tackle the risks associated with a changing environment

In several of the examples aforementioned the selective pressures exerted on pathogen populations by host domestication are intensified by secondary threats arising from anthropogenic activities. The risk and impact of factors related to the acceleration of anthropogenic activity on the diversity and evolution of plant pathogen populations has been extensively documented and discussed (Corredor-Moreno and Saunders 2020; Desprez-Loustau et al. 2007; Fisher et al. 2012; Hamelin and Roe 2020; Stukenbrock and Bataillon 2012). In theory, the impact of threats such as the introduction of invasive alien exotic species (IAES) and global climate change on domesticated crops and natural systems should be highly variable and will depend on factors associated with the host and pathogen affected.

One of the challenges when an invasive alien pathogen is introduced is that the host has either not co-evolved with the pathogen, and therefore resistance genes are at extremely low frequency or absent, or in the case of domesticated species, resistance has been integrated during traditional breeding cycles of the crop species or the host is simply not bred for resistance to that alien pathogen (Zhan et al. 2015). The introduction of sugarcane rust in the Americas and

wheat stripe rust in Australia are some examples (Purdy et al. 1985). Introduction of invasive alien forest pathogens have caused irreversible damages and changes at the landscape level largely due to the absence of any resistance in the hosts (Loo 2009). Natural forests are more heterogeneous and diverse, suggesting a high potential for resilience to biotic stresses. However, the effect of exotic pathogen introductions seems to be strongly dependent upon pathogen biological traits and host ecological features (Lovett et al. 2006).

1.7.1 The new domestication toolbox to counter pathogens in a changing environment

It is becoming clear that anthropogenic changes to the planet have to be countered with novel innovative approaches to reduce the threats on global food security and environment (Fones et al. 2020). Minimizing the effect of the changing environment on pathogens on crops and trees and mitigating their risk is becoming an urgent task as rapid increase in the global population is accelerating the need for increased agricultural production. New technologies are available to answer this need. While traditional breeding has increased yield or resistance to pests and pathogens, it remains a slow process, in particular for species with long reproductive cycles, that is unlikely to keep pace with the predicted demand. The new domestication toolbox comprises the recent advances in management practices, new sources of genetic diversity, genomics and biotechnology applied to breeding. This is broadening the possibilities of crop and tree growth and resilience optimization to find the right balance between productivity and sustainability in a changing environment. The lessons learned with the impact of plant domestication and management on crop and forest pathogen populations should guide the usage of this toolbox.

1.7.2 From plants to landscapes, diversity is the key

The anthropogenic impacts on crops and trees result in homogenization and reduction in species and genetic diversity. The future of sustainable crop and tree protection relies on increasing plant cropping and forest systems and landscape diversity (Hajjar et al. 2008; Storkey et al. 2019). The intuitive idea of a positive association between landscape complexity and pest control has developed into an agroecological paradigm (Chaplin-Kramer et al. 2011; Gurr et al. 2003). Species richness had a positive effect on both pollination and pest control and a negative effect of landscape simplification on these ecological services (Dainese et al. 2019; Karp et al.

2018; for conflicting view see Dainese et al. 2019). At the genetic level, increasing diversity by planting crop cultivars with different resistance genes or gene stacks can reduce or prevent pathogen attacks (Mundt 2002). Ultimately, a sustainable future for agroecosystems and forestry could lie in the combination of different techniques and technologies improving the plants as well as informed management practices increasing diversity thus promoting ecological services.

Forests can range from unexploited ecosystems with minimal human impact such old-growth - the late stage of forest development characterized by climax communities and large trees (Franklin et al. 1981), to planted forests and fragmented habitats with different levels of forest management practices for wood production and agroforestry ecosystems where trees are managed as perennial production systems around or among crops or pastureland. Agroforestry is a direct response to a need for more diverse and sustainable agroecosystems; this system provides direct benefits such as carbon sequestration (Banerjee et al. 2016) and can help reduce the loss of biodiversity and ecosystem services (food, wood, fiber) from intensive agricultural systems (Santos et al. 2019). In addition, the increase of diversity in agroforestry systems (higher genetic diversity, mixed planting, enhanced species richness) increases yields and pollinators and provides better weed and pest suppression (Isbell et al. 2017).

1.7.3 Modern breeding strategies: searching for new resistance and adapting to the changes

One way plant breeders have found to mitigate the pest and pathogen problems is the generation of durable and broad-spectrum disease resistant crop cultivars. Sequencing technologies coupled with the availability of annotated crop genomes have significantly increased the pace with which disease resistance genes are selected or cloned (Keller et al. 2018). So far, modern breeding practices have exploited a very limited fraction of the crop diversity (Wang et al. 2017). There are still many possible sources of diversity available, e.g. wild relatives, landraces and exotic germplasm accessions, that can be incorporated in breeding strategies and converted in long-term genetic gain (Dempewolf et al. 2014; Wang et al. 2017). They provide a promising basis to transit towards efficient crop breeding that will combine productivity and resilience to biotic and abiotic stresses.

1.7.4 Screening sources of diversity for pathogen resistance and adaptive traits

Identification of resistance and adaptive traits in different sources of diversity remains a challenge. Advanced high-throughput genomics, phenomics and biotechnology tools can be used to identify markers associated with important traits such as drought tolerance in maize seedlings (Wang et al. 2016), deep-sowing tolerance in rice (Zhao et al. 2018) or seedling heat tolerance in winter wheat (Maulana et al. 2018). The use of whole-genome prediction models incorporating identified and characterized resistance genes can be effectively applied to select for quantitative disease resistance (Poland and Rutkoski 2016). This approach has been tested for a variety of crop and tree fungal diseases such as Fusarium head blight of wheat and barley (Mirdita et al. 2015; Sallam et al. 2015), stripe and stem rust of wheat (Ornella et al. 2012), cassava anthracnose (Ly et al. 2013) and chestnut blight of American chestnut (Westbrook et al. 2019) with variable success.

Hardwood trees have large genomes, coming from multiple whole-genome duplication events and the presence of abundant repeated elements. Since the first assembly of the *Populus trichocarpa* genome in 2006, a growing number of association studies have emerged in a variety of wooden plant species such as poplar (*Populus trichocarpa* and *Populus deltoides*) (Chhetri et al. 2019; Fahrenkrog et al. 2017; McKown et al. 2014), lodgepole pine (Parchman et al. 2012) or white spruce (Lamara et al. 2018), identifying loci associated with a wide range of phenotypic traits. The number of forest tree breeding programs that are now including durable resistance (or ‘tolerance’) to pests and pathogens has drastically increased. In Norway spruce, a genome-wide association study discovered candidate SNPs associated with larger necrotic lesions from *H. parviporum* (Mukrimin et al. 2018). This study, and similar ones (e.g Liu et al. 2018; Resende et al. 2017; Vázquez-Lobo et al. 2017) provide information on the genetic architecture of traits and the identification of valuable markers involved in development, morphology, resistance to pathogens, or to harsh environmental conditions. One drawback of these approaches, however, is the lack of functional validation of promising candidate genes, due to the biological features particular to long-lived trees.

1.7.5 Engineering and editing the genomes of crops and trees to increase resistance to diseases

The availability of genetic engineering tools such as transgenic expression of host/pathogen genes, RNA interference (RNAi) in agroecosystems could present rapid approaches to improve disease resistance in crops. With better understanding of host-microbe interactions, these tools can be effectively used to develop durable disease resistance (Schweizer 2019). RNAi-based biopesticides are based on topical application of double-stranded RNAs (dsRNAs) complementary to pathogen genes and can induce gene silencing in a specific pest (Fletcher et al. 2020). Use of dsRNA-based silencing has been reported with variable success for various plant fungal pathogens (Fletcher et al. 2020; Gill et al. 2018; Höfle et al. 2020; Hu et al. 2020). This method also offers perspectives in forestry with promising results in insect pest management as tested with the devastating mountain pine beetle (Kyre et al. 2020) and emerald ash borer (Leelesh and Rieske 2020). Beside the apparent effectiveness of this technology, the commercial use of dsRNA based biopesticides could be restrained by excessive cost and lack of effective delivery methods on a commercial scale, especially in forest plantations.

However, the success of genetically modified crops (GMO) lies in finding target genes for genetic manipulation. Multiple disease resistance (MDR) genes (pleiotropic genes) such as Lr34 (in wheat) have great potential. Lr34 encodes an ATP-binding cassette (ABC) transporter and confers resistance to stem rust, stripe rust, leaf rust, and powdery mildew of wheat. In addition to wheat, Lr34 is also effective against biotrophic pathogens of rice, barley, sorghum, and maize when expressed transgenically (Sucher et al. 2017). Transgenic crops carrying single or multiple R genes may put strong selection pressure on the pathogen and increase chances of disease epidemics in monocultures. However, how transgenic crops impact natural plant pathogen populations has not been well studied yet.

Genome editing (GE) utilizes mechanisms discovered in nature to change specific nucleotides within the plant genome. Over the last decade, the use of targeted genome editing technologies such as CRISPR/Cas9 has exploded in a variety of organisms. GE is a powerful tool for crop improvement and has already been widely used to modify plant immunity and increase pathogen resistance (Andolfo et al. 2016). For instance, mildew resistant plants have been generated by manipulating host susceptibility genes in wheat (Wang et al. 2014) and resistance to *M. oryzae* was generated by modifying the ethylene pathway in rice (Liu et al. 2012).

Although GE using CRISPR/Cas9 has become a viable approach to knock-out genes in fruit trees, such as citrus, apple, grape, cassava, coffee, and kiwifruit, site-specific gene targeting or allele replacement remains a major challenge in tree species (Bewg et al. 2018). The use of CRISPR/Cas9 in forestry and in forest pathology is in its infancy, yet offers great potential for disease control (Dort et al. 2020). In poplar, CRISPR-based gene editing has successfully been used to knock-out 4CL genes, associated with lignin and flavonoid biosynthesis (Zhou et al. 2015).

1.7.6 Combining tree genetic improvement with adaptation to climate

Historically, foresters have taken the ‘local is best’ approach to selecting reforestation seed sources. Tree breeding programs usually apply the same approach by operating within breeding zones. Climate fluctuations are starting to disrupt historical local adaptation and tree populations are simultaneously challenged to withstand the consequences of novel climates, and unable to adapt or migrate rapidly enough to remain locally adapted (Aitken et al. 2008). Many current tree improvement programs are trying now to incorporate assisted gene flow to match reforestation with climates expected in the future. Tree improvement programs in Alberta and British Columbia (Canada) are designing climate-based seed transfer systems to mitigate adaptive discordance between tree genotype and climate (Gray et al. 2011; O’Neill and Ukrainetz 2008). Such an approach also necessitates a deep knowledge of genetic variation in climatically adaptive traits in breeding populations but also, genetic structure and pathogenicity profiles of present and future pathogen populations.

Epidemic outbreaks of the endemic pathogen Swiss needle cast (SNC), a foliar disease of Douglas fir caused by *Nothophaeocryptopus gaeumannii* have steadily increased in severity since the 1980’s in response to rising winter temperatures and spring/summer precipitations and changes in forestry practices (Agne et al. 2018; Mildrexler et al. 2019; Ritóková et al. 2016). Similarly, extensive tree mortality caused by DNB on lodgepole pine have been associated with key environmental factors that directly affected the life cycle components and biological traits of the pathogen, leading to recent increases in disease incidence and severity (Woods et al. 2005; Welsh et al. 2009). A population genomics study (Capron et al. this issue) identified four distinct DNB genetic lineages that diverged at the end of the last glacial maximum before recolonization of lodgepole pine in western North America. A unique genetic lineage is present in the areas most affected by the climate-driven outbreak. This knowledge on the DNB population structure

will inform future movements of planting material and assisted gene flow practices, preventing some secondary contact between genetically isolated DNB lineages that could result in new epidemics.

1.8 Take-home message

The planet's ecosystems have been changing during the anthropocene, with a global homogenization of plant species and genetic make-up and an intensification of production. This is a recipe for promoting the emergence of plant diseases or the adaptation and specialization of novel pathogens. In spite of the remarkably different characteristics of crop plants and forest trees, we find that similar processes such as hybridization, host jumps, selection, specialization and clonal expansion are shaping the populations of pathogen of both crops and forest trees, in particular in cases where trees have been domesticated and are grown in managed plantations. Genomic tools to monitor pathogen populations can help identify lineages with increased virulence, different geographic origin or mating types or other adaptive traits (Hamelin and Roe, 2020; Weldon et al., this issue). In some cases, this will directly influence disease management, for example to eradicate a lineage of *P. ramorum* and prevent potential mixing of different mating types (Grünwald et al. 2019). Genomics also provides the most promising solutions to discover and clone resistance genes or to develop biopesticides to rapidly address plant health issues. We propose that integrating these monitoring and management tools will lower the probability of global pathogen outbreaks so that we can envision better management strategies to sustain global food production as well as ecosystem services.

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Chapitre 2 Hybridization drives genome evolution of Dutch Elm Disease pathogens

2.1 Résumé

L'hybridation et l'introgression qui peut en résulter influencent le succès d'une espèce invasive en permettant l'acquisition rapide de traits adaptatifs. La pandémie de maladie hollandaise de l'orme des cent dernières années est provoquée par trois lignées de champignons possédant des barrières reproductive perméables : *Ophiostoma ulmi*, *Ophiostoma novo-ulmi* ssp *novo-ulmi* et *Ophiostoma novo-ulmi* ssp *americana*. Grâce aux données génomiques et phénotypiques d'une collection d'échantillons répartis dans le monde, nous montrons que l'introgression a été le principal moteur générant de la diversité génétique et qu'elle impactait des traits liés à la fitness. Les régions introgressées contiennent des gènes impliqués dans les interactions hôtes-agent pathogène ainsi que dans la reproduction. Les isolats introgressés présentent une croissance plus grande à une température élevée et produisent des tailles de nécroses différentes sur pommes. De plus, les différentes lignées génétiques possèdent des croissances différentes sur un milieu contenant une molécule de défense de l'hôte.

2.2 Abstract

Hybridization and the resulting introgression can drive the success of invasive species via the rapid acquisition of adaptive traits. The Dutch elm disease pandemics in the past 100 years were caused by three fungal lineages with permeable reproductive barriers: *Ophiostoma ulmi*, *Ophiostoma novo-ulmi* subspecies *novo-ulmi* and *Ophiostoma novo-ulmi* subspecies *americana*. Using whole-genome sequences and growth phenotyping of a worldwide collection of isolates, we show that introgression has been the main driver of genomic diversity and that it impacted fitness-related traits. Introgressed isolates have enhanced growth rate at high temperature and produce different necrosis sizes on an in vivo model for pathogenicity. In addition, lineages diverge in many pathogenicity-associated genes and exhibit differential mycelial growth in the presence of a proxy of a host defence compound, implying an important role of host trees in the molecular and functional differentiation of these pathogens.

2.3 Introduction

Genetic admixture has been associated with genetic admixture, from adaptation to high-altitude in humans (Yi et al. 2010) to bacterial adaptation to antibiotics in bacteria (Benett et al. 2008). Hybridization is one of the major drivers of evolution and adaptation in plant pathogens (Stukenbrock et al. 2016, Depotter et al. 2016, Barton 2001, Schardl and Craven 2003, Brasier 2001, Olson & Stenlid 2002), resulting in new combinations of alleles that increase diversity and provide new material for natural selection. Invasive plant pathogens have become a major burden in animal and plant conservation, in agriculture and public health (Fisher 2012, Fisher et al. 2018). An increase in interspecific hybridization events between fungal pathogens has been attributed in part to human trade bringing into contact related but previously geographically isolated taxa that have not evolved strong pre- or post-zygotic barriers (Paoletti et al. 2006, Brasier 2000).

Two fungal species of the genus *Ophiostoma* caused successive pandemics of Dutch Elm Disease (DED), one of the world's most damaging tree diseases (Brasier 1986a). The pathogens are vectored by scolytid bark beetles that feed in the crown of healthy elms, resulting in infections that lead to a vascular wilt, dieback and death. A first pandemic caused by the fungal pathogen *Ophiostoma ulmi* (Buisman) Melin & Nannf. (OU) spread across Europe and North America during the early 1900s with the help of human trade. In the 1940s a far more destructive pandemic began on both continents caused by *Ophiostoma novo-ulmi* Brasier (ONU), a more aggressive pathogen with a lower optimum growth temperature than OU, making it a better competitor in a wider span of ambient temperatures (Brasier et al. 1981, Brasier 1977).

ONU comprises the Eurasian and North American subspecies, ONU *subspecies novo-ulmi* and ONU *subspecies americana*, that differ in several traits such as sexual fruiting body morphology and aggressiveness (Pipe et al. 1995, Brasier 1986b , Brasier & Kirk 2001). ONU *subsp americana* - the most aggressive of these taxa - was discovered in North America and later in Europe in regions where its distribution overlaps with OU and ONU *subsp novo-ulmi* (Gibbs & Brasier 1973). A third species endemic to the Himalaya was identified as *Ophiostoma himal-ulmi* Brasier & M.D. Mehrotra (Brasier & Mehrotra 1995). Additional population structure was observed within ONU *subspecies americana* with the discovery of two dominant vegetative compatibility (vc) supergroups, and a large fraction of heterogeneous vc groups, suggesting clonal reproduction and admixture

enabled by sexual reproduction (Brasier & Kirk 2000). These investigations uncovered very dynamic population structures with frequent geographic overlap among migrating lineages.

OU and ONU are sexually outcrossing, requiring two distinct sexual compatibility types for sexual mating, MAT-1 (acting as recipient) and MAT-2 (acting as donor) (Brasier 2001, Brasier 1986a). During the saprophytic phase, mycelia of opposite sexual compatibility types can produce perithecia in beetle galleries (Brasier 1986a). The multilocus vegetative incompatibility (vic) system promotes outcrossing and territorial antagonism, and it also prevents fusion of the hyphae from genetically dissimilar individuals (Brasier 1983). Since a strong unidirectional prezygotic fertility barrier limits but does not prevent perithecial formation between OU and ONU, and only moderate unidirectional barrier exists between ONU *subsp americana* and ONU *subsp novo-ulmi*, reproductive isolation among taxa is not complete (Brasier 1986a). As a consequence, hybridization occurs between OU and ONU in North America and Europe (Brasier & Kirk 1998, Brasier 2001) as well as between ONU *subsp novo-ulmi* and ONU *subsp americana* in Europe. OU x ONU hybrids with MAT-1 sexual compatibility type are sterile and have poor competitive fitness, but hybrids were found at epidemic fronts (Brasier & Kirk 1998), and a remarkable change to high genetic variability was shown to involve widespread selective acquisition of both OU vic alleles and the OU MAT-1 locus in ONU (Paoletti & al. 2006). By the mid-1980s, complex ONU *subsp americana* x ONU *subsp novo-ulmi* hybrid swarms exhibiting high growth and pathogenic fitness comparable to that of ONU *subsp americana* were also emerging across Europe, possibly remodelling, via hybridization and natural selection, this pathogenic species complex (Brasier 2002, Brasier & Kirk 2010).

Some of the genes introgressed via hybridization have potential adaptive advantage. For instance, it has been hypothesized that *vic* genes are involved in protection against viral propagation, potentially impacting these species beyond their role in reproduction (Zhang et al. 2014). Other genomic regions, including OU pathogenicity and hydrophobin genes, were also acquired by hybridization in ONU (Et-Touil et al. 2019). The underlying genomic footprint behind these dynamic and environmentally significant hybridization events has yet to be characterized. Here we investigate the genomic diversity of a worldwide collection of isolates from the *Ophiostoma* species complex using population genomics, growth and virulence phenotyping. We show that hybridization among DED-causing *Ophiostoma* lineages creates a mosaic of distinct evolutionary histories across their genomes and is the major determinant of

genomic diversity that may have contributed to the success of these plant pathogens. We also detect molecular signatures of selection on genes involved in several key pathways such as membrane transport, binding of defence compounds and enzymes involved in plant cell wall degradation, suggesting that the fungi are adapting to tree defences. Introgressed isolates have a higher growth rate at high temperature and cause larger or smaller necroses on an in vivo system, depending on the introgressed region, suggesting that introgression from OU affects adaptation in ONU.

2.4 Results

2.4.1 Population structure and cryptic lineages

We sequenced the genomes of a worldwide collection of 97 haploid isolates of DED-causing fungi from North America ($n = 43$), Europe ($n = 41$), Asia ($n = 10$) and New Zealand ($n = 2$) (Fig. 2a, Supplementary Table 1, unknown ($n = 1$)). Genome sequences group isolates according to the two previously described species: OU and ONU (3.2% pairwise sequence divergence). Within ONU, isolates generally cluster according to the described groups and will be hereafter referred to as three different lineages rather than subspecies: NOV (*O. novo-ulmi* subsp *novo-ulmi*), AME1 and AME2 (*O. novo-ulmi* subsp *americana*) (Fig. 2b-d). Pairwise sequence divergence is 0.5% between NOV and each AME lineage and 0.3% between AME1 and AME2. The tree built using 8640 concatenated genes separates OU from the ONU lineages with strong bootstrap support (Fig. 2b). The clustering analysis ($K = 2$) and the first component of the Principal Component Analysis (PCA) delineate isolates in groups matching the previously defined OU and ONU species (Fig. 2b, Supplementary Table 1). Similarly, the second component of the PCA and the clustering analysis excluding OU ($K = 2$) differentiates the NOV and AME lineages. The third PCA axis and the clustering analysis ($K = 3$) identify AME1 and AME2 within AME (Fig. 2b, d). These clusters, based on SNPs, are also found using copy number variants (CNVs, Supplementary figure 1). We detect geographical structure within OU in the PCA and clustering (Supplementary figure 2); isolates from Northern Europe and the United Kingdom cluster largely distinctly from the rest of the European samples. OU isolates from North America are mostly overlapping on the PCA, consistent with clonality and low diversity in American OU population and possibly a European origin. There is no clear geographic structure within ONU in the samples analysed within each group, except that AME1 is widespread in North America and Europe, AME2 is mostly found in North America and

NOV is absent from North America (Fig. 2, Supplementary figure 2). NOV also appears to be more frequent in Eastern Europe and central Asia, while AME is more frequent in Western Europe (Brasier & Kirk 2010).

In spite of the general agreement with previous taxonomy, NOV, AME1 and AME2 do not form well supported monophyletic groups (Fig. 2b); this is likely due to inter-lineage admixture, as illustrated by multiple NOV, AME1 and AME2 individuals in the clustering analyses (Fig. 2b) or the intermediate PCA and DAPC values (Fig. 2c, d; Supplementary figure 1). In total, 33% and 44% of the samples from North America and Eurasia, respectively, have some admixture (Supplementary figure 2). Analysis of the mitochondrial genome (838 SNPs) provides support for the OU and ONU clades but not for the genetic structure observed within ONU using nuclear genomes (Supplementary figure 3).

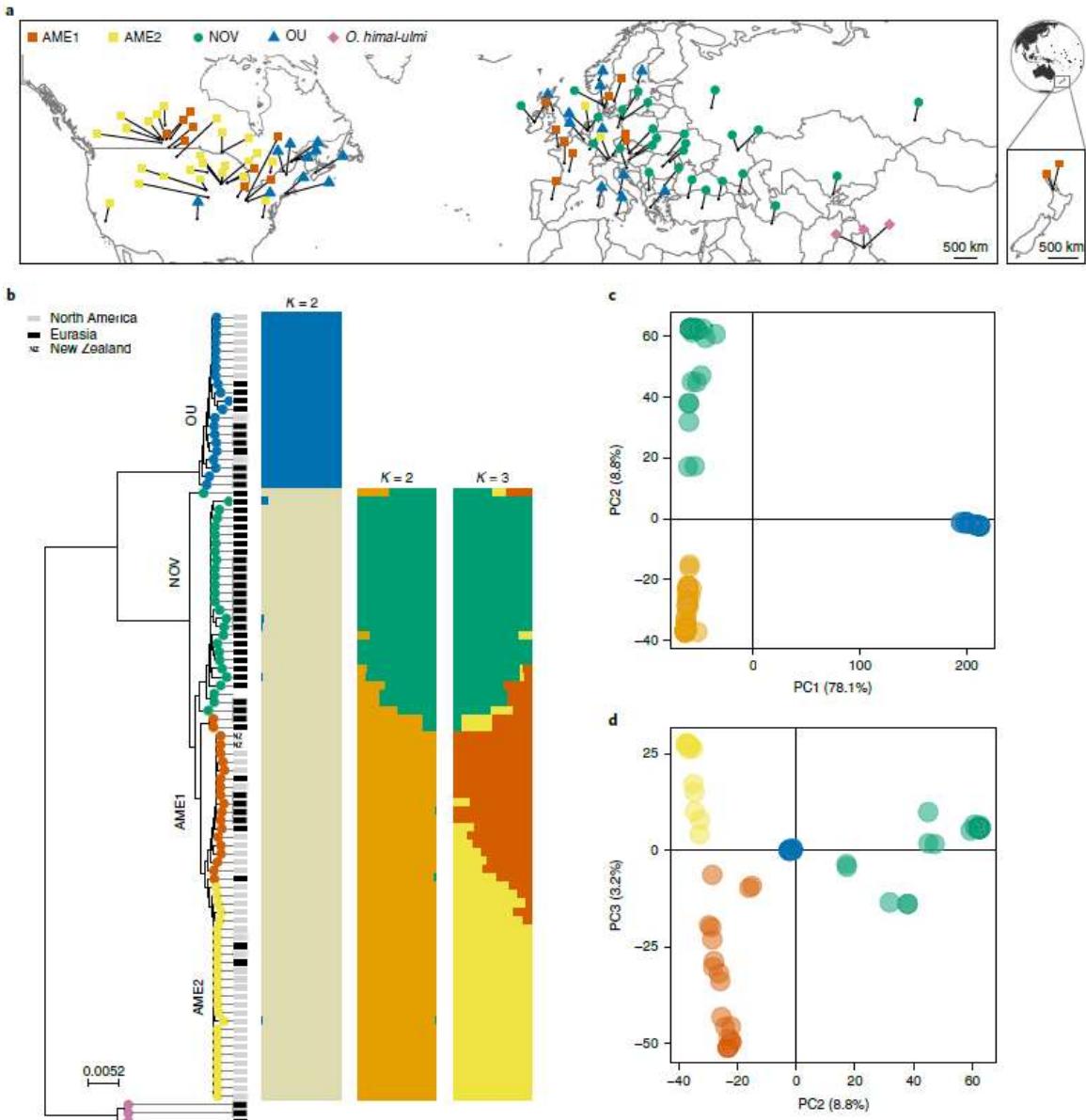


Figure 2. Clustering analyses based on genomic variation of worldwide *Ophiostoma* species samples show among-lineage admixture.

a, Sampling locations: three *O. himal-ulmi*, 21 OU, 26 NOV, 21 AME1 and 26 AME2 (the location of one NOV is unknown). The scale bar depicts a distance of 500 km. **b**, Neighbour-joining tree built using 8,640 concatenated genes and rooted in *O. himal-ulmi* (pink dots). Nodes with bootstrap support <75 are collapsed. Rectangles aligned to branch leaves indicate geographical location (black: Eurasia; grey: North America; NZ: New Zealand; empty for unknown location). Bar plots show the results of a Bayesian clustering analysis performed with STRUCTURE using: (1) OU, NOV and AME isolates with $K = 2$ clusters; and (2) NOV and AME with $K = 2$ and $K = 3$ clusters. Each isolate (bar) is partitioned into K clusters.

coloured segments displaying the estimated membership fraction in the K clusters. The analysis was based on a subset of 100,000 SNPs. **c,d**, Plots of the first and second axes (c) and the second and the third axes (d) of the PCA. PC, principal component.

2.4.2 Diversity and genomic signatures of introgression

2.4.2.1 Introgression shapes genetic diversity in ONU

We found the lowest nucleotide diversity (π) in AME2 (4.25E-04), followed by AME1 (1.60E-03), NOV (1.80E-03) and OU (2.75E-03, Fig. 3a). Population statistics and genotype clustering are consistent with rapid expansion and presence of many clonal isolates within NOV and AME2 (Supplementary figures 4-7). Nucleotide diversity in NOV, AME1 and AME2 is heterogeneous across the genome, which can be explained by inter-lineage introgression. Most non-overlapping 50 kb windows in ONU have nucleotide diversity below 0.001 (52%, 58% and 89% of 50 kb in NOV, AME1 and AME2, respectively), and others exceed the values observed in OU (0.03%, 0.03% and 0.005% 50 kb windows higher than maximum OU estimate in NOV, AME1 and AME2, respectively; Fig. 3a, Supplementary figure 4). Windows with high π are not randomly distributed across the genome but cluster together (e.g. peaks on chromosomes 2 and 8; Fig. 4, Supplementary figure 4). Clusters of high diversity in NOV, AME1 and AME2 also tightly overlap with regions of phylogenetic discordance (Supplementary figures 4 and 8) and are correlated with regions of low divergence and low differentiation with OU (Supplementary figures 6 and 9). In these regions, estimated topologies generated for sets of 100 adjacent SNPs (and 50 kb windows) cluster OU with one or two ONU lineages. These results suggest that introgression from OU increases nucleotide diversity in NOV and contributes to the differentiation between ONU lineages (NOV, AME1 and AME2).

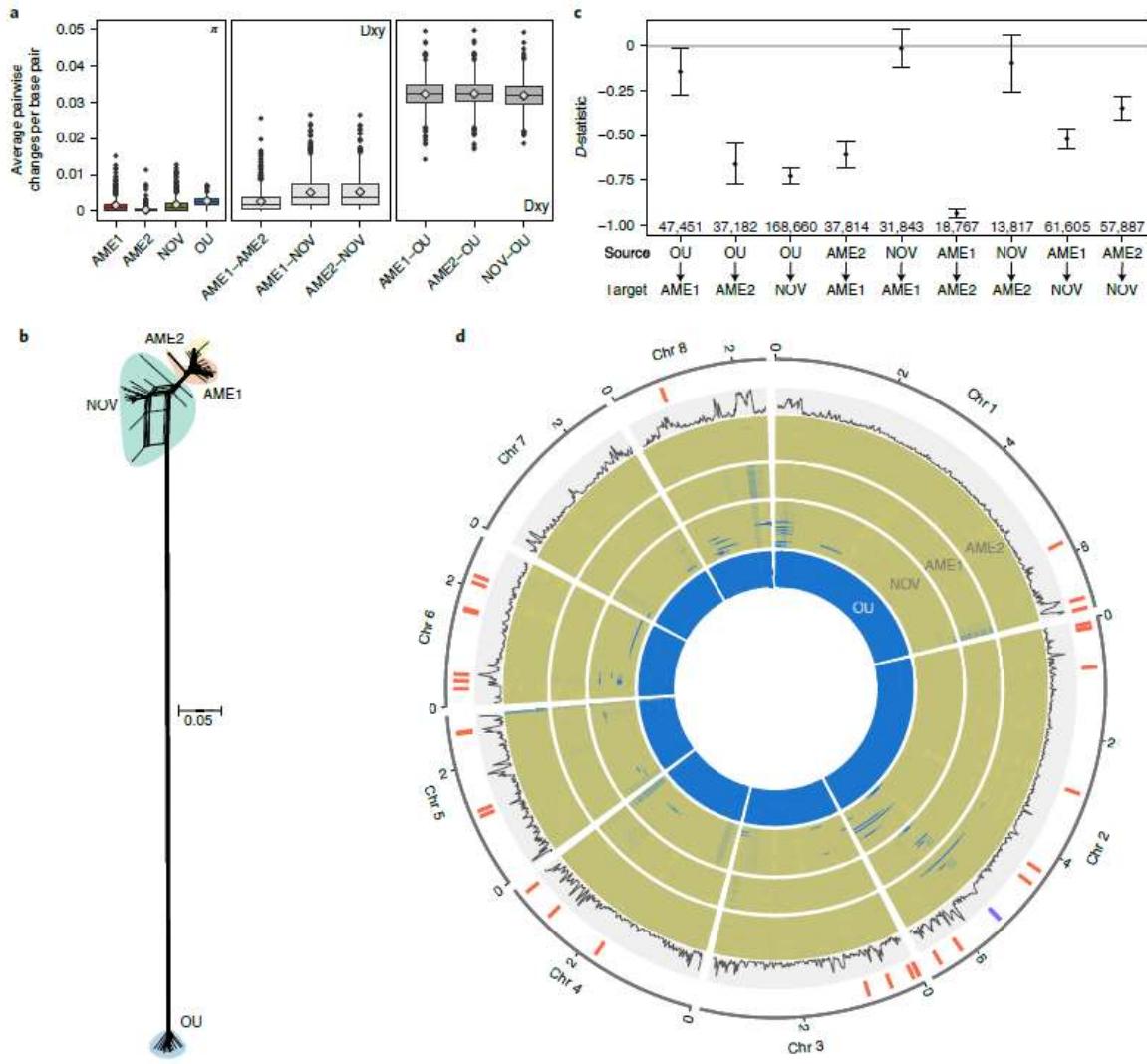


Figure 3. Introgession between DED-causing *Ophiostoma* lineages is frequent.

a, Box plots of the average pairwise nucleotide changes per site in 50-kb windows within lineages (nucleotide diversity (π)) and between lineages (Dxy). Diamonds indicate genome-wide mean estimates. For the box plots, the centre line is the median, the box limits represent the upper and lower quartiles and the whiskers show 1.5 \times the interquartile range. Data points indicate outliers. **b**, A phylogenetic network based on a subset of 68,061 SNPs with no missing genotypes across 94 genomes shows multiple network relationships between DED-causing *Ophiostoma* lineages. **c**, D-statistics calculated using genome-wide SNPs provide evidence for introgression from OU and among AME1, AME2 and NOV. Negative values indicate introgression from source to target lineages. Whiskers depict three standard errors of the D-statistic and values significantly different from 0 suggest introgression. The values at the bottom of the plot indicate the number of sites for which the D-statistic was calculated. **d**, Regions of OU (blue) introgression in ONU (green) isolates inferred from PCA in 200-kb non-overlapping windows. The level

of OU introgression in ONU is depicted by the colour gradient, where green indicates the longest, and blue the shortest, genetic distance to OU. The line plot shows the proportion of discordant topologies grouping OU with one or two ONU isolates estimated in 100-SNP windows. Red bars indicate the location of vic genes and the purple bar shows the location of MAT-1 loci. The scale of the chromogram is given in megabase pairs. Chr, chromosome.

2.4.2.2 Hybridization and introgression increase diversity in DED-causing fungi

We found signatures of introgression among all lineages of the DED-causing fungi. First, we constructed a phylogenetic network (Fig. 3b), which shows that ONU lineages are connected with each other and with OU by multiple reticulate patterns. Second, we tested for the presence and direction of introgression using D-statistics, dividing isolates into pure and putative hybrid groups based on PCA results (Fig. 2c, d). The selected pure isolates did not show genome-wide signature of introgression using D-statistic (Supplementary figure 10). We assessed introgression from each of the four pure groups into three groups of putative hybrids (AME1, AME2 and NOV). We detected introgression from OU into AME1, AME2 and NOV hybrid groups (Fig. 3c). Further, we detected introgression between AME1 and AME2, as well as from AME1 and AME2 to NOV, but not from NOV to AME1 or AME2. Overall, this confirms that introgression has occurred often between distant and closely related lineages.

To examine the extent of introgression from OU to NOV, AME1 and AME2, we inferred OU ancestry in 200 kb windows in ONU genomes. We performed a PCA for each window and computed the normalized distance of each isolate from the mean value of OU isolates along the first PC. This revealed that all ONU isolates are equally distant from the mean value of the OU group (normalized distance around 1, Fig. 3d). However, for 18 ONU isolates (13 NOV, 3 AME1 and 2 AME2), some windows, representing 0.6-8% of the genome, show a normalized distance close to 0 (arbitrary threshold, normalized distance < 0.25), suggesting that these genome regions are clustering with OU. A Bayesian clustering in the same non-overlapping windows gave qualitatively similar results to the first approach (Supplementary figure 11).

2.4.2.3 Potential adaptive consequences of introgression

Introgression has contributed extensively to genomic diversity in these pathogens. OU introgressed regions (IRs) represent in combination over 20% of the ONU genome length. IRs and non-IRs do not differ in terms of density of genes (mean proportion of coding sequence per window in IRs = 0.45, in non-IRs = 0.48, Mann-Whitney, U = 2603, P-value = 0.320, two-

tailed) or repeat elements (mean proportion per window in IR = 0.037, in non-IR = 0.022, Mann-Whitney, $U = 2294.5$, P-value = 0.830, two-tailed), but show a small, but significant difference in gene conservation, with introgressed genes being slightly less conserved (mean dN/dS in IRs = 0.226, in non-IRs = 0.219, Mann-Whitney, $U = 6,607,176.5$, P-value = 0.0008, two-tailed). IRs are enriched in genes that could impact fitness traits related to pathogenicity, including genes involved in host-pathogen interactions, vic genes, carbohydrate-active enzymes (CAZYmes) and ontology terms such as integral component of membrane (GO:0016021) and oxidation-reduction process (GO:0055114, Supplementary Table 2). A maximum of seven isolates carry IRs from OU in one window around the MAT-1 locus (chromosome 2). Three other IRs are present in 4 or 5 ONU isolates (chromosomes 1, 6 and 8, Fig. 3a). The four most frequent IRs (800 kb in total), are enriched for host-pathogen interacting genes (Fisher's exact test, four IRs merged, P-value = 0.019, and only chromosome 1, P-value = 0.008, two-tailed) and vic genes (Fisher's exact test, chromosome 6, P-value = 0.025, two-tailed). This suggests that most introgressions may be driven by loci related to sexual reproduction, vegetative incompatibility and interactions with the host tree. In isolates having both rare (present in 1 isolate) and frequent (present in >1 isolate) IRs, frequent IRs tend to be younger (Supplementary figure 12), except for IR with the MAT-1 locus, which carries more or less diverged ONU haplotypes. Different ONU haplotypes in the same IR group with different OU genetic clusters and IR show individually distinct genetic distance with OU (Supplementary figure 12), suggesting multiple events of introgression.

We investigated the ancestry of the two sexual compatibility types (MAT-1 and MAT-2). The frequency of MAT-1 was 0.67 in OU, 0.07 in NOV, 0.20 in AME1 and 0.19 in AME2 in our samples (Supplementary Table 1, Supplementary figure 13). Analysis of admixture in 200 kb windows spanning these loci shows that all ONU MAT-1 isolates have OU admixture. Maximum likelihood phylogeny of genes flanking the MAT-1 locus supports this result by showing ONU MAT-1 isolates grouping together with one OU group corresponding to MAT-1 isolates (Supplementary figure 13). This shows that all ONU MAT-1 isolates acquired their mating type locus via hybridization with OU. As a consequence, recombination within and among ONU lineages most likely was made possible by hybridization of ONU with OU.

2.4.3 Genetic basis of lineage divergence

2.4.3.1 Signatures of selection and divergence

Genetic differences between ONU lineages are largely driven by regions introgressed from OU. To find genes contributing to ONU lineage differentiation and remove the impact of introgression from OU, for each gene we excluded ONU isolates with sequences identical or very similar to OU (see Methods). Numerous genes differentiating AME1 from AME2 and AME from NOV were found (1% top Dxy, Fig. 4, Supplementary Table 3). Predicted genes with unknown function, as well as genes coding for transporters, and isoflavone reductase contribute most to AME1 and AME2 differentiation (Supplementary Table 3). Gene ontology terms were not enriched in the top 1% genes in either of the two comparisons, although AME1 vs. AME2 show marginally significant divergence in ABC transporters ($w = 119,750$, corrected P-value = 0.085). Analysis of genetic distance as estimated with Dxy along the genome identifies outliers between AME1 and AME2 or AME and NOV that often cluster together (Fig. 4). Some Dxy clusters, such as at the end of chromosome 1, 8 and the beginning of chromosome 4 overlap with regions of apparent genetic structure among ONU lineages and discordant phylogenies (Supplementary figures 11 and 14), suggesting increased mutation rate, past events of introgression or both. Clusters at the beginning of chromosome 8 overlap an unsorted or introgressed 100 kb inversion which separates AME2 and NOV from AME1 and OU (Supplementary figure 15).

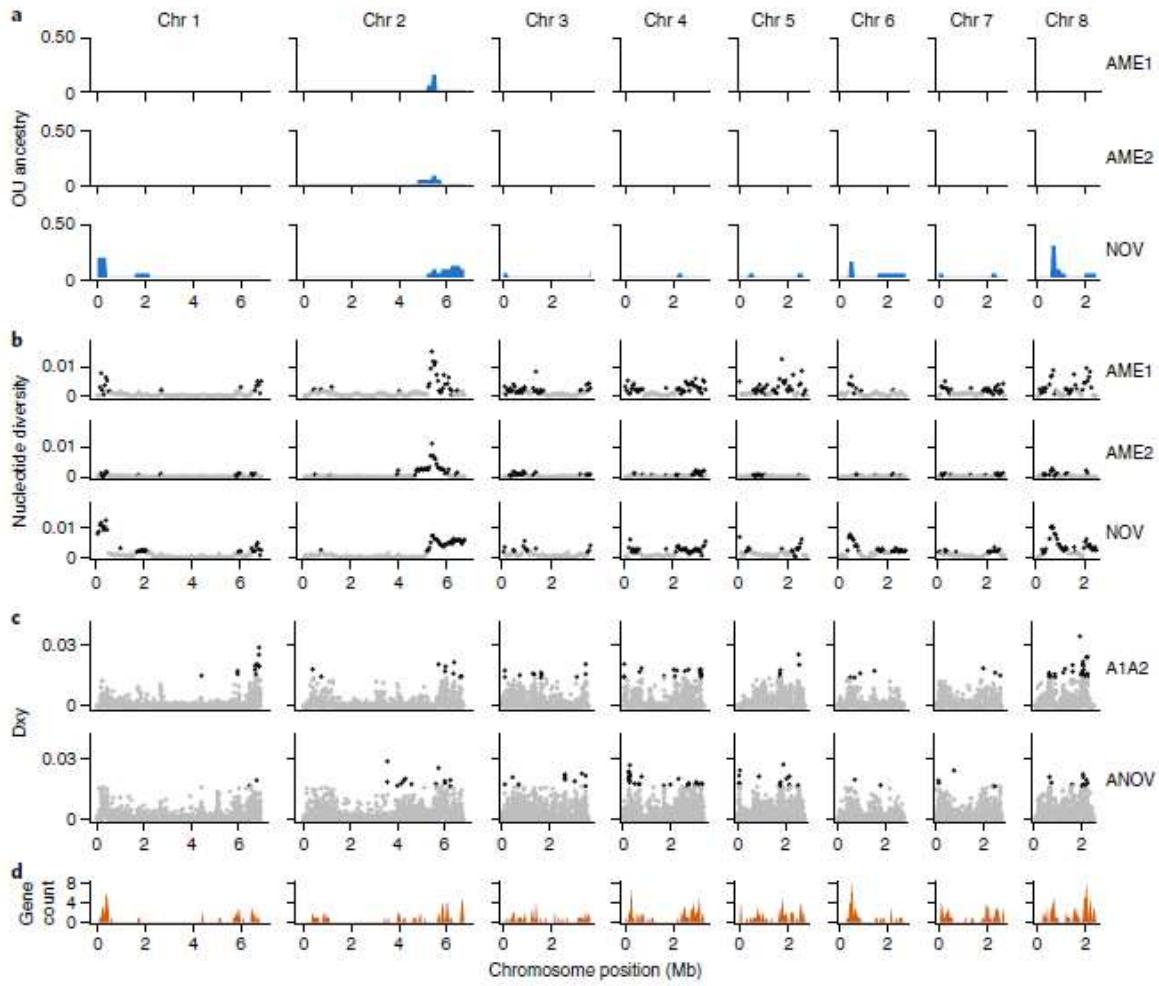


Figure 4. The most diverse genomic regions in ONU derive from recent introgression from OU.

a, Proportions of isolates with OU ancestry in three ONU lineages (blue). Ancestry was estimated using PCA in 200-kb non-overlapping windows. **b**, High-diversity regions in three ONU lineages are depicted with black dots. Nucleotide diversity was estimated in 50-kb non-overlapping windows. Windows of high diversity were identified as those above the 99th percentile of the bootstrapped distribution of nucleotide diversity. **c**, Per-gene Dxy between AME1 versus AME2 (A1A2) and AME versus NOV (ANOV). Black dots correspond to the top 1% of genes with the highest Dxy values, after excluding ONU with recent OU ancestry. **d**, Counts of genes within 50-kb windows that were found to be under positive selection in ONU by comparing synonymous and non-synonymous variants of ONU with *O. bimal-ulmi* (McDonald–Kreitman-like approach; see Methods and Supplementary Table 5). Mb, megabases. We find significantly higher divergence in genes overexpressed during the yeast phase in the AME1 vs. NOV and AME2 vs NOV comparisons (Nigg et al. 2016, Nigg et al. 2015) compared with the rest of the genes ($w = 200,440$, corrected P-value = 0.046; Supplementary Table 5). CAZYmes also exhibit a pattern of high divergence in both the AME vs. NOV and AME1 vs. AME2 comparisons, although they are not

significant after multiple test correction ($w = 1,188,100$, corrected P-value = 0.188 and $w = 1,206,800$, corrected P-value = 0.295, respectively).

Positive selection was detected in a large fraction of genes (436 genes, 5.2%) in ONU (Fig. 4, Supplementary figure 16, Supplementary Table 5), using synonymous and nonsynonymous, polymorphic and fixed variants (Eilertson et al. 2012) with *O. himal-ulmi* as an outgroup (5.2% overall nucleotide divergence from OU and ONU). Transporters are the most enriched group of genes among the targets of selection (Supplementary Table 6). Genes related to transmembrane transport are also overrepresented among genes with CNVs of length below 1 kb (corrected P-value = 0.0429), suggesting that copy-number variation could also play an adaptive role in these functional groups. Among positively selected genes we find genes involved in pathogenicity of fungi, such as 15 CAZymes, 5 cytochromes P450s, 103 genes differentially expressed in yeast or mycelium stage, 1 predicted effector gene, 5 vic and 47 genes with predicted signal peptides. The last two categories are significantly enriched among selected targets (5 out of 33 vic, P-value = 0.014, and 47 out of 621 genes encoding signal peptides, P-value = 0.0049). Finally, genes under positive selection are enriched in quinone binding functions (Supplementary Table 6).

2.4.4 Phenotypic differences between lineages

To investigate phenotypic differences among the genetic lineages and examine the potential fitness consequences of introgressions, we estimated growth rates of sequenced isolates ($n = 89$) at different temperatures, which is a major determinant of fungal geographic distributions (Newsham et al. 2016). We measured growth on standard media (MEA) and on media supplemented with a molecule (1,2-naphthoquinone) that mimics the host defensive compound mansonone E (MEA+N). Growth rate is significantly affected by the interactions of lineage, media and temperature (lineage:temperature P-value = 1.88e-8, lineage:media P-value = 1.53e-5, media:temperature P-value = 0.0001, Supplementary Table 7), suggesting extensive variation in growth rate and complex interactions with the growth conditions. All isolates exhibit a reduced mycelial growth in MEA+N compared to MEA but temperature affects the growth of some lineages differentially. There are few differences in growth rate among the lineages at 20°C or 25°C, except that OU grows slower and AME1 faster (Supplementary figure 17, Supplementary Tables 8 and 9). At 30°C in MEA+N, OU is the fastest growing lineage and

there is a significant growth difference between AME1 and AME2 despite their high level of similarity at the genome level.

Introgression impacts fitness. The extent of hybridization per isolate, measured by PC1 (P-value = 0.005, PC1:temperature P-value = 0.0175) and PC3 scores (P-value = 1.534e-7, Fig. 1c, d), has a significant effect on growth in interaction with temperature (Supplementary Table 7); PC1 scores (high scores mean high OU genome content) is positively correlated with growth at 30°C, but negatively correlated at 25° and 20°C (Fig. 5a). PC3, which quantifies the proportion of admixture between AME1 and AME2 (Fig. 2d), is negatively correlated with growth, suggesting that the purest AME1 isolates grow fastest at all temperatures. To estimate the potential effect of introgression on fitness, we fitted a new model using only ONU isolates (Fig. 5b). Growth rate was affected by the interactions of lineage and media, temperature and media (P-value = 4.201e-5 and P-value < 2.2e-16, respectively), but also by introgression from OU depending on the temperature (P-value = 0.0349, Supplementary Tables 10 and 11). Introgressed isolates have a similar growth rate as non-introgressed ones at 20°C (estimate = -44.9 ± 31.1, P-value = 0.1482) and 25°C (estimate = -35.7 ± 30.9, P-value = 0.2483), but have significantly higher growth rate at 30°C (estimate = -104.8 ± 31.7, P-value = 0.0010, Supplementary Table 12), suggesting that introgressions could provide a significant ecological advantage at this temperature.

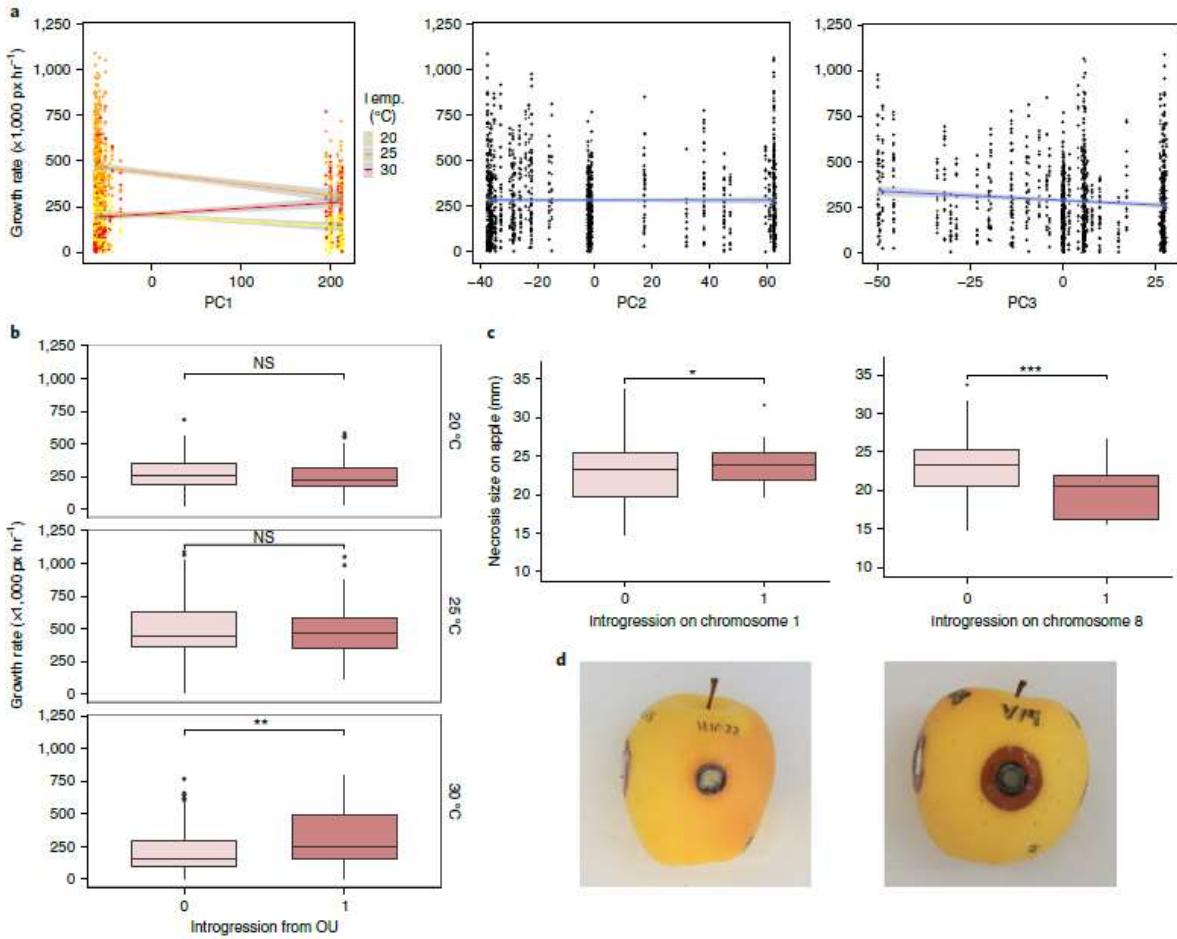


Figure 5. Hybridization and introgression have a strong impact on growth rate and virulence.

a, Impact of hybridization on growth, estimated by principal components of genetic differentiation. PC1, which measures OU genome content, has a significant effect on growth, in interaction with temperature (temp.). PC2 has no effect on growth. PC3, which quantifies the proportion of admixture between AME1 and AME2, has a significant negative effect on growth. **b**, Introgessed isolates have a higher mean growth rate at 30 °C than those without introgression. ONU isolates with introgression from OU ($n = 18$, with replicates) are designated by 1, whereas non-introgessed isolates are designated by 0 ($n = 50$, with replicates). **c**, Introgression on chromosome 1 (0: $n = 78$; 1: $n = 7$, with replicates) is positively correlated with necrosis size on apples. Introgression on chromosome 8 (0: $n = 79$; 1: $n = 6$, with replicates) is negatively correlated with necrosis size on apples. In the box plots, the centre line is the median, the box limits represent the upper and lower quartiles and the whiskers show 1.5 \times the interquartile range. Data points indicate outliers. **d**, Examples of necroses on apples inoculated with OU (isolate H1022; left) and ONU (isolate V19; right) 14 days after inoculation. NS, not significant. Significance was determined by post hoc tests on pairwise comparisons. Tukey adjusted P values: *P ≤ 0.05, **P ≤ 0.001, ***P ≤ 0.0005.

We assessed virulence by measuring necrosis size on apples, which is correlated with defoliation in elms (Plourde & Bernier 2014). OU had smaller necroses than ONU (Student's test: $t = 5.2327$, $df = 66.741$, $P\text{-value} = 1.819e-6$, Supplementary figure 18), reflecting the virulence levels of these two species. We found a positive effect of introgression on chromosome 1 ($P\text{-value} = 0.020794$) and negative effect of introgression from OU on chromosome 8 ($P\text{-value} = 0.001067$) on necrosis size (Supplementary Table 13, Fig. 5c, d). Isolates carrying an introgression on chromosome 1 induced larger necroses than those without introgression (non introgressed vs introgressed estimate = 2.3467 ± 1.13 , $P\text{-value} = 0.0387$, Supplementary Table 14); an opposite effect of introgression was observed on chromosome 8: isolates carrying an introgression on chromosome 8 caused smaller necroses than those without introgression (non introgressed vs introgressed estimate = -3.91 ± 1.11 , $P\text{-value} = 0.0005$, Supplementary Table 14), suggesting a contrasting effect of introgression on virulence.

2.5 Discussion

The pathogens responsible for DED have a complex population history (Brasier 2001, Brasier 1991). Species barriers are permeable and allow for hybridization and introgression, possibly enabled by global migration and widespread overlap of the species and subspecies (Brasier 2001, Brasier 1991). We detected four lineages corresponding to two distinct species and three groups of isolates in the incipient stage of speciation, likely corresponding to previously identified groups of predominantly vegetatively compatible isolates (Brasier & Kirk 2000, Mitchell & Brasier 1994), which spread rapidly in Eurasia and North America, and hybridized at the confluence of their ranges (Bates et al. 1993, Konrad et al. 2002). Our study substantially extends these findings by revealing the extent of introgression from OU to ONU, as well as pervasive hybridization and introgression among ONU lineages.

The success of invasive species in the face of low genetic diversity has been called the genetic paradox of invasion (Estoup et al. 2016). Hybridization could be particularly important in this context because admixture could promote adaptation by rapidly creating novel allelic combinations (Gladieux et al. 2015). We show that more than one third of the DED isolates studied have some admixture from one or several of the other lineages. We confirmed gene flow from OU to ONU and from AME to NOV (Bates et al. 1993), but not from NOV to AME. This pattern could be explained by the presence of a unidirectional prezygotic mating barrier

and by the relative frequency of the groups in the locations where hybridization takes place. In controlled laboratory crosses MAT-1 AME and MAT-2 NOV produce viable offspring while the reverse crosses with MAT-1 NOV had reduced fecundity, supporting what was observed in natural hybrids (Brasier & Kirk 2010). In Oomycetes and Basidiomycetes, vegetative karyogamy and sexual reproduction with meiosis are mechanisms leading to the formation of hybrids (Schardl & Craven 2003). The latter mechanism is likely in DED-causing species, as they are closely related, have the same number of chromosomes and share the same ecological niche, including host and vector. We showed that introgressed regions likely originate from different isolates, thus hybridization events might have occurred several times during the evolution of these fungi.

We show that the extensive and previously reported hybridization among DED-causing *Ophiostoma* lineages has created a mosaic of distinct evolutionary histories across their genomes that can have important consequences, including rapid increase of adaptive potential to hosts and environments (Gladieux et al. 2015, Menardo et al. 2016). One of the important outcomes of hybridization is the repeated acquisition of the complementary MAT-1 gene from OU by different ONU lineages (Paoletti et al. 2006, Brasier 1988). At the epidemic front European ONU populations were largely clonal and composed of MAT-2, making sexual recombination impossible. ONU with original, not introgressed MAT-1 system was found to be limited to western parts of Russia (Paoletti et al. 2006). The transfer of the MAT-1 gene from OU to ONU during recent expansion of ONU and multiple hybridization events with resident OU, reshaped the genomic composition of the DED-causing fungi by restoring a mixed mating system and generating highly admixed populations in Europe (Paoletti et al. 2006, Brasier & Kirk 2010). In contrast, in North America AME populations have low diversity of vegetative compatibility types (Brasier 1996, Milgroom & Brasier 1997). We show that introgression of OU MAT-1 locus occurred in several AME isolates, but unlike in European ONU, it is solely restricted to the sexual compatibility type locus and its surrounding regions. Models (Philibert et al. 2011) and simulations (Bazin et al. 2014) show that mixed reproduction systems have a positive impact on invasion success. Although asexual reproduction may lead to larger genetic load in its source environment, it may increase the success of invasion of a species with mixed system in a new environment (Bazin et al. 2014) and speed up adaptation. This can be particularly important in natural ecosystems, where, unlike in agricultural pathosystems, invasive pathogens are facing

more genetically diverse hosts, with which they may not have coevolved (Desprez-Loustau et al. 2007).

The combined introgression of the mating type and vic genes across species and lineages enables sexual reproduction, which in turn promotes vegetative incompatibility (Milgroom & Brasier 1997). Vegetative incompatibility keeps different genotypes separated and may limit the spread of deleterious viruses in populations of DED-causing fungi (Paoletti et al. 2006, Brasier 1983, Brasier 1988, Rogers et al. 2017). Our population genomic analyses confirm that vic alleles readily cross species barriers (from OU to mainly NOV). Vic genes act as an immune system of DED-causing fungi, and tend to introgress between the species as was shown in pathogen-recognition genes and genes involved in immune response in vertebrates (Fijarczyk et al. 2018, Enard& Petrov 2018). Similarly, introgressed host-pathogen interacting genes could provide a selective advantage. We find enrichment of these genes in the chromosome 1 introgressed region, a region linked to higher virulence, suggesting a possible mechanism of adaptation to local host. Introgressed CAZYmes, enzymes critical for the degradation of plant polysaccharides to facilitate nutrition and infection in pathogens (Zhao et al. 2014, Lah et al. 2017), could also be important for adaptation to local host.

Fungal diversity is largely determined by climatic conditions such as ambient temperatures (Newsham et al. 2016). The ability to grow at high or low temperature may therefore be a strong determinant of the invasive success of the *Ophiostoma* lineages. Our results show that introgression between lineages could influence adaptation to climatic conditions. We find that ONU with introgression from OU have higher growth rate at high temperature, which may enable the exploitation of different geographical ranges than non-introgressed isolates. Introgressed isolates are not solely constrained to the southern part of *Ophiostoma* distribution, but despite rapid human-aided dispersal hindering location of original introgression events, such introgression can eventually determine which isolates will prove successful in warmer parts of the distribution.

However, there may be some trade-offs to hybridization. Introgression of beneficial reproductive alleles from less to more aggressive species could result in the transfer of less adaptive linked alleles. We found that introgression on chromosome 8 reduced virulence as measured on apples. Introgression of pathogenicity genes and linked quantitative loci influencing

mycelial growth rate from ONU could actually result in lower aggressiveness of ONU populations (Et-Touil et al. 2019, Et-Touil et al. 1999) but may provide yet to be detected fitness benefits in other contexts, for instance at higher temperature. Growth at high temperature is a complex trait in fungi that likely involves many loci with small effects (Sinha et al. 2008, Eberlein et al. 2017). This may explain why the introgressed lineages as a group show faster growth at high temperature even if they do not share the exact same introgressions. Further studies will be needed to identify the causal loci. However, efficiency of plant invasion may not be the only fitness component determining *Ophiostoma* success. The interactions with the vector, or other unidentified environmental factors not related to the host could impact the performance of *Ophiostoma*.

We show that since the split with the common ancestor of ONU, OU and *O. himal-ulmi*, selective pressures on host-fungus interactions have shaped the evolution of many genes in DED-causing *Ophiostoma*. Several lines of evidence show that the fastest evolving genes in ONU encode transporters as well as genes with unknown function. Major Facilitator Superfamily (MFS) transporters play a role in drug and fungicide resistance in several fungi (Wu et al. 2016, Lin et al. 2018, Chen et al. 2014, Sanglard 2016). Also, ABC transporters are involved in transmembrane transport and multi drug resistance in the mycoparasite *Trichoderma atroviride* and the plant pathogens *Mycosphaerella graminicola* and *Magnaporthe grisea* (Ruocco et al. 2009). The ability to efflux host compounds such as the plant defence phytoalexins by ABC transporters has been reported as a major virulence factor for instance in *Botrytis cinerea* (Stefanato et al 2009). In *Grosmannia clavigera*, another *Ophiostomales* fungus, deleting a transporter increased sensitivity to monoterpenes, a tree defence compound, and delayed symptom development in pine trees (DiGuistini et al. 2011). The rapidly evolving genes of unknown function could play key roles as well and our results should motivate work on the characterization of their functions.

Resistance to antifungal or host defence molecules, and protection against other host-inhabiting microorganisms could be important driving forces of genome evolution in DED-causing fungi. 1,2-naphthoquinone, a compound closely related to the phytoalexin mansonone E, isolated from elm trees (Duchesne et al. 1985, Overeem & Elgersma 1970), decreased growth of all lineages, but this effect was modulated by temperature. This interaction between temperature and host defence compound detoxification could point to niche adaptation in these lineages and could explain the maintenance of multiple lineages within this multipartite symbiosis (Ojeda

Alayon et al. 2017). Although tolerance in ONU to naphtoquinone and mansonone E has not been shown to have direct influence on virulence towards American (susceptible) or Siberian (moderately resistant) elms (Proctor et al. 1994), the increased ability to withstand tree defences can be crucial for successful establishment and expansion of fungal populations.

Additional fastest-evolving genes in ONU include cytochrome P450s, an important family of enzymes associated with host compound detoxification in plant pathogens. One of the top candidates under positive selection in our study is a cytochrome (O-methylsterigmatocystin oxidoreductase) responsible for conversion of O-methylsterigmatocystin to aflatoxins, a family of toxins produced by molds and fungal pathogens of agricultural crops. An isoflavone reductase was among the fastest diverging genes in the comparison of AME1 and AME2 and was upregulated in *Aspergillus nidulans* in presence of bacterial antibiotics (Melin et al. 2002). Mannan endo-1,6-alpha-mannosidase, a CAZYme with a signal peptide, which allows secretion outside the fungal cells, was also under selection. These genes could be involved in fungal morphology determination, as was shown in the case of related alpha-1,2-mannosidase in ONU (Eades 2001). The ability to efficiently diffuse as yeast form in the xylem vessel is believed to be one of the mechanisms important during the infection process (Nigg et al. 2016, Gauthier 2015). Genes overexpressed during the yeast growth phase (Nigg et al. 2015, Nigg et al. 2016) are also strongly differentiated between AME from NOV, supporting the observations of AME being a more aggressive pathogen on some elm varieties (Brasier 1986b).

Our study provides new evidence that support the contribution of hybridization to genome evolution in invasive tree pathogens that results in increased adaptability and capacity to overcome host and environmental challenges. In spite of an initially low diversity within lineages linked to predominantly clonal reproduction, the introduced DED-causing fungi expanded rapidly and hybridized, resulting in new genetic combinations and phenotypes (Gladieux et al. 2015). However, nucleotide substitutions are not the only source of variation relevant in pathogen evolution. Many aspects of genome architecture, such as expansion of transposable elements, gene duplications or genomic rearrangements can have an enormous and immediate impact on pathogen fitness (Möller & Stukenbrock 2017) and need to be characterized further in DED causing fungi. This first draft of the genome-wide and distribution-wide diversity of the DED-causing fungi is a first major step in this direction.

2.6 Methods

2.6.1 Sampling, DNA extraction and whole-genome sequencing

We obtained 97 isolates of *O. himal-ulmi* (n = 3), *O. ulmi*, OU (n = 21) and *O. novo-ulmi*, ONU (n = 73) from a collection that covers more than 50 years of the outbreak (Fig. 1, Supplementary Table 1). Import permits of *Ophiostoma* samples were obtained from the Canadian Food Inspection Agency (CFIA). The isolates were handled in a facility that has been awarded Plant Pest Containment Level 1 (PPC-1) certification by the CFIA. Isolates were grown on Malt Extract Agar (MEA) (15 g/L agar, 30 g/L malt extract and 5g/L mycological peptone) and the mycelium harvested for DNA extraction using a CTAB chloroform protocol (Zolan & Pukkila 1986). Library preparation and sequencing was performed at the Genome Quebec Innovation Center (Montreal, Canada). Samples were pooled into a single lane for genome sequencing on the Illumina HighSeqX (PE 150bp) platform. The data generated an average coverage of 40X. Reads quality was assessed using Fastqc v0.11.869.

2.6.2 SNP and genotype calling

Raw reads were cleaned using Trimmomatic v0.36 (Bolger et al. 2014). OU and ONU reads were mapped onto the reference genome H327 (ONU, NOV lineage) (Comeau et al. 2014, Forgetta et al. 2013) using BWA v0.7.17 (Li & Durbin 2009). Transposable elements (TEs) were identified on H327 using Repet (Quesneville et al. 2005). SNPs overlapping TEs were filtered out. Duplicate reads were removed using Picard v2.16 (<http://broadinstitute.github.io/picard/>) and reads were realigned around INDELs using calmd option in SAMtools v1.3 (Li 2011). Genotypes were called with samtools mpileup and bcftools call options using bcftools multiallelic caller in BCFtools v1.4.1 (Li 2001). To generate the pileup format, we filtered reads with mapping quality less than 4 (-q 4), adjusted mapping quality for reads with excessive mismatches (-C50), and recalculated base alignment quality (-E). We only focused on SNPs and applied downstream filters using VCFtools v0.1.12b (Danecek et al. 2011). vcf-annotate was used to annotate bad quality SNPs using default filter settings, increasing the minimum allowed read depth per site to 10. Positions with the minimum depth below 2 reads or genotype quality less than 20 were considered as missing data. Finally, we considered only SNPs with less than 50% of missing data across all isolates. In total this resulted in 1,137,353 SNPs. To create genome alignments, we used vcf file with genotypes called at all positions and applied the same genotype

quality, coverage and missing data filters as for SNPs. The genotypes of each isolate in the remaining positions were transferred on the reference genome H327 using seqtk with mutfa option (seqtk v1.2, <https://github.com/lh3/seqtk>), where all bases were encoded as 'N'. Isolate genotypes were extracted from vcf file using the bcftools query option in BCFtools v1.4.1 (Li 2011).

2.6.3 Whole-genome alignments with *O. himal-ulmi*

O. himal-ulmi isolates were used as an outgroup to root phylogenies and polarize variants. Isolate HP32 was sequenced to high coverage (Illumina HighSeqX, PE150bp reads, coverage 400X). Adaptors were trimmed using Trimmomatic v0.3370 and the overlapping reads were merged using the BBMerge program in the BBMap package v37.47 (Bushnell et al. 2017). Merged and unmerged reads were assembled using SPAdes assembler v3.9.1 (Bankevich et al. 2012). We used careful option to reduce the number of mismatches and short INDELs, and performed the assembly using multiple k-mer sizes: 21, 33, 55, 77 and 99. We further mapped the reads of HP32 isolate as well as low-coverage Illumina PE150 reads of two other isolates (HP30 and HP31) to the assembled genome using BWA-MEM v0.7.13 (Li 2013). Three *O. himal-ulmi* genome sequences were obtained as described in the previous section. The genome sequences were then aligned to the reference genome H327 and SNPs were identified using Mauve v2015-02-13 (Darling et al. 2004). *O. himal-ulmi* SNPs were transferred onto H327 genome sequences using seqtk. Regions with transposable element and regions that did not align to H327 genome, were masked using BEDtools v2.26.0 (Quinlan & Hall 2010).

2.6.4 Population structure and identification of lineages

We reconstructed the phylogenetic relationships among 94 isolates (OU and ONU) for 8640 concatenated gene alignments using the double-precision version of FastTree v2.1.11 (Price et al. 2010). Support values for the best tree topology were assessed by nonparametric bootstrapping with 100 replicates.

We ran STRUCTURE v2.3.4 (Pritchard et al. 2000) on a subset of 15,203 SNPs distributed randomly in OU and ONU samples ($n = 94$) first and then only in ONU lineages ($n = 73$). We set a burn-in period of 50,000 followed by 100,000 MCMC replications, and no prior on geographic affiliation of the samples. Because our samples may not meet some of the assumptions of STRUCTURE, we performed additional analyses. We performed a discriminant

analysis of principal components (DAPC) and a principal component analysis (PCA) with the R package adegenet (Jombart 2008).

2.6.5 CNVs

Cleaned reads were aligned to the reference genome H327 using the speedseq v0.1.0 (Chiang et al. 2015), which uses the BWA-MEM v0.7.10 (Li 2013) algorithm for mapping reads and extracts discordant-reads and split-reads using SAMBLASTER v0.1.22 (Faust & Hall 2014). The command speedseq-sv was used to call structural variants using LUMPY-SV v0.2.13 (Layer et al. 2014), CNVnator v0.3 (Abyzov et al. 2011) and SVTyper v0.04 (Chiang et al. 2015) and get genotypes for each variant in each sample. This yielded 9271 CNVs including duplications, deletions, inversions and all other generic breakpoints tagged as BND. CNVs with a minimum size of 100 bp and a minimum of 80% known genotypes were retained, for a total of 1891 high quality CNVs.

2.6.6 Mitochondrial DNA

Illumina reads were mapped to the mitochondrial genome of H327 (NCBI assembly accession number GCA_000317715.1). Read mapping, SNP calling, and filtering were done as for the nuclear genome. A phylogenetic network was reconstructed with SplitsTree v4.14.5 (Huson & Bryant 2006) using NeighborNet algorithm, after removing positions with any missing genotypes ($n = 269$ SNPs). A maximum likelihood tree on 838 SNPs was built using RAxML v8.2.9 (Stamatakis 2014), under the GTRCAT model of nucleotide substitution without rate heterogeneity, and 100 bootstraps. Nodes with bootstrap support below 50% were collapsed with TreeCollapseCL v4 (<http://emmahodcroft.com/TreeCollapseCL.html>).

2.6.7 Genetic diversity and divergence

We calculated nucleotide diversity within lineages (π), Tajima's D, weighted FST and divergence (Dxy) between lineages in non-overlapping windows of 50 kb. Global and per window π , Tajima's D and FST were calculated from bam files using ANGSD (Korneliussen et al. 2014). Genotype likelihood model of SAMtools was used (-GL 1) and site allele frequency likelihood was calculated using -doSaf 1 option. Basic filtering was performed by discarding reads not mapping uniquely (-uniqueOnly 1), with SAM flag higher or equal 256 (-remove_bads 1), with mate unmapped (-only_proper_pairs 1) or with mapping quality below 1 (-minMapQ 1). Base qualities were adjusted around INDELS (-baq 1) and mapping quality for reads with

excessive mismatches was adjusted (-C 50). To estimate Dxy, we used calcDxy.R script supplied with ngs-tools program (Fumagalli et al. 2014). To visualize diversity in each lineage, we generated a heatmap for 2324 SNPs with no missing genotypes colouring two states: major and minor allele. Genotypes were clustered using hierarchical cluster analysis based on euclidean distances between pairs of genotypes in R (R Core Development Team).

2.6.8 Phylogenetic discordances across the genome

We used Twisst v.0.2 (Martin & Belleghem 2010) to explore the extent of phylogenetic discordance. Twisst subsamples topologies of a small number of samples from a tree, where each sample is a single representative of a predefined group, and calculates (weights) proportions of possible topologies. We weighed 15 possible topologies of 4 lineages and an outgroup (AME1, AME2, NOV, OU and *O. himal-ulmi*) in non-overlapping windows of 100 SNPs across the genome (corresponding to 1411 bp windows on average). Windows with missing information for at least one isolate were excluded. Per-window trees were built with RAxML v8.2.9 (Stamatakis 2014), with GTRCAT model of nucleotide substitution without rate heterogeneity. To eliminate the potential errors in the tree, we averaged weighed topologies across 10 bootstrap trees for each window. We also checked the tree weighting using only windows containing 3 or more segregating sites in either OU or ONU. We used the complete method, in which the number of sampled subtrees of 5 taxa is equal to the total number of possible subtrees. The proportions of each topology in consecutive windows were smoothed using loess function in R93. The analysis was repeated for windows of 50 kb for comparison.

2.6.9 Gene flow between DED-causing *Ophiostoma* lineages

We built a phylogenetic network using set of SNPs without any missing information ($n = 68,061$ SNPs), as described for the mitochondrial DNA. To detect gene flow among AME1, AME2, NOV and OU, we calculated D-statistic (Green et al. 2010, Durand et al. 2011) using *O. himal-ulmi* as an outgroup. Given a topology of four taxa, $((P1,P2),P3),O$, where O is an outgroup, the expected amount of shared variation between P3 and P1 and between P3 and P2 is the same and gives D-statistic equal 0. Gene flow between any of the two pairs will skew the D-statistic towards negative (gene flow between P3 and P2) or positive values (gene flow between P3 and P1). As gene flow is expected to occur between any pair of the 4 *Ophiostoma* lineages, we first identified the source populations comprising isolates from each lineage with

least admixture from any other lineage based on genome wide PCA results (Fig. 1c, d). 8 AME1, 5 AME2, 8 NOV and all (21) OU isolates were selected. None of the D-statistics testing gene flow between these source populations was different from 0 (Supplementary figure 10). Then, we designed 9 tests, 3 of which tested for gene flow from OU to AME1, AME2 or NOV, and 4 of which tested for gene flow among AME1, AME2 and NOV. In each test, the target population was always set as taxon P2, the source population as taxon P3, and P1 was another source population belonging to the same lineage as P2. In this setting, gene flow between P1 and P3 is absent, thus we can expect either D-statistic equal 0 in case of no gene flow between P2 and P3, or negative D-statistic in case of gene flow between P2 and P3. To detect gene flow between AME1, AME2 and NOV, we excluded any potential regions of introgression from OU, based on the analysis in the following paragraph. D-statistic was estimated for the filtered set of biallelic SNPs using scikit-allel v1.2.0 package in python v3.6. In each performed test, SNPs which were fixed for the same allele in a pair (P1,P2) or (P3,O) were removed. Standard error was estimated with block-jackknife procedure using block sizes of 200 sites. Estimated of D-statistic with more than 3 standard errors from 0 ($|Z\text{-score}| > 3$), were regarded as significantly different from 0, and therefore indicate gene flow.

2.6.10 Identification of regions of OU introgression

Across the genome, the most likely value of K identified using Bayesian clustering analysis is $K = 2$, the two groups reflecting the two species OU and ONU. In such cases, we expect that ONU isolates harboring introgression from OU cluster with OU isolates in introgressed regions, while on the rest of their genome they cluster with other ONU isolates. To test this, we performed a Bayesian clustering analysis, implemented in the STRUCTURE software v2.3.483 (Falush et al. 2003), in 200 kb non-overlapping windows along the genome. For each window, the number of potential K clusters was assessed from 10 different runs of K ranking from 1 to 5. To ascertain adequate convergence of the Markov Chain Monte Carlo (MCMC) model, we allowed a burn-in of 50,000 iterations, followed by 100,000 MCMC replications without any prior geographic information on the putative affiliation of individuals. To identify the most likely value of K, the ad hoc statistic ΔK was calculated as described in (Evanno et al. 2005). CLUMPP v1.1.2 (Jakobsson & Rosenberg 2007) was subsequently used to find the optimal alignment of independent runs by averaging the 10 runs. The resulting most likely grouping of populations for each window was plotted using circos v0.69-6 (Krzywinski et al. 2009). VCFtools v0.1.13

(Danecek et al. 2011) was used to split the vcf file into 200 kb windows, Plink v1.9 (Purcell et al. 2007) (<http://pngu.mgh.harvard.edu/purcell/plink/>) was used to convert each window vcf into STRUCTURE format, both steps using GNU Parallel (Tange et al. 2011). We also performed a PCA, which has less assumptions, in the same 200 kb non-overlapping windows along the genome. For each window, we computed the distance of each isolate from the mean value of OU isolates along the first principal component. This distance was then divided by the maximum distance obtained in the window, so that the value is normalized across windows. VCFtools v0.1.13 (Danecek et al. 2011) was used to split the vcf file into 200 kb windows, Plink v1.9 (Purcell et al. 2007) (<http://pngu.mgh.harvard.edu/purcell/plink/>) was used to convert each window vcf into a plink format used by the R package adegenet (Jombart 2008, Jombart & Ahmed 2011) to perform the PCA. The normalized distance of each isolate from the mean value of OU isolate along the first principal component was then visualized using circos v0.69-6 (Krzywinski et al. 2009).

2.6.11 Functional analysis of IRs

To search for enrichment of functional categories (Gene Ontology, GO) regions introgressed from OU, we annotated proteins of *Ophiostoma novo-ulmi novo-ulmi* H327 isolate using current nr database (release 22-01-2018). To find homologous proteins, we used DIAMOND blastp v0.9.22 (Buchfink et al. 2015) with default e-value 0.001, sensitive option and salltitles option to print subject description. We used Blast2GO Basic v5.2 (Götz et al. 2008) to obtain GO terms and InterProScan v69.0 (Jones et al. 2014) to assign protein families, protein domains, and detect signal peptides. We used EffectorP v1.0 (Sperschneider et al. 2016) to search for effector proteins among identified signal peptide proteins. For enrichment of differentially expressed genes, we used two expression profiles (DEG39 and DEG9) found by (Nigg et al. 2016), comprising genes with increasing expression during yeast to hyphae transition (DEG39) and with decreasing expression during the yeast to hyphae transition (DEG9). Annotations of carbohydrate-active enzymes (CAZYmes), cytochromes and mitochondria related genes were obtained from jgi Mycocosm database (31-01-2019). Overrepresentation of functional categories was tested using two-sided Fisher's Exact test, and P-values were adjusted using FDR correction (Benjamini & Hochberg 1995) in R93.

2.6.12 Introgression of the mating type

To identify the mating type in each isolate, we measured read coverage in three sexual compatibility type loci: MAT1-1-1, MAT1-1-2 and MAT1-1-3. MAT1 isolates harbour all three loci, whereas MAT2 isolates harbour only a truncated version of locus MAT1-1-1 (Comeau et al. 2014). We estimated mean coverage as the mean number of reads covering one bp in the locus divided by the mean number of reads covering one bp in the whole genome. Since the reference genome H327 is a MAT1, isolates with standardized coverage in MAT1-1-2 and MAT1-1-3 above 0.1 were classified as MAT1 and below 0.1 as MAT2.

ML phylogenies for two genes adjacent to both sides of the sexual compatibility type loci ($n = 216$ and $n = 111$ SNPs, respectively) were built with RAxML v8.2.12 (Stamatakis 2014), with GTRCAT model of nucleotide substitution without the rate heterogeneity model and with 100 rapid bootstraps. The same settings were used to recover phylogeny of a 200 kb window in the distal part of chromosome 8 ($n = 16262$ SNPs). In both cases, nodes with bootstrap support less than 50% were collapsed using TreeCollapseCL v4 (Hodcroft Emma, <http://emmahodcroft.com/TreeCollapseCL.html>). Phylogenies were drawn using ETE v3 (Huerta-Cepas et al. 2016).

2.6.13 High-diversity regions in ONU

To identify high diversity regions in each *Ophiostoma* lineage, we reshuffled all positions in the genome 1000 times, and with each reshuffling, we recalculated average nucleotide diversity (π) for 50 kb non-overlapping windows. 99th percentile of the resulting π distribution was selected as a threshold for selecting outlier windows with exceptionally high π . High diversity windows separated by no more than 2 low diversity windows (maximum of 100 kb) were joined to form regions of high diversity.

2.6.14 Identification of ONU with OU ancestry within genes

We used the gene annotations from the H327 assembly (Comeau et al. 2014) to extract coding sequences from alignments. We used gene topologies to determine ONU isolates which are identical to OU or are clustering with other OU. Maximum likelihood gene phylogenies were generated with RAxML v8.2.12 (Stamatakis 2014), with GTRCAT model of nucleotide substitution without rate heterogeneity and with 100 rapid bootstraps. Identification of OU-like ONU was done using ETE v3 (Huerta-Cepas et al. 2016). For each ONU isolate we identified

on the gene tree at least eight most closely situated leaves (isolate). The isolate was labelled as OU-like if for the selected group of related leaves, the proportion of OU isolates was higher than 50%. All OU-like isolates were subsequently removed from fasta alignments. In most genes we removed at most 11 ONU isolates, however in the case of 21 genes almost all AME1 isolates had to be removed. In these genes' topologies AME1 clustered with OU. We removed these genes from further tests.

2.6.15 Selection test

We looked for strength and sign of natural selection in ONU genes using a non-parametric method based on the McDonald-Kreitman test (McDonald & Kreitman 1991) implemented in SnIPRE (Eilertson et al. 2012). Polymorphic and fixed, synonymous and nonsynonymous mutations in all genes were counted in PopGenome v2.6.1 (Pfeifer et al. 2014), for all ONU isolates together (after removing OU-like isolates) and with *O. bimal-ulmi* as an outgroup. Synonymous and nonsynonymous sites per gene were calculated in mstatspop v0.1 (<https://github.com/CRAVENOMICA/mstatspop>). 8432 genes with at least one polymorphic or fixed substitution were included. Selection and constraint effects and coefficients were estimated with Bayesian implementation of the method. We ran 50,000 iterations after a burn-in of 10,000 and sampled every fourth value. The priors for random effects had a normal distribution with mean equal 0 and precision equal 0.1 and multivariate normal distribution with mean equal (0,0,0,0) and precision being a hyperparameter with Wishart distribution W(S4x4, 10), where S4x4 stands for identity matrix. MCMC samples were used to estimate credible intervals of selection and constraint effects.

2.6.16 Dxy outliers

R package PopGenome (Pfeifer et al. 2014) was used to compute Dxy in pairwise comparisons between NOV, AME1 and AME2. We looked at genes with highest Dxy among pairs of ONU. Jgi Mycocosm website (Grigoriev et al. 2012) was used to download lists of genes involved in following GOs: ABC transporters, major facilitator superfamily (MFS), cytochromes P450 and extracellular carbohydrate-active enzymes (CAZYmes). Lists of genes overexpressed in yeast and mycelial form were retrieved from (Nigg et al. 2016). Pairwise comparisons for GOs against the rest of the genes were tested using two-way Wilcoxon-Mann-Whitney nonparametric ranking test.

2.6.17 Inversion between AME1 (and OU) and AME2 (and NOV)

Several isolates of AME1 ($n = 9$) and AME2 ($n = 6$) were assembled using SPAdes v3.9.1 (Bankevich et al. 2012) as described for *O. himal-ulmi*. All pairs of assembled genomes were aligned and compared visually using D-Genies v1.2.0 (Cabanettes & Klopp 2018). The exact coordinates of inverted regions were identified by doing pairwise alignments with NUCmer v3 (Kurtz et al. 2004). ML phylogeny of 29 concatenated genes ($n = 3190$ SNPs) located in the inversion was built with RAxML v8.2.12 (Stamatakis 2014), with GTRCAT model of nucleotide substitution without rate heterogeneity and with 100 rapid bootstraps.

2.6.18 Growth assay

We measured growth of 89 isolates in all four lineages. Liquid cultures of the isolates were maintained in a solution of glycerol 50% at -80°C in a 96 well plate. From those stocks, 2 μ l were used to inoculate MEA media, isolates were grown for 3 days at room temperature (RT) in 6-well plates (Greiner Bio-one, Kremsmünster, Austria). After growth, 4 to 9 mm² of mycelium were transferred to 4 mL of liquid minimal medium (Bernier & Hubbes 1990) (MM; MM recipe can be found in Supplementary Table 15) and grown for 3 days at RT in 24 deep well plates (Corning, Tewksbury MA, USA). Optical density of the cultures was estimated using 96 well plates (Greiner Bio-one, Kremsmünster, Austria) and a Tecan infinite 200F pro platform (Tecan Männedorf Switzerland). Optical density of the cultures was adjusted to approximately 1.0 OD600. 5 μ l of these dilutions were spotted on two Omnitray plates, one containing MEA and the other containing MEA supplemented with 0.2 mM of 1,2-naphthoquinone (Sigma-Aldrich, St-Louis, MO, USA). Plates were incubated for 4 days at 20°C, 25°C and 30°C in a spImager custom platform (S&P Robotics Inc, Toronto, Canada) and pictures were taken every 2 hour with an EOS Rebel T5i camera (Canon, Tokyo, Japan). For each temperature, the screen was replicated 3 times with randomized positions. Images were processed with a custom script using the R package EBImage (Pau et al. 2010). Pixel number was extracted from each isolate position. Maximum growth rate was estimated as the second highest slope value of the regressions performed on sliding windows using two measurements per window. We fitted several linear mixed models using the package lme4 (Bates et al. 2007) while including growth as a response variable and media, lineage, temperature, PCs and their interaction as explanatory variables. Replicates were included as a random effect. Model fit was checked using graphical diagnostics and we defined the best model as the one with the lowest Aikake Information Criterion (AIC).

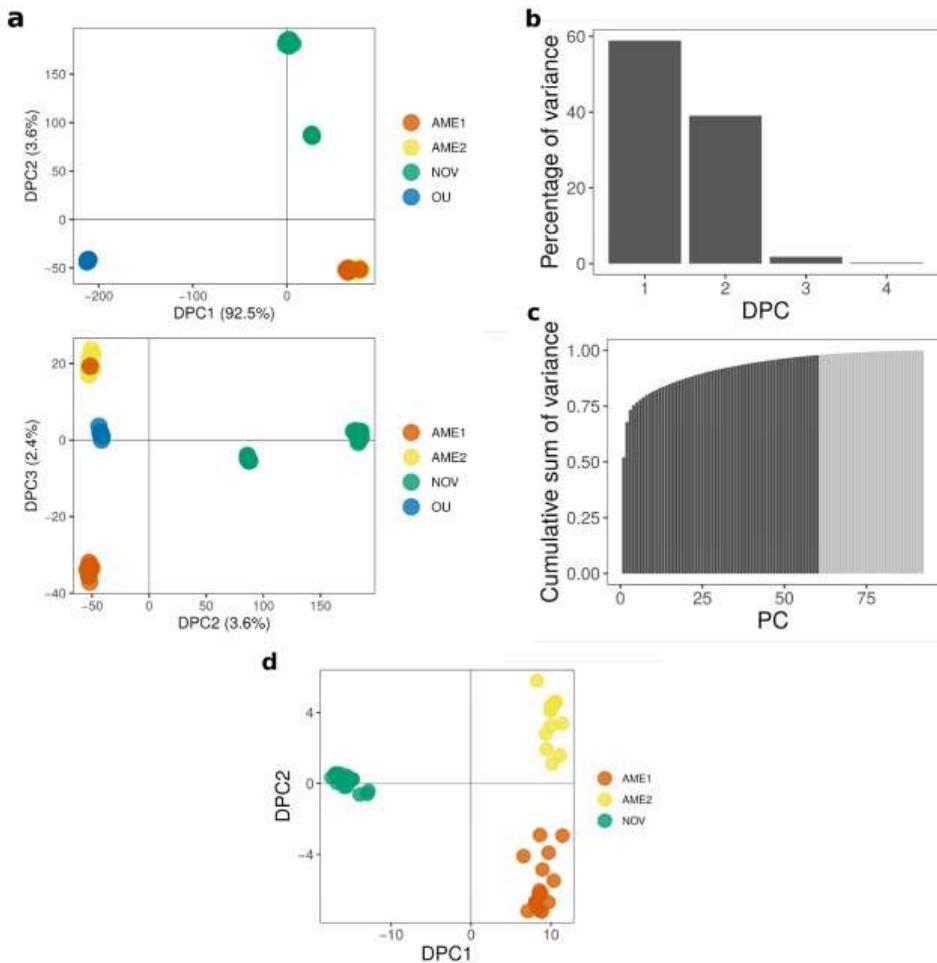
(Akaike 1974). To test specifically for the effect of introgression on growth in ONU, we constructed a mixed model comprising media, temperature, lineage, introgression (0 = introgressed isolates, 1 = introgressed isolate) and their interaction while excluding OU isolates. Post-hoc tests were conducted using emmeans package (Length 2018).

2.6.19 Virulence assay

Golden Delicious (Plourde & Bernier 2014) apples were inspected visually to eliminate damaged or bruised fruits, washed with soft soap in warm water and surface-disinfected with 70% ethanol. Apple tissue was removed with a 9 mm diameter metal cork borer. The 10 mm deep hole was filled with a MEA plug bearing fungal mycelium, with mycelium facing inwards. A piece of transparent masking tape was placed over the inoculation site to prevent outside contamination and desiccation. Apples were kept in the dark at 25°C in a growth chamber for 28 days. Necrosis diameter was measured 14- and 28-days post-inoculation (14 dpi and 28 dpi). Each measurement of necrosis was taken twice (vertically and horizontally) at both 14 dpi and 28 dpi. The virulence of 85 isolates (AME1: n = 18, AME2: n = 25, NOV: n = 23, OU: n = 19) was evaluated on four different apple replicates per sample. Each apple suspected of contamination was removed from the dataset, resulting in a mean inoculation success of 65%. Given the large number of apparent contaminations at 28 dpi, we retained only the measures taken at 14 dpi. Measurements of necrosis size at 14 dpi were strongly correlated (estimate = 0.7274 ± 0.03577 , P-value < 2.2e-16) and a linear model was fitted between the two sets of measures. The predicted values were retained as a more precise evaluation of necrosis size than mean between measures. To assess the validity of the test, we verified that necrosis size was not correlated with growth rate in MEA (Pearson's correlation test: t = 0.02353, df = 81, P-value = 0.7851)31. To investigate the effect of introgression, we built a mixed linear model with only ONU isolates to test for the effect of genetic lineages and introgression on chromosomes 1, 2, 6 and 8, while adding the apple number as a random effect (representing the replicate).

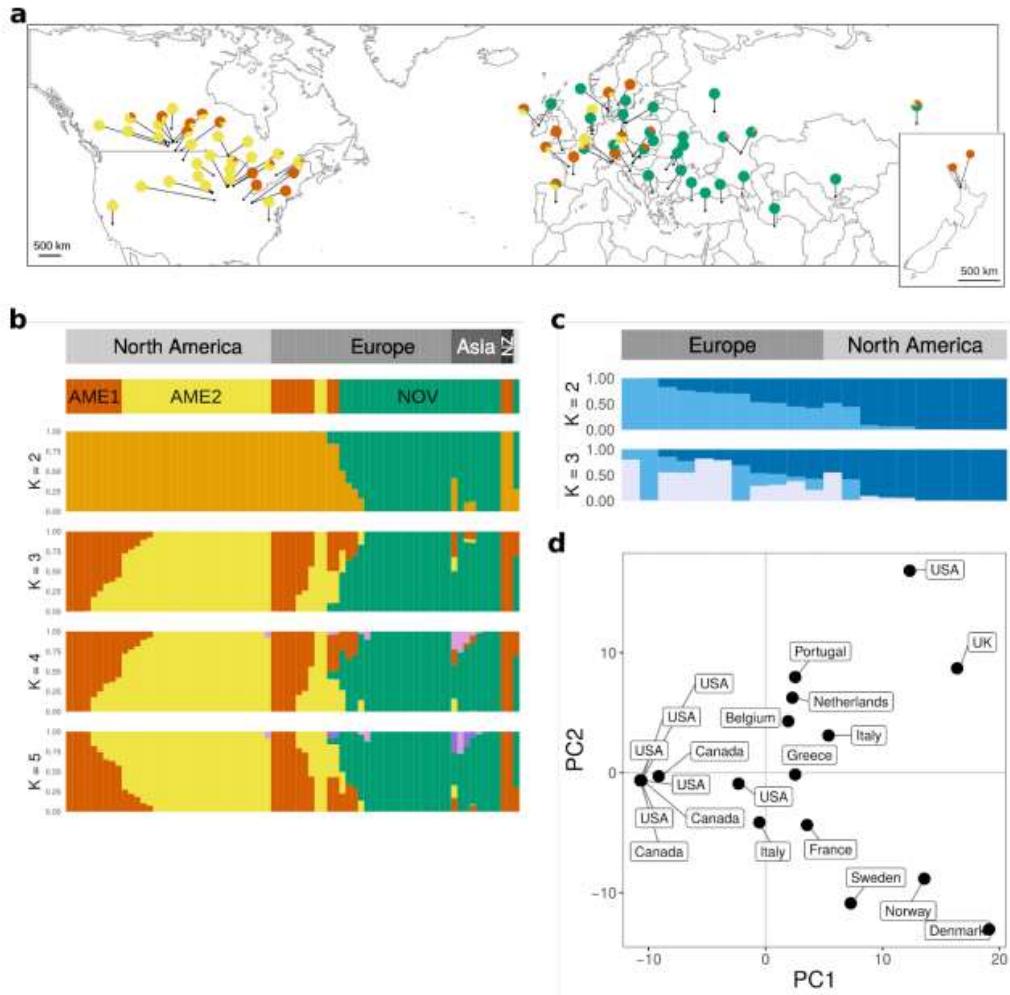
2.7 Supplementary materials

All supplementary materials for this study can be found at
<https://www.nature.com/articles/s41559-020-1133-6>



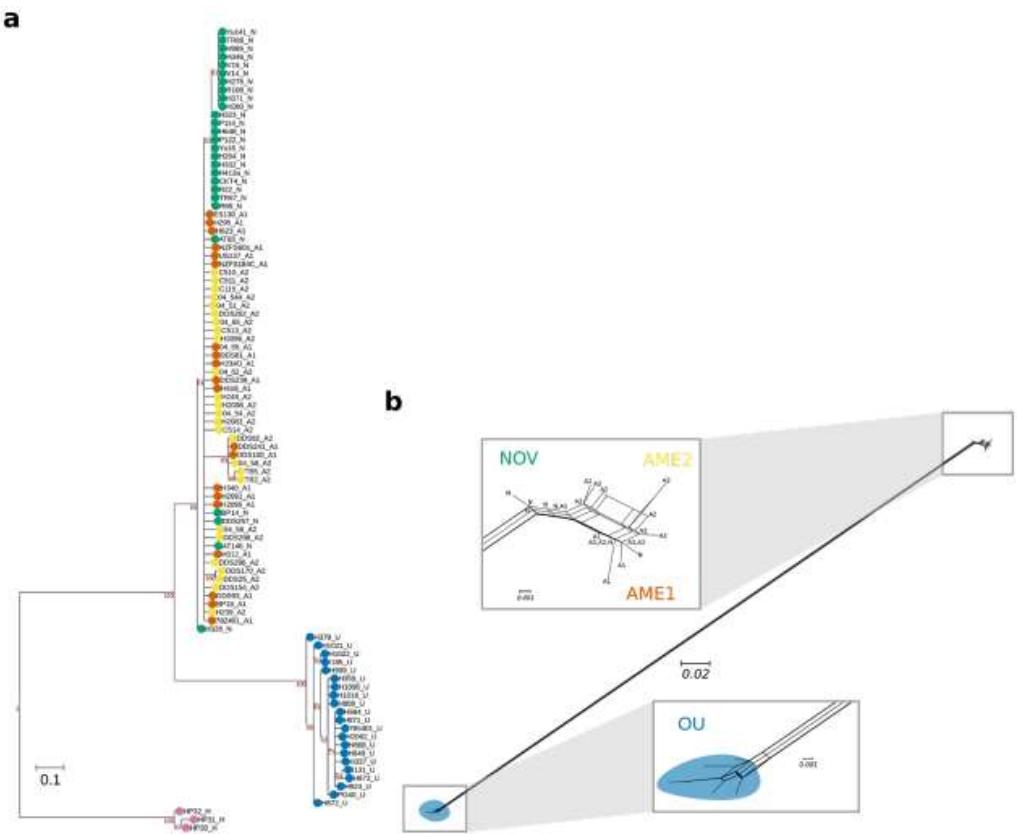
Supplementary figure 1. Discriminant analysis of principal components (DAPC) based on small copy number variants (**a-c**) and single nucleotide polymorphisms (**d**) supports clustering of *Ophiostoma* isolates into four lineages.

a, Copy number variants principal component (PC) 1 separates ONU from ONU, whereas PC 2 separates AME1, AME2 and NOV, except for two isolates. **b**, Percentage of variance explained by the first four PCs. Most variance in copy number variants comes from differences between ONU and ONU. **c**, Cumulative distribution of PCs shows that PCs higher than four do not add substantial information. **d**, Identification of three ONU lineages with DAPC. AME1, AME2 and NOV are clearly distinguished with first and second PCs.



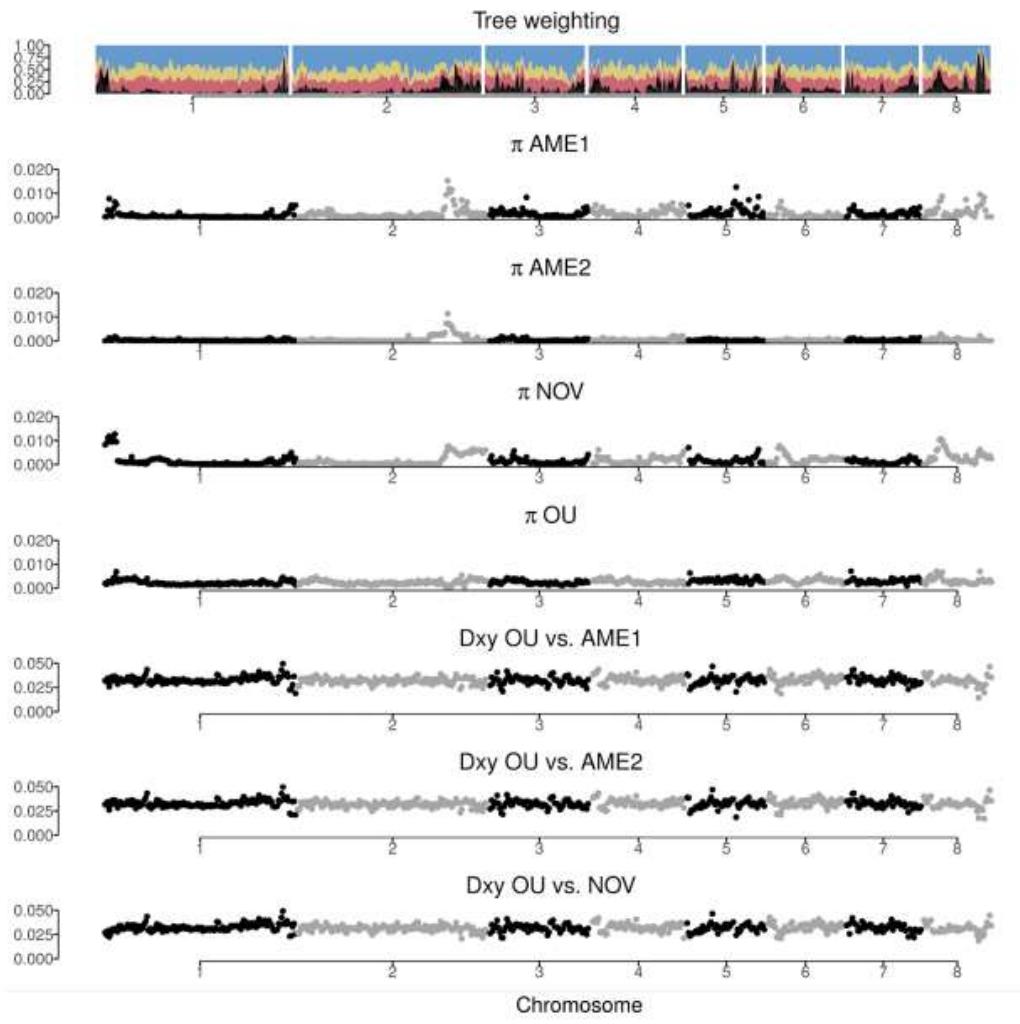
Supplementary figure 2. Population genetic structure of ONU and OU.

a, Bayesian clustering of isolates of ONU with $K = 3$ clusters. The bottom right rectangle shows isolates from New Zealand. **b**, Bayesian clustering of isolates of ONU for $K = 2$ to 5. Isolates are sorted according to geographic sampling location. NZ refers to New Zealand and the last isolate has unknown location. The second band shows assignment to 3 lineages: AME1 (red), AME2 (yellow) and NOV (green). NOV is found only in Europe and Asia, and AME is found mostly in North America. Most interlineage admixture is found in Europe. Several NOV and AME isolates show a unique genetic component, suggesting introgression from a distinct, unsampled isolate (or/and OU), or unique genetic composition. **c**, Bayesian clustering of isolates of OU for $K = 2$ and 3. **d**, First two principal components obtained using genome-wide nucleotide variants in OU separate most North American and European samples.



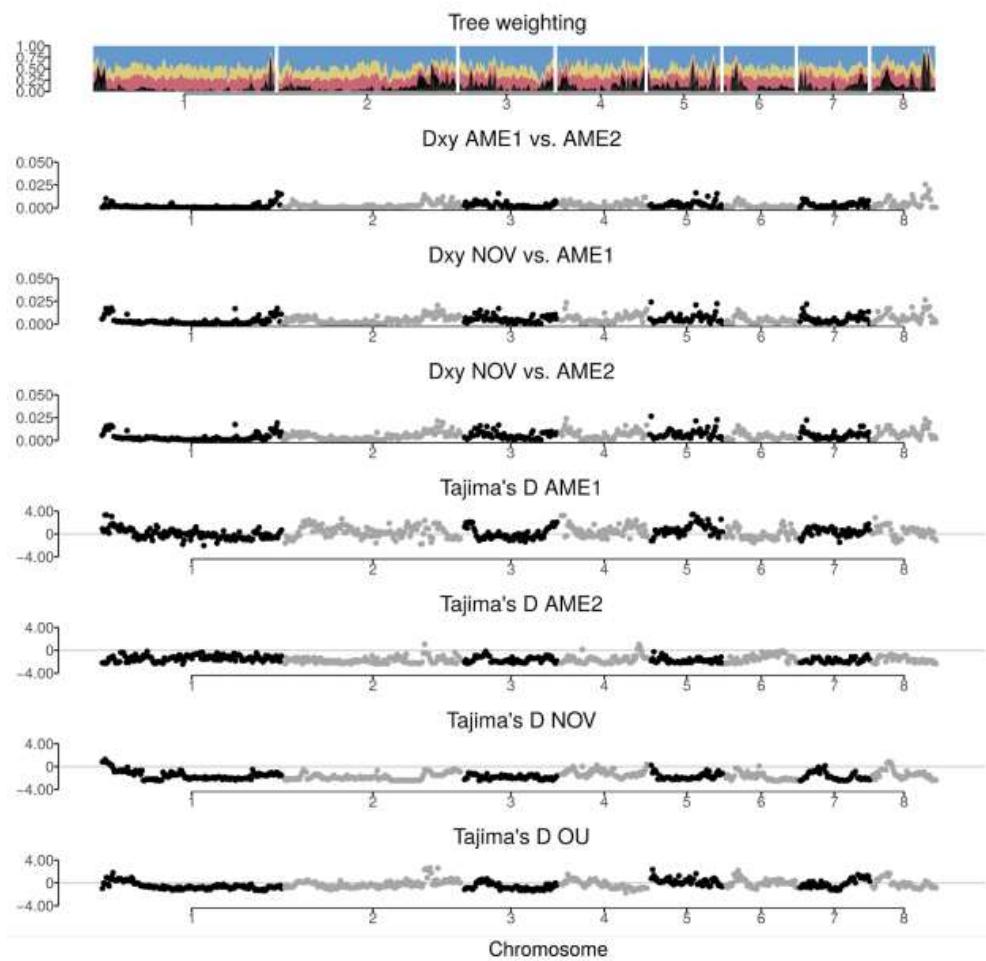
Supplementary figure 3. Maximum likelihood phylogeny and phylogenetic network of mitochondrial genome discriminate between *Ophiostoma* species, but not among ONU lineages.

a, Maximum likelihood tree based on 838 filtered SNPs from the mtDNA genome shows no strong support for NOV, AME1 and AME2 lineages. Isolates with pink colour correspond to *O. bimai-ulmi*. **b**, A phylogenetic network based on a subset of 269 SNPs with no missing genotypes from the mtDNA genome shows dense reticulation among NOV, AME1 and AME2 lineages.



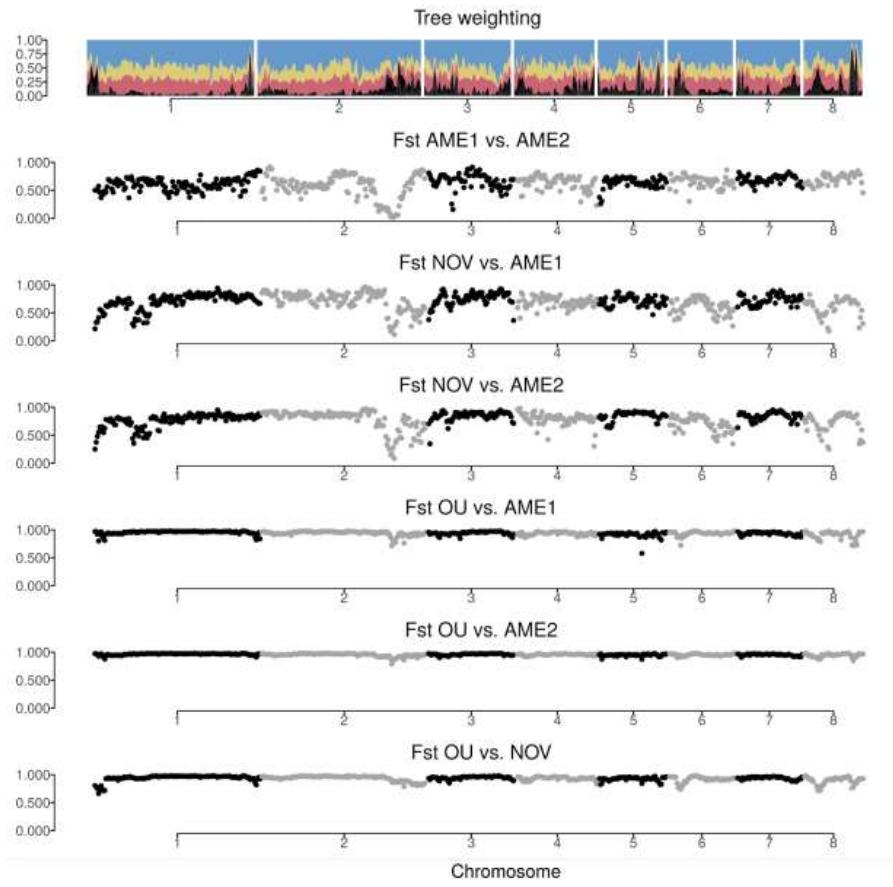
Supplementary figure 4. Uneven distribution of genetic diversity among ONU and divergence between ONU and OU.

Tree weighting corresponds to proportions of different topologies calculated for non-overlapping windows of 100 SNPs with *O. bimaculata* as an outgroup. Black topologies indicate discordant trees with unexpected branching of OU. Blue topologies reflect the species tree (Fig. 1), whereas yellow and pink topologies put NOV with AME2 or NOV with AME1 as sister lineages, respectively. Nucleotide diversity (π) and divergence (Dxy) were calculated in non-overlapping windows of 50 kb across eight chromosomes.



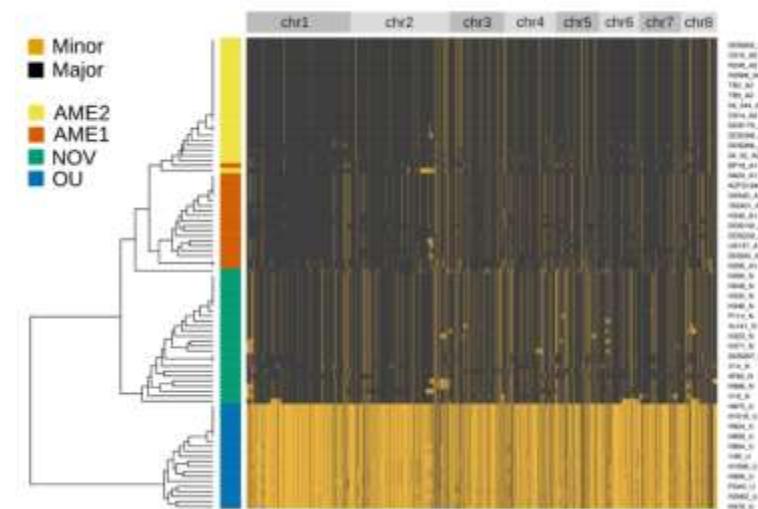
Supplementary figure 5. Genetic divergence among ONU is elevated in regions of phylogenetic discordance.

Tree weighting corresponds to proportions of different topologies calculated for non-overlapping windows of 100 SNPs with *O. himal-ulmi* as an outgroup. Black topologies indicate discordant trees with unexpected branching of OU. Blue topologies reflect the species tree (Fig. 1), whereas yellow and pink topologies put NOV with AME2 or NOV with AME1 as sister lineages, respectively. Negative Tajima's D in AME2 and NOV suggest population expansion, background selection or widespread selective sweeps. Positive Tajima's D in AME1 could be explained by some genetic structure within the lineage. Genetic divergence (Dxy) and Tajima's D were calculated in non-overlapping windows of 50 kb across eight chromosomes. On the Tajima's D plots, horizontal line indicates 0.



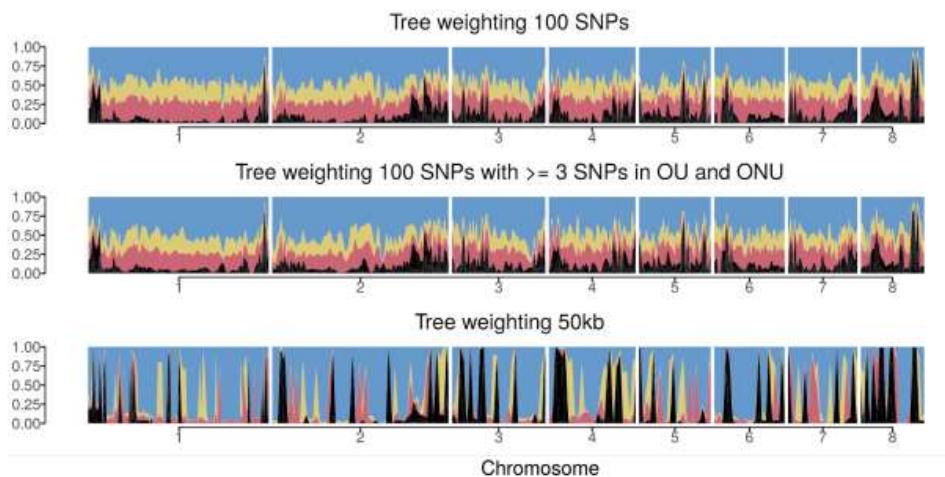
Supplementary figure 6. FST decreases in regions of phylogenetic discordance.

Tree weighting corresponds to proportions of different topologies calculated for non-overlapping windows of 100 SNPs with *O. bimaculata* as an outgroup. Black topologies indicate discordant trees with unexpected branching of OU. Blue topologies reflect the species tree (Fig. 1), whereas yellow and pink topologies put NOV with AME2 or NOV with AME1 as sister lineages, respectively. FST was calculated in non-overlapping windows of 50 kb across eight chromosomes.



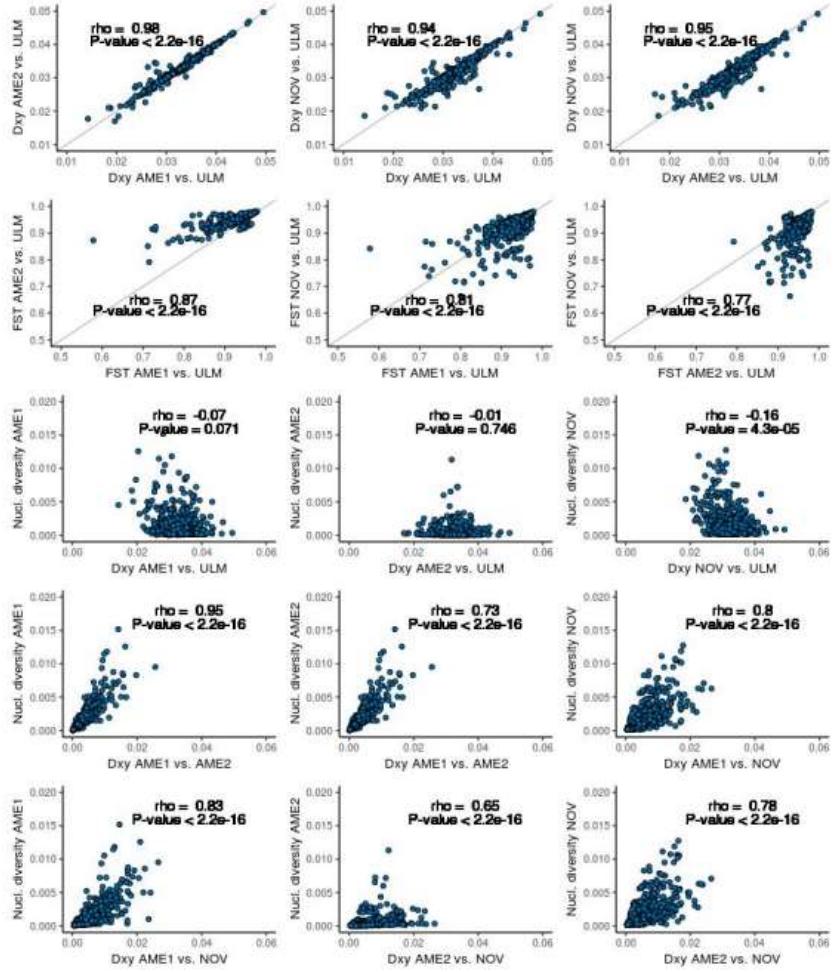
Supplementary figure 7. Genotypes of all isolates cluster into 4 lineages.

Haploid genotype heatmaps are determined according to major (black) and minor (orange) alleles for a subsample of 2324 genomic SNPs with no missing data mapped to a reference genome H327 (NOV). Lineage assignment is shown with colours in row labels, and is based on genome-wide DPCA analysis. The grey bar on the top shows chromosomes.

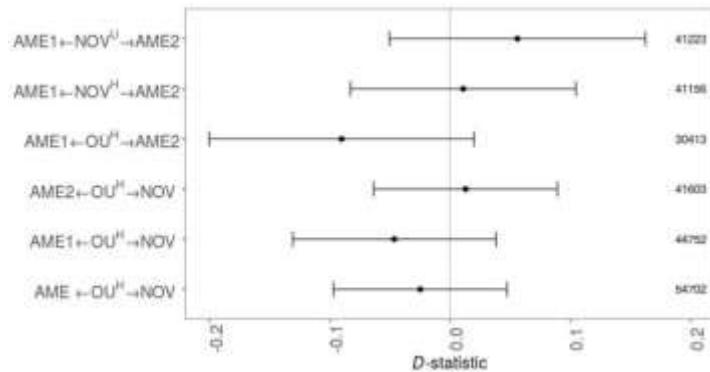


Supplementary figure 8. Phylogenetic discordance across the genome.

Tree weighting corresponds to proportions of discordant trees in windows of 100 SNPs (top and middle) and 50 kb (bottom). Middle row shows tree weighting for 100 SNP windows, with windows having less than 3 SNPs in OU or ONU removed. Black topologies indicate discordant trees with unexpected branching of OU. Blue topologies reflect the species tree, whereas yellow and pink topologies put NOV with AME2 or NOV with AME1 as sister lineages, respectively. Larger windows include more variation discerning relationships between NOV, AME1 and AME2.

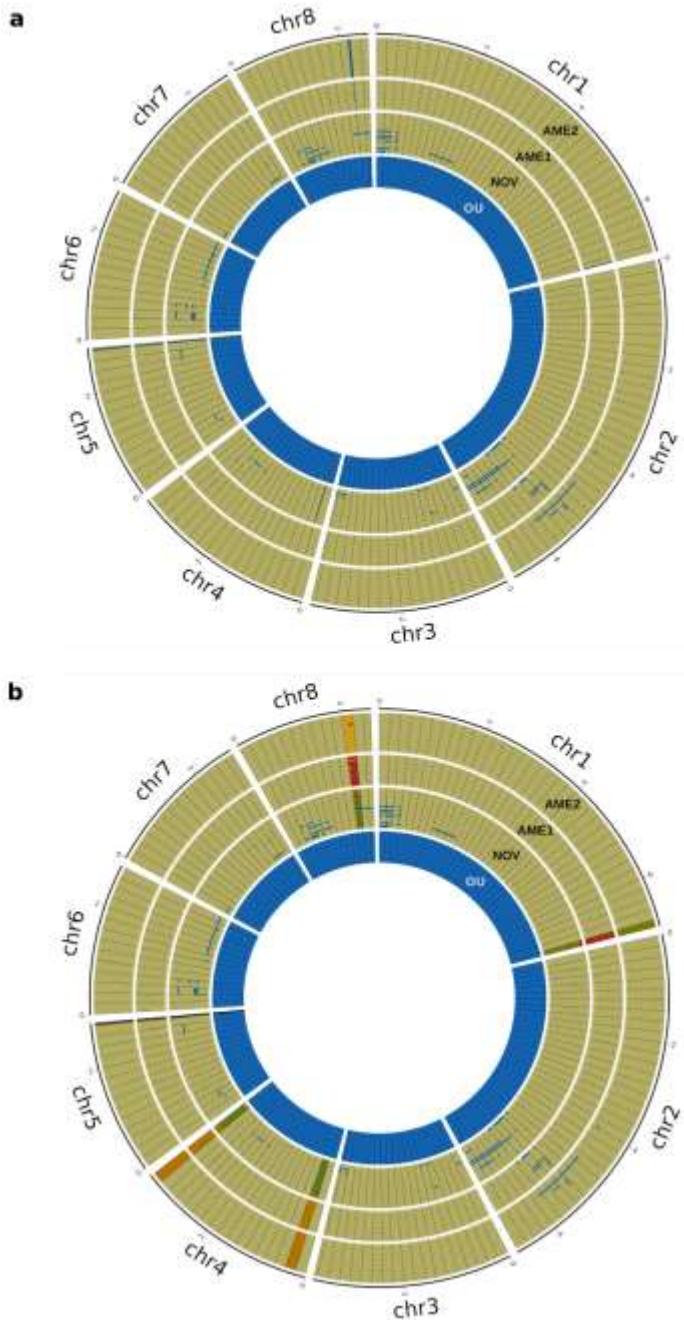


Supplementary figure 9. Genetic divergence and diversity are strongly correlated in *Ophiostoma* genomes. Spearman rank correlation was estimated between pairs of non-overlapping 50 kb windows. Strong correlation in Dxy (top row) and FST (second row) between OU and all ONU are expected if divergence of three ONU lineages was very recent. Dxy between NOV and OU is negatively correlated with diversity in NOV, suggesting introgression from OU to NOV. This is not the case for other ONU (third row). On the other hand, nucleotide diversity in ONU positively correlates with divergence between ONU lineages (two last rows).



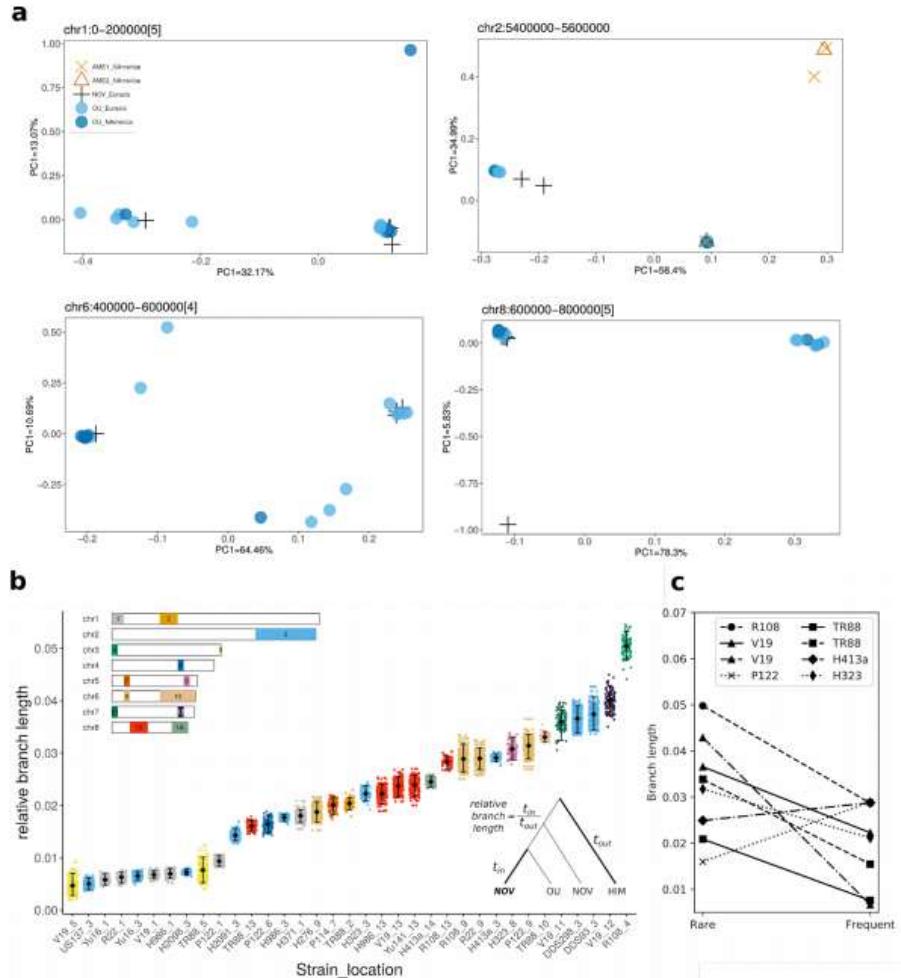
Supplementary figure 10. D-statistics calculated using genome-wide SNPs provide no evidence for gene-flow between pure (non-admixed) populations of AME1, AME2, NOV and OU.

In each test either *O. himal-ulmi* (H) or OU (U) was selected as an outgroup species (shown as a superscript of the second lineage in y-axis labels). Whiskers depict three standard errors of D-statistic ($|Z\text{-score}| > 3$), all of which overlap values of 0, suggesting absence of gene-flow. Values at the right side of the plot indicate number of sites for which statistic was calculated.



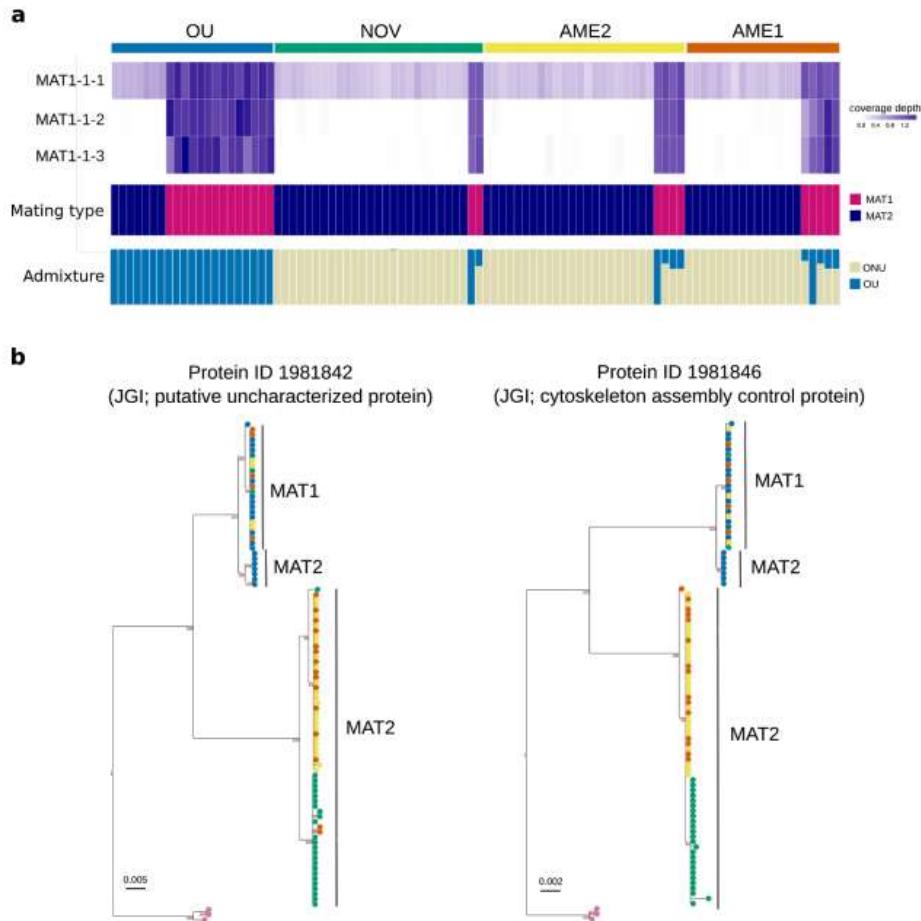
Supplementary figure 11. Regions of introgression from OU to ONU identified with Bayesian clustering in 200 bp non-overlapping windows.

a, Inference of region ancestry performed with $K = 2$ clusters and b, the most likely number of clusters determined in each window. OU ancestry is depicted with blue colour, and ONU ancestry is depicted with green colour. Several windows (chromosome 1, 4 and 8) have ancestry inferred for $K > 2$ clusters, which are shown with orange, green and red colours in b.



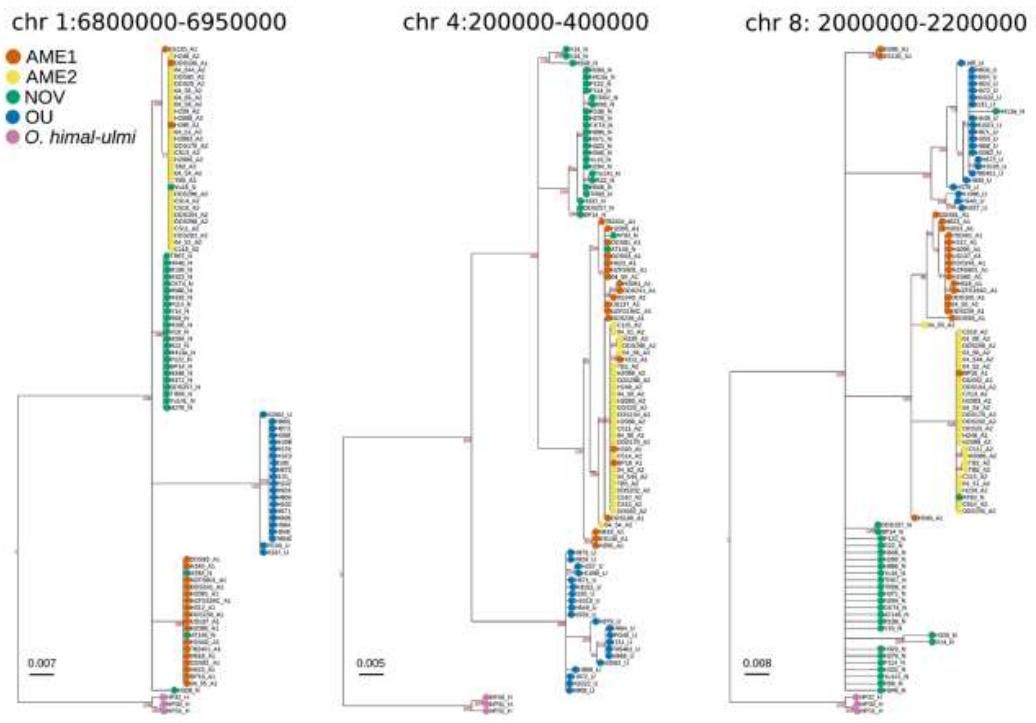
Supplementary figure 12. Introgression from ONU to NOV likely occurred multiple times.

a, ONU isolates group with different clusters of OU in the same introgressed regions in SNP-based PCA. **b**, Relative branch length coloured by location on chromosome (shown on the inset). Means, 95% confidence intervals, and point estimates were obtained by empirical bootstrap (100x). Relative branch length was estimated in all introgressed regions (IR) as an average number of derived mutations in a focal lineage since the split with an average OU relative to the number of mutations since the split with an outgroup. **c**, For the isolates having both rare (single occurrence) and frequent (at least 2 isolates) IR, rare IR are marginally significantly older than frequent IR (Wilcoxon signed-rank test, $W = 4$, P -value = 0.049, two-tailed).

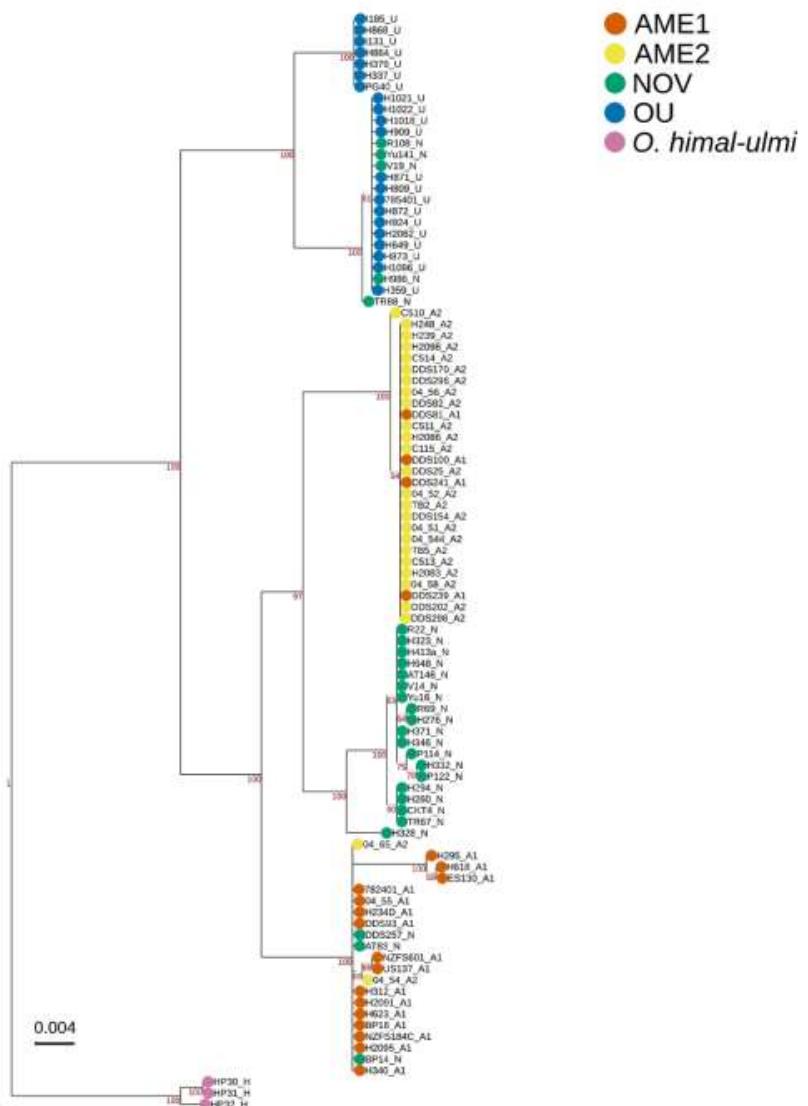


Supplementary figure 13. Regions of introgression from OU to ONU include the mating type and other loci.

a, OU admixture estimated for 94 isolates in 200 kb region, where three MAT-1 loci are located. First three rows show depth of coverage in three MAT-1 loci. Three loci are present only in MAT1 isolates including reference genome H327. The fourth row shows classification of all isolates to one of the two mating types. The last row shows Bayesian clustering analysis using $K = 2$ number of clusters in 200 kb window on chromosome 2, where three MAT-1 loci are located. The blue colour corresponds to OU ancestry and the beige colour corresponds to ONU ancestry (AME1, AME2 and NOV). **b**, Two maximum likelihood trees for genes on both sides of three MAT-1 loci support introgression of the sexual compatibility type region from OU to all ONU lineages. Colours depict lineages as in a. Pink corresponds to *O. himal-ulmi* isolates, which are used as outgroups.

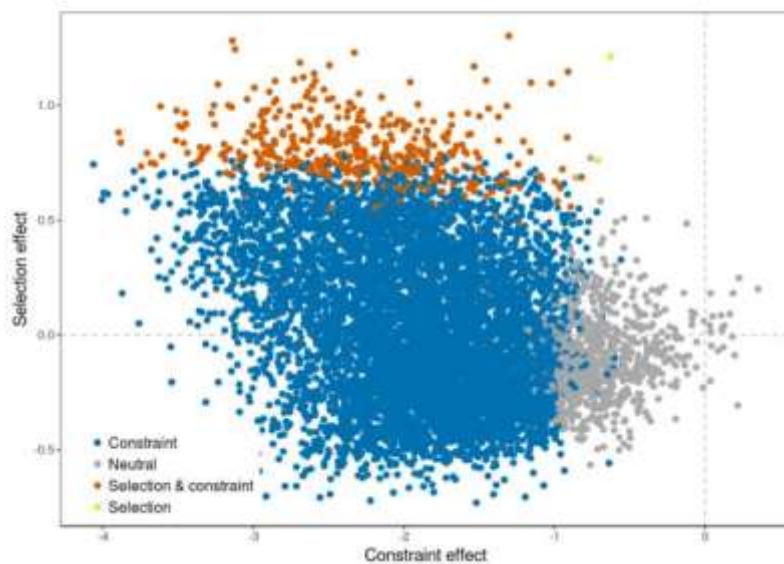


Supplementary figure 14. Maximum likelihood tree of 200 kb regions on chromosomes 1, 4 and 8.
 Isolate colours depict lineages: red - AME1, yellow - AME2, green - NOV, blue - OU and pink - *O.himal-ulmi*.



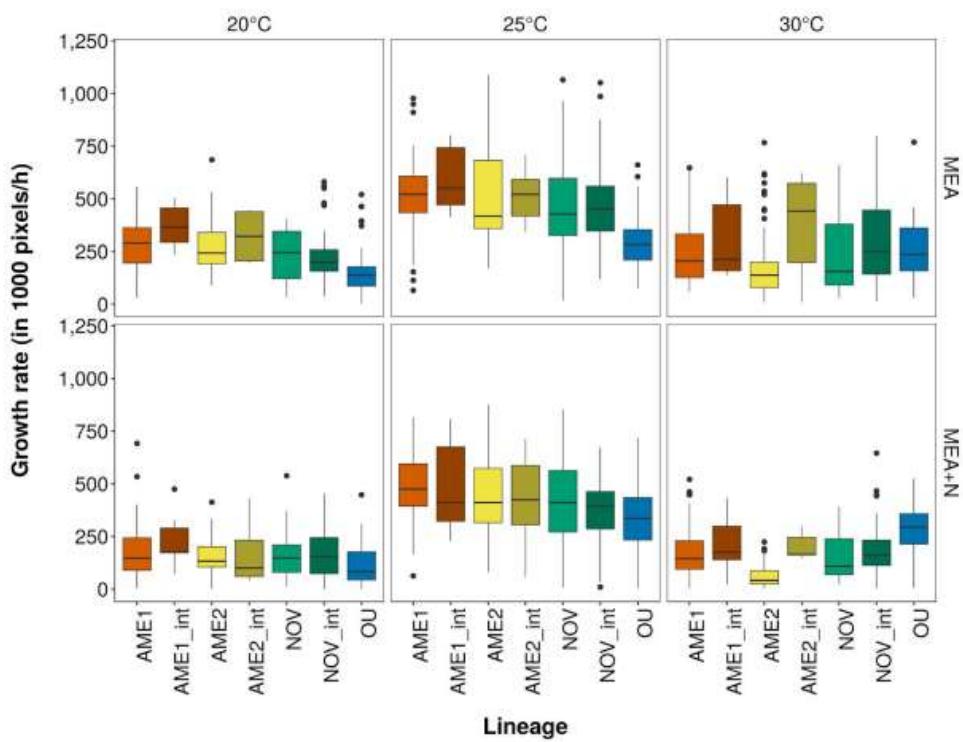
Supplementary figure 15. Maximum likelihood tree built for concatenated alignment of 29 genes within the inversion on chromosome 8.

Isolate colours depict lineages: red - AME1, yellow - AME2, green - NOV, blue - OU and pink - *O. himal-ulmi*.



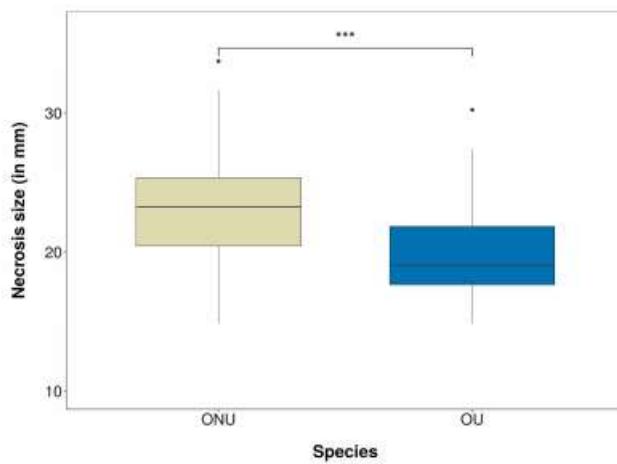
Supplementary figure 16. Estimates of selection and constraint effects in 8432 genes in ONU.

SnIPRE method was used to infer selection across 8432 genes in combined lineages of ONU with *O. himal-ulmi* as an outgroup. For each gene a selection and constraint effect were inferred based on polymorphism and divergence of synonymous and nonsynonymous variants, accounting for across-genome variation. Negative constraint indicates that nonsynonymous polymorphic mutations are fixed or eliminated at a higher rate than synonymous mutations, whereas positive constraint indicates that they are eliminated at lower rate. 78 genes have both positive selection effect and negative constraint, suggesting strong cases of selection.



Supplementary figure 17. Lineage and introgression have a strong impact on growth performance.

Growth rate in 1000 pixels per hour for isolates from each group: AME1 ($n = 16$), introgressed AME1 (AME1_int, $n = 2$), AME2 ($n = 13$), introgressed AME2 (AME2_int, $n = 2$), NOV ($n = 12$), introgressed NOV (NOV_int, $n = 13$) and OU ($n = 21$) with replicates in media MEA and MEA+N at 20°C, 25°C and 30°C. In boxplots, center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range; points, outliers.



Supplementary figure 18. ONU, the most virulent species, has larger necroses than OU, the less virulent species.

Necrosis size measured at 14 days post-inoculation are represented in millimetres and compared between the two species ONU ($n = 66$, with replicates) and OU ($n = 19$, with replicates). In boxplots, center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range; points, outliers.

2.8 Acknowledgements

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Chapitre 3 Adaptation and virulence in Dutch Elm Disease pathogens

3.1 Résumé

La Maladie Hollandaise de l'Orme est une maladie dévastatrice toujours active dans l'hémisphère Nord. Deux espèces d'*Ophiostoma* présentant différents degrés de virulence sont responsables de la maladie : *O. ulmi* (OU) et *O. novo-ulmi* (ONU). Les mécanismes génétiques sous-jacents à la virulence et à la croissance sont mal connus chez les champignons responsables de la MHO. Nous réalisons l'identification de gènes potentiellement associés avec les variations de croissance et de virulence chez OU et ONU en utilisant une approche de type « *Genome Wide Association* » (GWA). Nous trouvons plusieurs gènes candidats associés à la virulence, comme une protéine contenant un domaine CFEM et une pompe d'efflux de type « *HC-toxin* ». Nous identifions également des transporteurs ABC et MFS, des cytochromes et des facteurs de transcription associés à la croissance.

3.2 Abstract

Dutch elm disease (DED) is a highly destructive tree disease currently active in the Northern hemisphere. Two species from the *Ophiostoma* genus, *O. ulmi* (OU) and *O. novo-ulmi* (ONU) displaying different virulence levels are responsible for the disease. The genetic determinants underlying virulence and growth in DED are yet not well known. We performed the identification of OU and ONU genes potentially associated with variations in virulence and growth using Genome-Wide Association (GWA) analysis. We measured necrosis size induced on apples as a proxy for fungal virulence and measured growth rates at three different temperatures and two different media. We found several candidate genes for virulence, such as a CFEM domain containing protein and a HC-toxin efflux carrier. For growth, we identified several important gene families such as ABC and MFS transporters, cytochromes and transcription factors. This study is the first GWA analysis performed in DED pathogens and allowed the discovery of new candidate genes potentially associated with growth and virulence variations in this tree disease.

3.3 Introduction

Fungal diseases have devastating effects both on agricultural, natural and urban ecosystems, resulting in lower crop yields and reduction of biodiversity and ecosystem services (Freer-Smith and Webber 2017; Gurr et al. 2011; Paini et al. 2016). It is becoming increasingly clear that plant diseases can be affected by climate: changes in environmental conditions can affect the pathogen ability to infect a host or sporulate and can also increase the host susceptibility; in some cases, this can result in new disease emergence (Garrett et al. 2016; Velásquez et al. 2018). As the world's climate is changing, we are witnessing modifications in host pathogen interactions, pathogen distribution, host susceptibility or pathogen growth (Eastburn et al. 2011; Simler-Williamson et al. 2019). Understanding the underlying mechanisms involved in fungal pathogen adaptation to their environment is key to model, predict and ultimately prevent the spread and emergence of plant diseases (Ikegami and Jenkins 2018).

There are several methods allowing the identification of loci underlying adaptation in plant pathogens, such as scanning genomes for signatures of selection through population genomics, comparative genomics approaches (Plissonneau et al. 2017) and Quantitative Trait Loci (QTL) mapping (Bamba et al. 2019; Lendenmann et al. 2016; Möller and Stukenbrock 2017; Plissonneau et al. 2017). QTL mapping is a powerful tool for the identification of genomic regions associated with the variation in a trait of interest by using F1 hybrids of a cross between two parents presenting different phenotypes. However QTL has two main limitations : the only genetic diversity that can be screened is the one segregating between the two parents, and second, the amount of recombination occurring during the cross can limit the resolution of the analysis (Korte and Farlow 2013). Genome-Wide Association (GWA) overcome these limitations by using the natural genetic variation present in a large number of individuals. This type of analysis has been widely used to detect resistance and climate-related adaptive loci in plant hosts both in crops and in natural forest (Chhetri et al. 2019; Jorge et al. 2005; Kang et al. 2016; Maulana et al. 2018; Mukrimin et al. 2018). But there are far fewer GWA in fungi due to different factors : the efficiency of using comparative genomics to identify candidate genes, the long linkage disequilibrium often observed in pathogen species due to the mixed mating system, the strong population structure that has to be taken into account to avoid the generation of false positives and lastly the difficulty of phenotyping the pathogenic trait of interest (Bartoli and Roux 2017). While in recent years there has been considerable progress in high-throughput plant phenotyping

(Mutka and Bart 2014; Singh et al. 2016), fungi phenotyping remains complex to dissect (Cairns et al. 2016). Nevertheless, a growing number of studies have overcome these difficulties and have been using these methods to study adaptation in pathogens (Vieira et al. 2019; Zeng et al. 2018). These analyses provide powerful tools to identify candidate genes or genome regions involved in virulence as they allow to link genetic variants with the phenotype of interest, whereas comparative methods identify the variants but cannot link them directly with a phenotype. GWA analyses have now been successfully performed in crop (e.g. *Zymoseptoria tritici* see (Hartmann et al. 2017)) and forest pathogens (e.g. *Heterobasidion annosum* s.s see (Dalman et al. 2013)).

Dutch Elm Disease (DED) is a well-known devastating tree disease, occurring in Europe and in North America (Brasier 2000; Brasier 1991). Two fungal species from the *Ophiostoma* genus are responsible for the two successive destructive pandemic waves observed: *Ophiostoma ulmi* (Buisman) Melin and Nannf. (OU) and *Ophiostoma novo-ulmi* Brasier (ONU) (Brasier et al. 1986). These two species present different aggressiveness and temperature optima (Brasier 1977; Brasier et al. 1981). ONU is organized into three different genetic lineages, NOV occurring in Europe and AME1 and AME2 occurring in both Europe and North America (Hessenauer et al. 2020). While population genetic structure has been extensively described (Brasier 1991; Hessenauer et al. 2020; Mitchell and Brasier 1994), the genes and mechanisms involved in adaptation and virulence are still not well identified (Bernier et al. 2015; Et-Touil et al. 1999). DED is a highly destructive tree disease that is still active in North America and in some regions of Europe (Katanić et al. 2020; Łakomy et al. 2016; Tziros et al. 2017), it is thus of primary importance to identify genes and pathways underlying virulence in these pathogens.

In this study, we use high throughput sequencing and phenotypic data to identify new genes potentially associated with virulence and growth at different temperatures and in different media for the first time in DED. We perform a GWA analysis to explore genetic variation underlying adaptation and discover new candidate genes for functional characterisation. We also find a few gene families potentially associated with growth and virulence phenotypes. We use available RNAseq data and prediction programs to investigate the expression pattern of the candidate genes and gene families we discovered and predict their functional impact on the phenotype.

3.4 Material and methods

3.4.1 Sample preparation and extraction

We used the sequences of 91 of the isolates published in (Hessenauer et al. 2020), and added 78 new isolates collected in North America and 3 collected from Europe from 2018 to 2020. We obtained a total of 169 isolates of OU ($n = 21$) and ONU ($n = 165$) from a collection that comprises samples from Eurasia (25 countries), North America (4 Canadian provinces and 10 states of America) and New Zealand and encompasses more than 50 years of the outbreak (Fig. 1, Table 1). Import permits of *Ophiostoma* samples were obtained from the Canadian Food Inspection Agency (CFIA). The isolates were handled in a Plant Pest Containment Level 1 (PPC-1) CFIA-approved facility. The 78 new isolates were grown on malt agar extract (MEA 5%) and the mycelium was harvested for DNA extraction using a CTAB chloroform protocol. Illumina Nextera XT library preparation kit was used to perform library preparation. Samples were pooled into a single lane for genome sequencing on the Illumina HighSeqX (PE 150bp) platform. The data generated an average coverage of 40X. Read quality was assessed using Fastqc v0.11.8.

3.4.2 Reads mapping and SNP calling

Raw reads obtained for the 78 new isolates were cleaned using Trimmomatic v0.36. OU and ONU reads were mapped onto the reference genome H327 (ONU, NOV lineage) using BWA v0.7.17. BAM files were retrieved for the 91 samples from (Hessenauer et al. 2020) and added to the 92 mapped isolates. SNPs were called from the 169 samples using samtools mpileup v1.10 and bcftools 1.10.2 call (Danecek et al. 2021). Several conservative filters were applied to the SNPs dataset to keep only high quality SNPs. SNPs with minimum depth lower than 2 and missing data higher than 50% were excluded. SNPs were pruned based on linkage disequilibrium using plink v1.90b5.3 (Purcell et al. 2007) --indep command using default parameters. The final dataset included a total of 57128 SNPs. VCF file was annotated using Snpeff v5 (Cingolani et al. 2012).

3.4.3 Genomic analyses

We performed a discriminant analysis of principal components (DAPC) and a principal component analysis (PCA) with the R package adegenet (Jombart 2008) on the entire dataset (169 isolates) to investigate population structure in the 78 new isolates (Table 1).

The GAPIT v3 (Wang and Zhang, 2020) package was used to compare methods and kinship matrix calculation methods. Several different methods with variable complexity and including different covariates (population structure, kinship) exist in the GAPIT v3 package (Wang and Zhang, 2020). We assessed the fit of the method by using the quantile-quantile plot (QQ-plot) as a graphical diagnostic. We chose the parameters in order to avoid strong inflation in the p-values or overcorrection. The best results were obtained using the FarmCPU model (Liu et al. 2016) and Loiselle kinship matrix (Loiselle et al. 1995). Correction for population structure was not included to avoid overcorrection. For each tested trait, we extracted the top 5% outliers. We used the functional annotation of the reference genome published in (Comeau et al. 2014) to find gene functions and descriptions. Within these, we compared genes that were annotated by SNPeff v5 (Cingolani et al. 2012) as having a high impact between traits, search for gene ontologies enrichment using a custom script, and identified genes overexpressed in yeast and mycelium growth phase in *O.novo-ulmi* using RNAseq data published in (Nigg et al. 2015). We marked SNPs as being in an introgressed region if we detected at least one sample exhibiting OU introgression in this particular region (Hessenauer et al. 2020). For each trait, we searched for gene ontology (GO) enrichment among outliers by performing a Fisher's exact test using a custom script.

3.4.4 Phenotyping

We used the phenotypic data from (Hessenauer et al. 2020) for the 91 previously studied isolates and measured growth for the 78 new ones (Table 1). Liquid cultures of the isolates were kept in a solution of glycerol 50% at -80°C in 96 well plates. From those stocks, 2 μ l were used to inoculate Malt Extract Agar (MEA) media (15 g/L agar, 30 g/L malt extract and 5g/L mycological peptone), isolates were grown for 3 days at room temperature (RT) in 6-well plates (Greiner Bio-one, Kremsmünster, Austria). After growth, 4 to 9 mm² of mycelium were transferred to 4 mL of liquid minimal medium (Bernier and Hubbes, 1990) (MM) (MM contained the following per liter distilled water: KH₂P0₄, 1.0 g; MgSO₄ 7H₂O, 0.5 g; CaCl₂ 2H₂O, 0.1 g; L-proline, 1.15 g; H₃B0₃, 500 μ g; ZnSo₄ 7H₂O, 400 μ g; MnSO₄ 7H₂O, 400 μ g; Na₂MoO₄ 2H₂O, 200 μ g; FeCl₃ 6H₂O, 200 μ g; CuSO₄ 5H₂O, 40 μ g; pyridoxine HCl, 100 μ g; sucrose, 20.0 g. The pH of the medium was 5.7) and grown for 3 days at RT in deep well plate 24 (Corning Tewksbury MA, USA). Optical density of the cultures was estimated using 96 well plates (Greiner Bio-one, Kremsmünster, Austria) and a Tecan infinite 200F pro platform (Tecan

Männedorf Switzerland). Optical density of the cultures was adjusted to approximately 1.0 OD₆₀₀. 5µl of these dilutions were spotted on two Omnitray plates, one containing MEA and the other containing MEA supplemented with 0.2 mM of 1,2-naphtoquinone. 1,2-naphtoquinone is a compound similar in structure to mansonone E, a defense molecule found in elm xylem. Plates were incubated for 4 days at 20°C, 25°C and 30°C in a spImager custom platform (S&P Robotics, Toronto, Canada) and the pictures were taken every 2 hours. For each temperature, the screen was replicated 3 times with randomized isolate positions. Images were processed with a custom script using the R package EBImage (Pau et al. 2010). Pixel intensity parameter was extracted from each isolate position on each picture, allowing us to draw growth curves. Linear models were then fitted in windows, and slopes were extracted. The second best slopes were kept for each isolate.

As a surrogate to pathogenicity, Golden Delicious apples were inspected visually to eliminate damaged or bruised fruits, then washed with soft soap in warm water and surface disinfected with 70% ethanol. Apple tissue was removed with a 9-mm-diameter metal cork borer. The 10-mm-deep hole was filled with an MEA plug bearing fungal mycelium, with the mycelium facing inwards. A piece of transparent masking tape was placed over the inoculation site to prevent outside contamination and desiccation. The apples were kept in the dark at 25 °C in a growth chamber for 28 days. The necrosis diameter (mm) was measured 14 and 28 days post inoculation. Each measurement of necrosis was taken twice (vertically and horizontally) on the 14th and 28th days post-inoculation. The virulence of the 169 isolates was evaluated on four different apple replicates per sample. Each apple suspected of contamination was removed from the dataset, resulting in a mean inoculation success of 65%. Given the large number of apparent contaminations on the 28th day post inoculation, we retained only the measures taken on the 14th day post-inoculation. A linear model was fitted between the two measurements of necrosis size on the 14th day post-inoculation. Measurements were strongly correlated (Linear model, slope ± standard error = 0.7274 ± 0.03577; P < 2.2 × 10−16). The predicted values were retained as a more precise evaluation of necrosis size than the mean between measures. To assess the validity of the test, we verified that necrosis size was not correlated with growth rate in MEA (Pearson's correlation test: t = 0.02353; d.f. = 81; P = 0.7851).

3.5 Results

3.5.1 Population structure

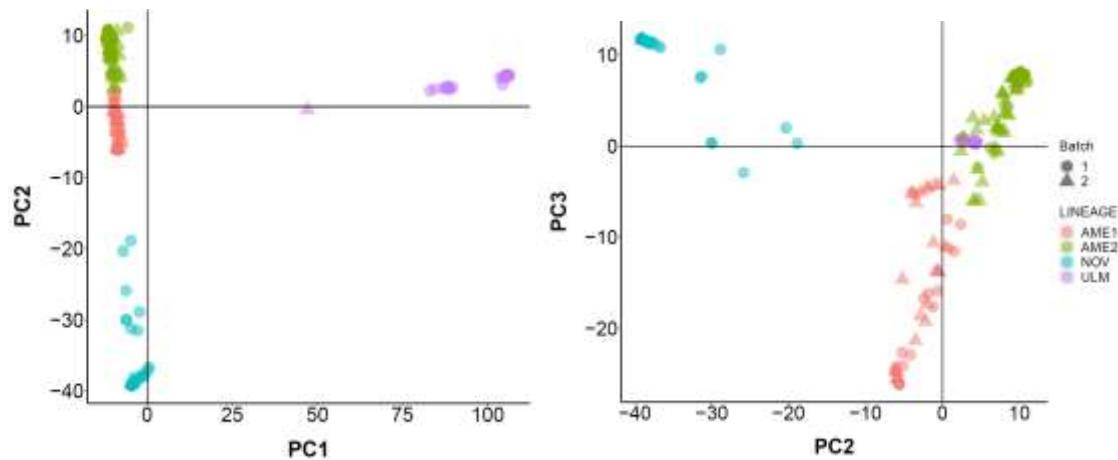


Figure 6. Plots of the first and second axes (left) and the second and the third axes (right) of the PCA. PC, principal component. Batch 1 corresponds to samples retrieved from (Hessenauer et al. 2020) and batch 2 to new samples.

The PCA and DAPC analysis performed on the pooled batches allowed us to determine the genetic lineages of the new isolates. As expected, OU and ONU formed two distinct groups along PC1 (Fig. 6), with the exception of sample 101478, which was placed between the OU and ONU clusters and is likely a OU x ONU hybrid. The three European samples clustered within NOV genetic lineages, and the other 75 new isolates grouped within the previously defined North American genetic lineages in ONU: AME1 ($n = 21$) and AME2 ($n = 53$). There was no batch effect detected in the dataset (Fig 6).

3.5.2 Phenotyping

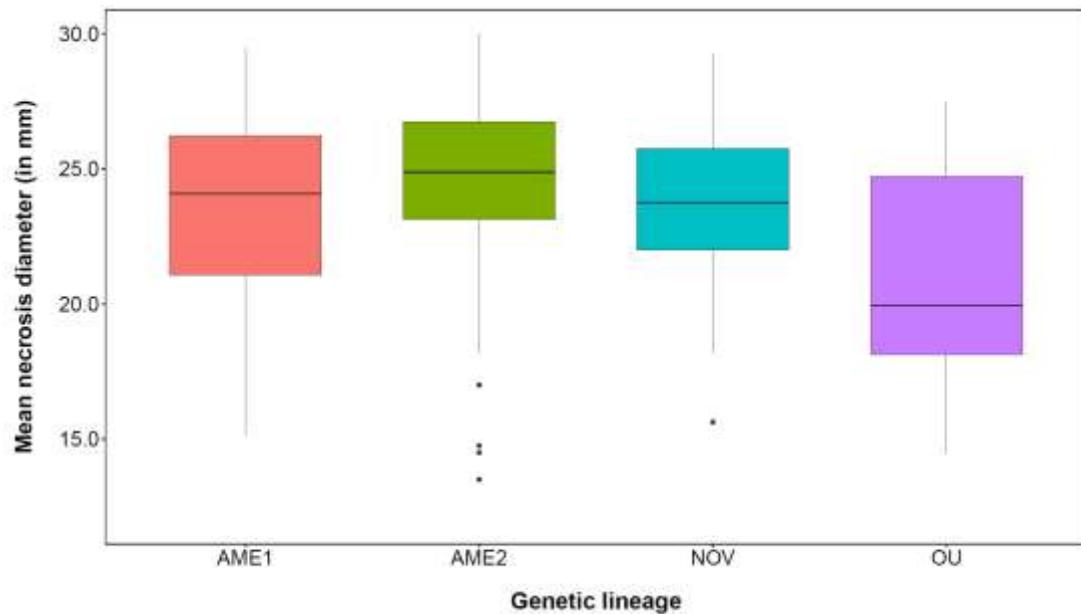


Figure 7. Differences in necrosis size depends on genetic lineage ($n = 169$). Y axis indicates the genetic lineage and X axis the diameter of the necrosis on apple in mm.

Our sampling presented phenotypic variation. OU samples have a smaller mean necrosis size than any ONU genetic lineage (Fig 7). We also observed a higher growth rate at 20°C and 25°C for ONU samples, whereas OU samples had the highest growth rate at 30°C, as shown in (Hessenauer et al. 2020) (Sup fig 19).

3.5.3 Association analyses

3.5.3.1 SNPs impact annotations

We found 12 putative high impact SNPs identified by the genetic variant annotation program SNPeff v5.0e (Cingolani et al. 2012) in genes associated with growth in MEA at 20°C, 15 with growth in MEA at 25°C, 6 with growth in MEA at 30°C, 13 with growth in MEAN at 20°C, 3 with growth in MEAN at 25°C, 4 with growth in MEAN at 30°C and 3 with necrosis size (Sup table 2). Some of these were shared between different traits and were identified in the H327 genome annotation (Comeau et al. 2014) as an ABC transporter CDR4, a choline transporter, a developmental factor flbA and a probable rhamnogalacturonate lyase.

3.5.3.2 Top SNPs candidates associated with virulence on apple

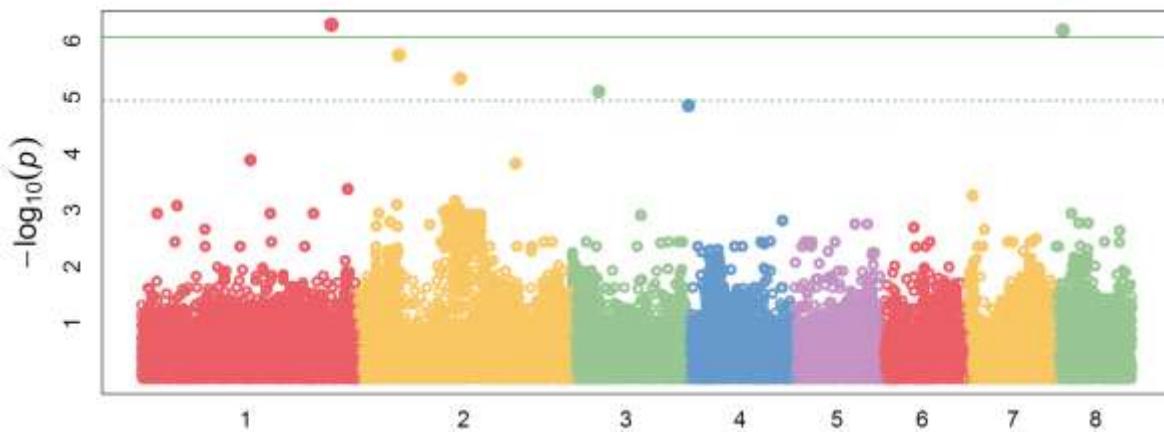


Figure 8. Manhattan plot of the GWA performed for the necrosis size on apple.

Numbers on x axis represent the chromosome number and y axis represents the p-value for each tested SNP. The dashed line shows the significance threshold ($p\text{-value} < 0.05$) and solid line ($p\text{-value} < 0.01$).

The top outliers associated with necrosis size on apple included a CFEM domain containing protein, a putative HC-toxin efflux carrier, an actin-related protein and a target of rapamycin complex (TORC) subunit LST8 (Fig 8). 27 of the top 40 outliers were around the position 3 000 000 on chromosome 2, which includes several transporters, cytochromes, as well as the putative MAT1-2-1 mating type protein (Sup table 3). Manhattan plots for growth phenotypes can be found in Supplementary figures (Sup fig. 20 to 25).

3.5.3.3 Genes, gene families and gene ontologies associated with phenotypes

Several important gene families were associated with some or all of the traits with various predicted impacts, such as ABC and MFS transporters, bZIP and zinc finger transcription factors, cytochromes, heat shock and thermotolerance proteins and vegetative incompatibility proteins (Sup table 2).

The GOs regulation of transcription and transcription factor activity were the only GOs enriched that was common to all phenotypes (Sup table 4). Other enriched GOs such as zinc ion binding, DNA binding, ribosome and metabolic process were shared by 5-6 phenotypic traits. Some GOs were unique to a trait, such as helicase activity associated with necrosis size, or oxydoreductase activity enriched with MEAN at 20°C. However, no GOs were unique to a specific temperature or media.

3.5.3.4 Gene expression levels in yeast and mycelium forms of ONU and level of introgression from OU

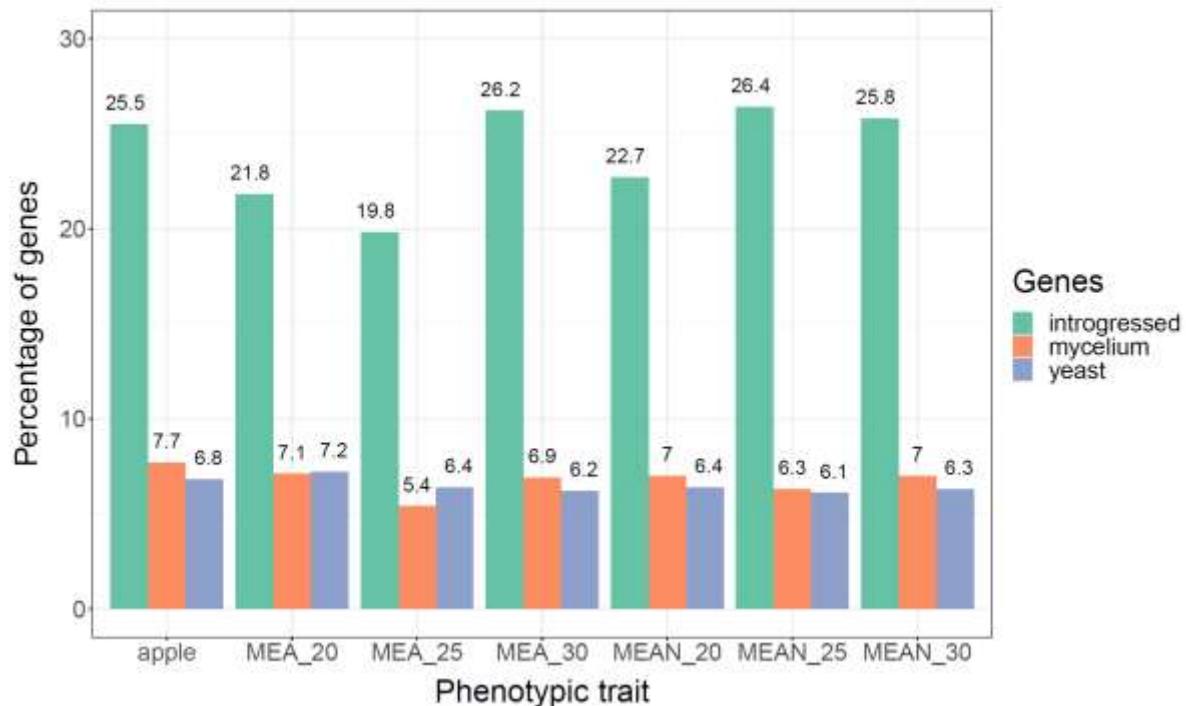


Figure 9. Number of genes associated with a SNP outlier being in an introgressed region (green), overexpressed in mycelium phase in RNAseq data (orange) and overexpressed in yeast phase (blue) in each phenotypic trait.

To further characterize outlier SNPs, we investigated their expression levels in RNAseq experiments (Nigg et al. 2015) and assessed whether they were in a genome region identified as introgressed from Hessenauer et al. 2020. The number of introgressed genes varied significantly between the traits (chi test : $\chi^2 = 47.7$, p-value = $1.35 \cdot 10^{-8}$), representing a portion of ~19.7% to ~26.3% of the outliers associated with regions subject to introgression events (Fig 10). Genes overexpressed in mycelium phase in the RNAseq data represented between 5.4% to 7.6% (chi test : $\chi^2 = 15.1$, p-value = 0.04) of the outliers and genes overexpressed in yeast between 6.1% and 6.8% (chi test : $\chi^2 = 3.9$, p-value = 0.68) (Fig 9).

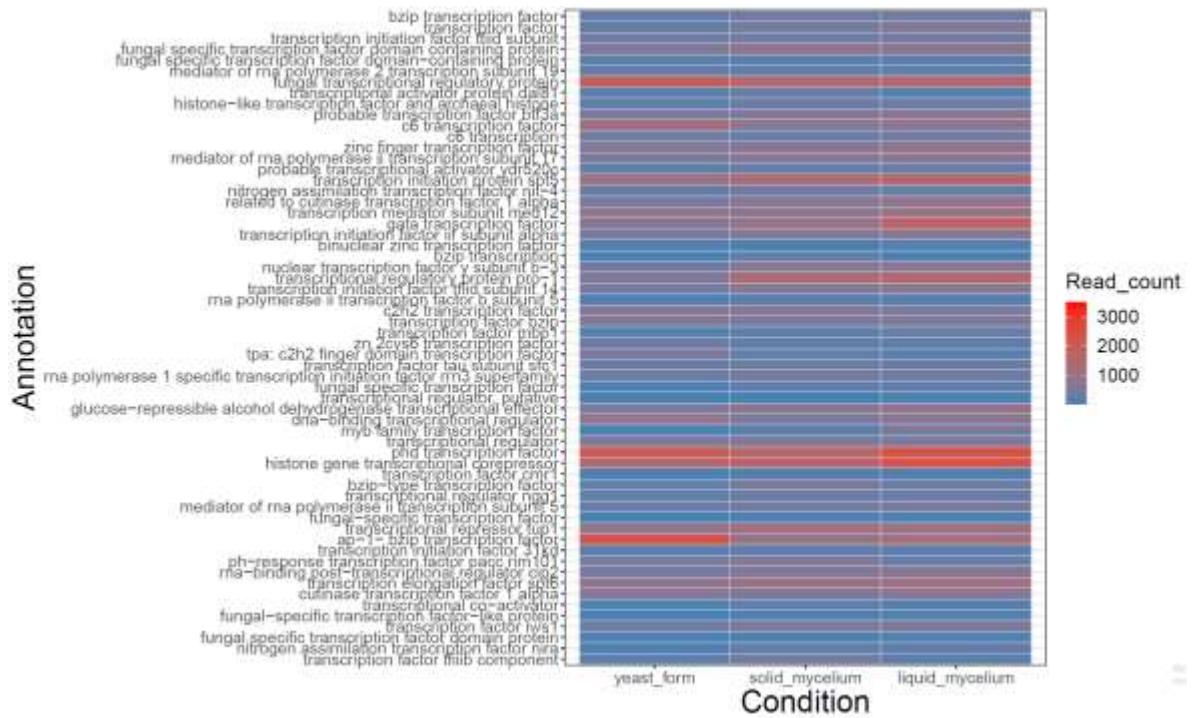


Figure 10. Expression levels of transcription factors associated with growth and virulence combined. Blue color means low number of read count observed for a particular gene in a condition, red color indicates a high number of reads for that condition.

We looked at the expression levels of genes belonging to specific gene families using raw read count from RNAseq data (Nigg et al. 2015). We found that several transcription factors (Fig 10) and cytochromes (Fig 11) were highly expressed when the fungus was growing either in liquid media in yeast and mycelium form or in solid media as a mycelium form.

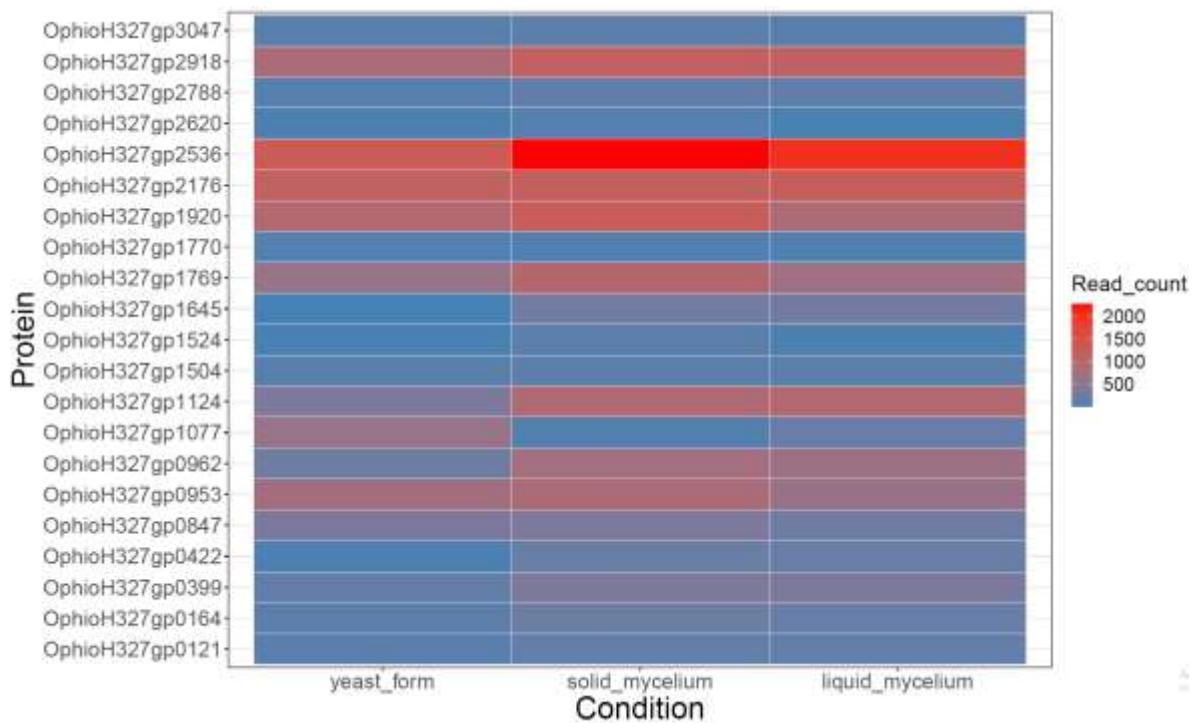


Figure 11. Expression levels of cytochromes associated with growth and virulence combined.

Blue color means low number of read count observed for a particular gene in a condition, red color indicates a high number of reads for that condition.

3.6 Discussion

In a context of environmental change, it is of primary importance to characterize loci involved in adaptation in fungal pathogens. DED is one of the most destructive tree diseases in the world. However, genes and mechanisms underlying virulence and growth remain poorly characterised. The objective of this study was to identify new candidate genes associated with virulence and growth in DED fungal pathogens. We used a GWA approach to explore genetic variation and identify a list of gene candidates and gene families putatively associated with growth and virulence phenotypes.

3.6.1 Identification of candidate genes associated with virulence on apple

GWA provides a powerful approach to identify loci involved in resistance to pathogens and economically important traits in plants and crops. These past few years have seen the emergence of GWA analyses applied to fungal pathogens to identify genes associated with virulence or other traits relevant to disease such as *Z. tritici* (Hartmann et al. 2017), *F. graminearum*

(Talas et al. 2016) or *P. nodorum* (Gao et al. 2016). These GWA presented a sampling number similar to our study (ranging from 106 to 191 samples) and identified several candidate genes potentially associated with the measured phenotypes. In the case of the wheat pathogen *Z. tritici* for instance, significant SNPs were located in genes associated with plant cell wall degradation, cell transport, and fungal metabolism (Hartmann et al. 2017).

The genes and loci responsible for virulence in *Ophiostoma* have so far been identified using comparative genomics and transcriptomics, and association studies using a limited number of markers (Comeau et al. 2014; Et-Touil et al. 1999; Temple and Horgen 2000). Earlier studies often lacked power and resulted in some genes being falsely associated with pathogenicity. This was the case of the cerato-ulmin protein which was initially identified as a pathogenicity related gene but was later refuted by the production of deletion mutants (Temple and Horgen 2000). This protein was later suggested to be associated with the ability of the pathogen spores to adhere to the beetle exoskeleton. The most promising candidate prior to our study was pat1, detected by RAPD markers association (Et-Touil et al. 1999), and identified as a possible ammonium transporter localized on the first chromosome (Plourde 2010). However, the exact phenotypic impact of pat1 has not yet been fully characterized.

In our study, we were able to identify several interesting new candidate genes associated with necrosis size on apples in OU and ONU. The top SNPs outliers were in regulating regions of several important genes. The top outlier is a SNP annotated as an upstream regulating variant for a protein containing a CFEM domain. CFEM domains are unique to fungi, and they are overrepresented in pathogenic fungi compared to non pathogenic fungi (Zhang et al. 2015). Some fungal proteins that contain CFEM are proposed to play an important role in pathogenesis: appressorium development in *Magnaporthe grisea* (DeZwaan et al. 1999), high expression in mycelium and spherule stages in *C. albicans* (Lamarre et al. 2000). Overall, CFEM-containing proteins are believed to function as cell-surface receptors or signal transducers, or as adhesion molecules in host-pathogen interactions (Zhang et al. 2015). The second highest outlier is an upstream regulator of an HC-toxin efflux pump. HC-toxin is a virulence determinant for the plant pathogenic fungus *Cochliobolus carbonum*. It was also discovered in another fungus, *Alternaria jesenskiae* (Walton 2006; Wight et al. 2013). Another interesting candidate within the top 10 SNPs associated with necrosis size on apple was a target of rapamycin protein kinase (TORC) subunit LST8. In yeast, TORC1 regulates cellular processes to control cell growth in response to environmental signals while LST8 is involved in maintenance of cell wall integrity (Wang and

Proud 2009). Interestingly, 27 of the top 40 outliers were around the position 3 000 000 on chromosome 2, suggesting an important impact of this region on the phenotype. This part of the chromosome does not correspond to a known introgressed region. Genes present in this portion of the chromosome include transporters, cytochromes and the putative MAT1-2-1 mating type protein.

3.6.2 Identification of gene families associated with fungal growth and virulence on apple

A major group of genes associated with all phenotypes were ABC and MFS transporters. SNPs were annotated as having an impact from low to high. Some of the SNPs were vastly spread in the population with a MAF up to 47%. Several of these genes were also associated with an overexpression in yeast or mycelium form. ABC transporters are well-known for their role as antifungal drug efflux pumps. For instance, the ABC transporter CDR4 is the major contributor to azole resistance in *Neurospora crassa* (Zhang et al. 2012). In yeast they are also characterized as proteins performing many critical functions, from xenobiotic cleansing to translocating various structurally unrelated cargoes, including lipids, fatty acids, ions, peptides, sterols, metabolites and toxins (Kumari et al. 2021). In plant pathogens, these transporters can play an essential role in protection against plant defense compounds during pathogenesis, it is thus not surprising to find them associated with growth in MEA supplemented in naphtoquinone, a molecule similar to a host defense compound, or on apples. Deletion of these genes can cause reduction of virulence in several different pathogenic species such as *F. graminearum* (Abou Ammar et al. 2013) or *M. grisea* (Urban 1999). Also, some MFS transporters actively secrete host-specific and non-host-specific toxins, such as observed in the plant pathogens *C. carbonum*, *Cercospora kikuchii* or *Fusarium sporotrichioides* (Del Sorbo et al. 2000).

All traits displayed association with transcription factors (TFs), mostly belonging to the basic leucine zipper (bZIP) and zinc finger protein families. Some of these genes appeared as overexpressed in yeast or mycelium phase in the transcriptomic data (Nigg et al. 2015), and were predicted as having from “LOW” (e.g. synonymous mutation) to “HIGH” (e.g. stop gained) impact. TFs are proteins that can bind to the promoter regions of target genes and can act as activators or repressors of gene expression. Importantly, many TFs are crucial for fungal pathogenicity, as shown in the crop pathogens *M. grisea* (Kong et al. 2015), *Alternaria brassicicola* (Srivastava et al. 2012), *Verticillium dahliae* (Wang et al. 2018), or *Valsa pyri* (Kange et al. 2019).

TFs are also required for plant invasion in *Ustilago maydis* (Flor-Parra et al. 2006). Transcription factors can play an important role in forest pathogens too. A GWA analysis in *H. annosum* s.s. identified a marker located near a SWI5 transcription factor as associated with fungal growth on pine (Dalman et al. 2013). The zinc finger transcription factor gene, cpst12, was shown to be required for *Cryphonectria parasitica* female fertility and virulence (Sun et al. 2009).

Several associated SNPs in all phenotypes were in genes or in the regulatory region of genes coding for cytochromes (CYPs), some of them overexpressed in mycelium or yeast phase in RNA-seq data (Nigg et al. 2015). This indicates that our candidates may be really important for fungal growth in different conditions. Fungal CYPs are known to be involved in diverse biological processes, including production of primary and secondary metabolites and degradation of toxic substances such as environmental pollutants, xenobiotics and plant-derived toxins (Shin et al. 2018). Several CYPs have been implicated in pathogen virulence because they neutralize antifungal compounds produced by hosts (Moktali et al. 2012). Recent genetic evidence suggests that CYP enzyme reactions are involved in fungal developmental processes and pathogenesis (Shin et al. 2018). It is not surprising to detect CYPs associated with our phenotypes, as they are very important genes for vascular pathogens such as OU and ONU in order to degrade toxins present in the tree sap.

Another important family of genes represented in the outliers was heat shock and thermotolerance proteins. The role of heat shock proteins is to facilitate the survival of the cell in response to stress-related changes such as misfolding of proteins or protein aggregation caused by high temperatures (Tiwari et al. 2015). These proteins have been shown to be involved in stress response and virulence in pathogens such as *C. albicans*, *F. graminearum*, *M. oryzae* and *U. maydis* (Bui et al. 2016; Ghosh 2014; Mayer et al. 2012; Yang et al. 2018). Null mutants for the protein CpHsp24 showed enhanced sensitivity to heat shocks and a decrease in the necrotic area in *C. parasitica* (Baek et al. 2014). Interestingly, thermotolerance proteins (30°C) have a missense variant in ONU that was exclusively associated with growth at the highest temperature. Moreover, the portion of chromosome carrying this gene is introgressed from OU to ONU (Hessenauer et al. 2020). OU grows at higher temperature than ONU and ONU isolates with introgression from OU exhibit a higher growth rate at 30°C than non introgressed ones (Hessenauer et al. 2020). Altogether, these results suggest that this thermotolerance protein could be participating in the higher growth rate of introgressed ONU isolates at 30°C.

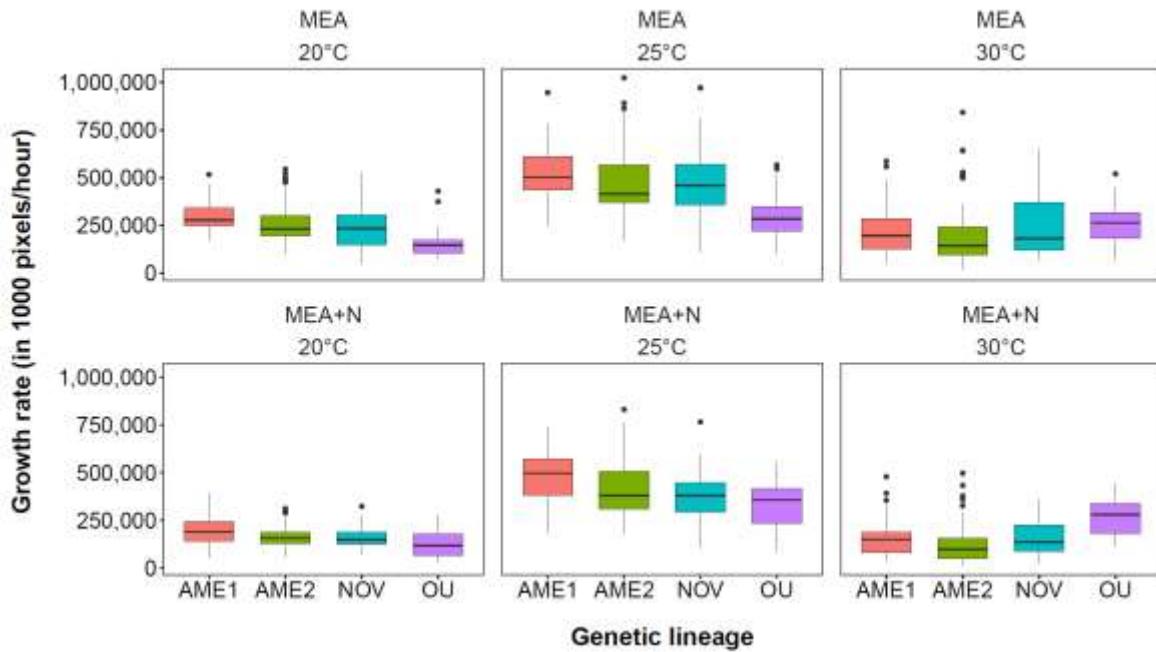
Lastly, several vegetative incompatibility proteins were found to be associated mostly with growth in MEAN and necrosis size on apple. Some of them were located in introgressed regions identified in (Hessenauer et al. 2020) and have missense mutations. In OU and ONU, about 20 loci spread in the genome are predicted to code for vegetative incompatibility proteins (Comeau et al. 2015). They are fast evolving genes, involved in self recognition (Paoletti 2016). They are believed to be involved in resistance to mycoviruses, as they operate by confining viable hyphal fusions to genetically identical vic genotypes (Paoletti et al. 2006). Fusions between hyphae of different vic genotypes result in cell death. So, these genes represent important adaptations for fitness in these fungi.

3.7 Conclusion

In this study we use GWA for dissecting important complex traits such as growth and virulence in a non-model organism. We provide several candidate genes associated with virulence that could be future prospects for functional genomics such a CFEM domain containing protein and a LST8 subunit protein. We also characterized several gene families associated with growth and virulence in DED pathogens such as ABC and MFS transporters, transcription factors and cytochromes. We also identified an introgressed thermotolerance gene involved in growth at high temperatures. Altogether, our results offer a better understanding of the genes and processes involved in growth and virulence in *Ophiostoma ulmi* and *Ophiostoma novo-ulmi*.

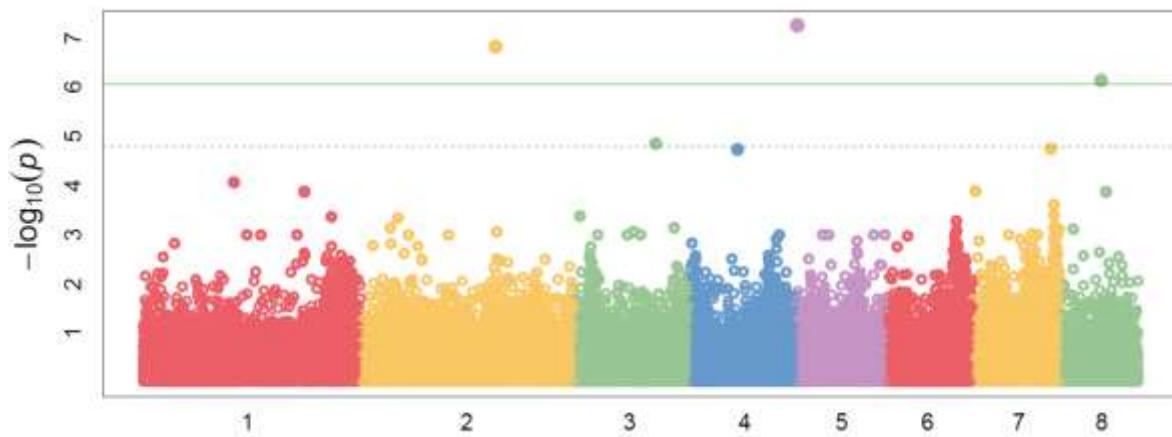
3.8 Supplementary materials

All supplementary tables can be found online at https://github.com/PaulineSnor/gwas_tables.git

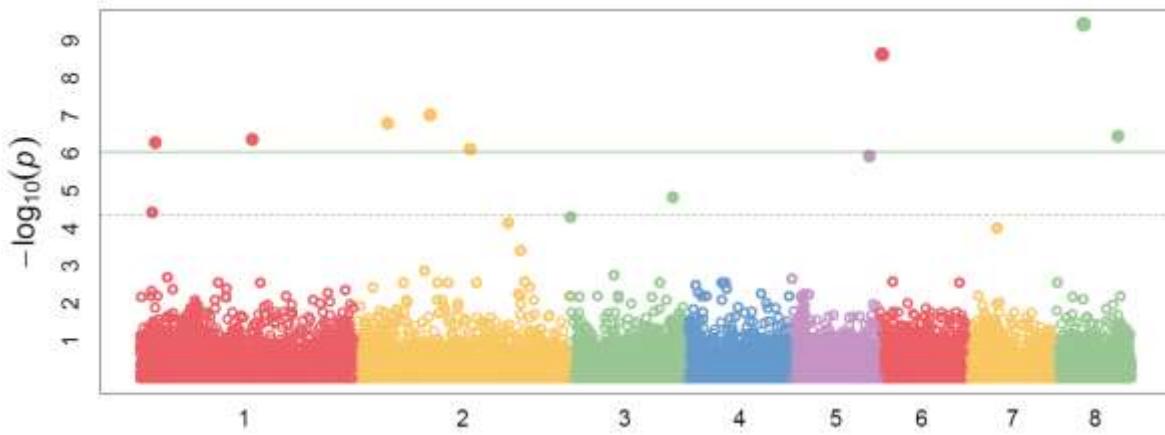


Supplementary figure 19. Lineages have different growth performance.

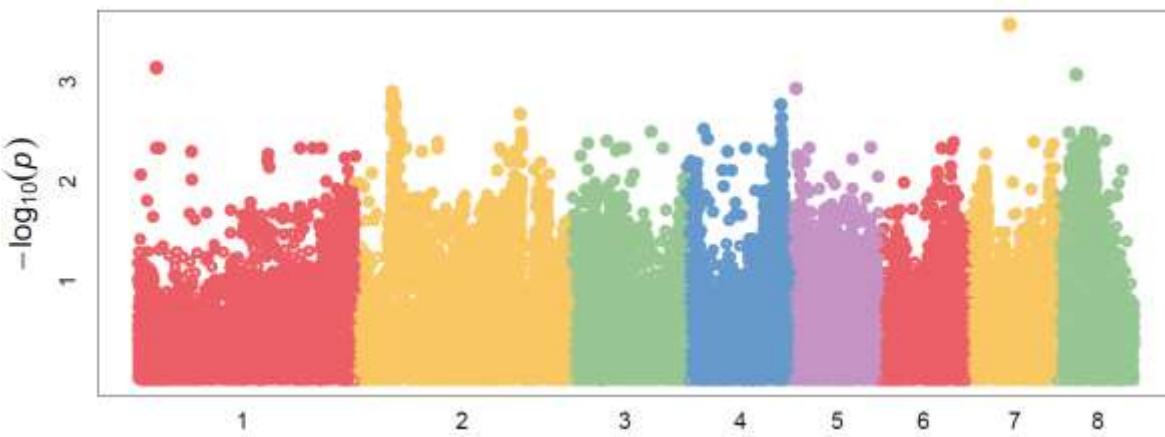
Growth rate in 1000 pixels per hour for isolates from each genetic group in media MEA and MEA+N at 20°C, 25°C and 30°C. In boxplots, center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range; points, outliers.



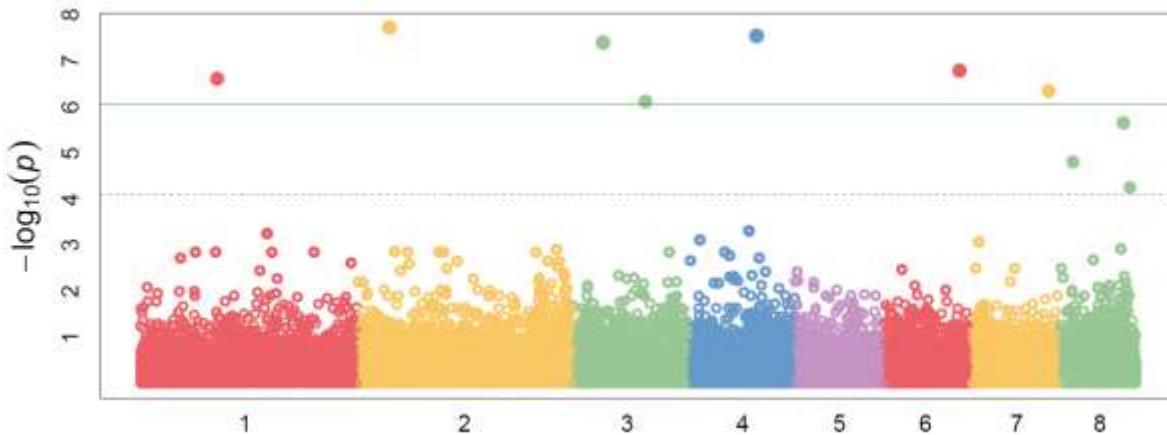
Supplementary figure 20. Manhattan plot of the GWA performed for growth in MEA at 20°C. Numbers on x axis represent the chromosome number and y axis represents the p-value for each tested SNP. The dashed line shows the significance threshold (p -value < 0.05) and solid line (p -value < 0.01).



Supplementary figure 21. Manhattan plot of the GWA performed for growth in MEA at 25°C.
Numbers on x axis represent the chromosome number and y axis represents the p-value for each tested SNP. The dashed line shows the significance threshold ($p\text{-value} < 0.05$) and solid line ($p\text{-value} < 0.01$).

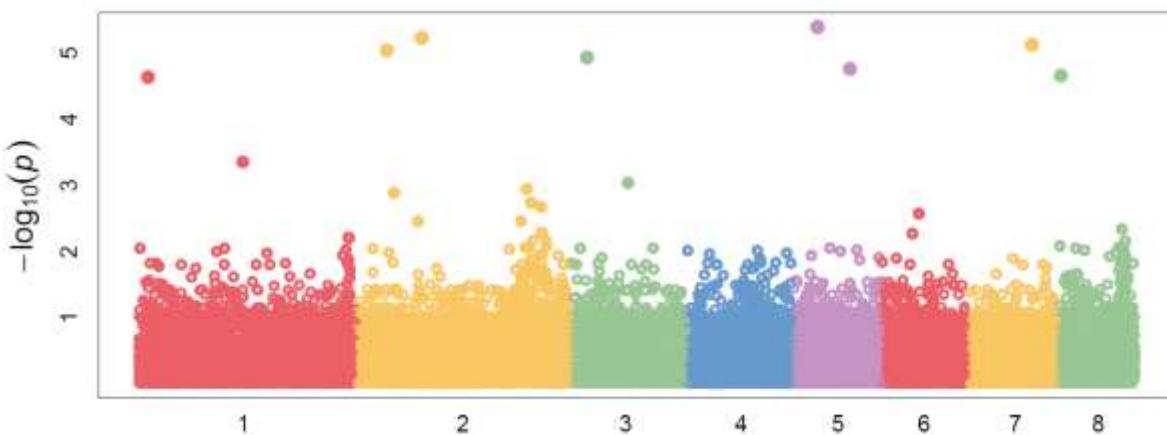


Supplementary figure 22. Manhattan plot of the GWA performed for growth in MEA at 30°C.
Numbers on x axis represent the chromosome number and y axis represents the p-value for each tested SNP.



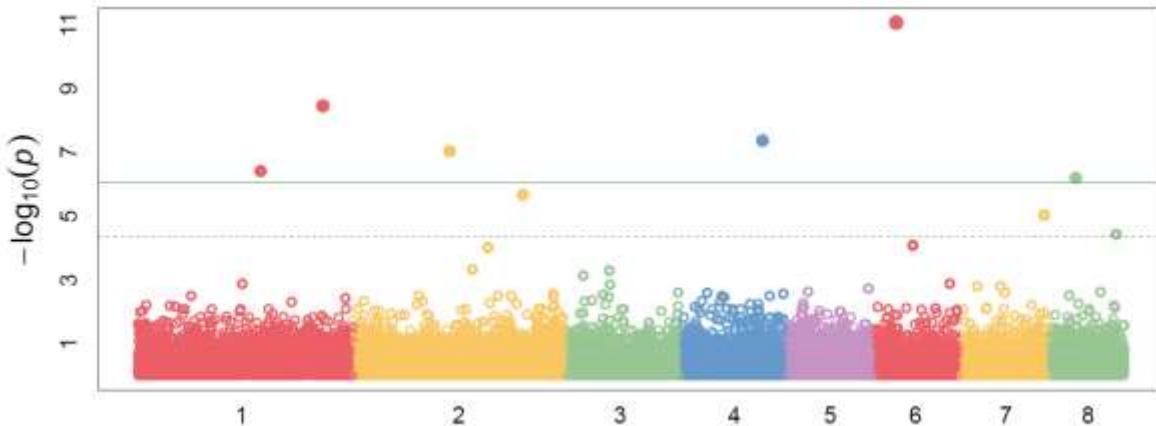
Supplementary figure 23. Manhattan plot of the GWA performed for growth in MEA supplemented with naphtoquinone at 20°C.

Numbers on x axis represent the chromosome number and y axis represents the p-value for each tested SNP. The dashed line shows the significance threshold (p -value < 0.05) and solid line (p -value < 0.01).



Supplementary figure 24. Manhattan plot of the GWA performed for growth in MEA supplemented with naphtoquinone at 25°C.

Numbers on x axis represent the chromosome number and y axis represents the p-value for each tested SNP.



Supplementary figure 25. Manhattan plot of the GWA performed for growth in MEA supplemented with naphtoquinone at 30°C.

Numbers on x axis represent the chromosome number and y axis represents the p-value for each tested SNP. The dashed line shows the significance threshold ($p\text{-value} < 0.05$) and solid line ($p\text{-value} < 0.01$).

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Conclusion

Les pathogènes sont une menace pour les milieux forestiers, urbains, et agricoles, en particulier lorsque leurs effets sur les écosystèmes peuvent être exacerbés par un contexte de changement climatique. La MHO est une maladie dévastatrice pour les ormes et est toujours active en Europe et en Amérique du Nord de nos jours. En dépit de l'importance de cette maladie, beaucoup de questions demeurent sur la biologie, l'évolution et l'adaptation des pathogènes qui causent la MHO. Dans ce thème, les objectifs étaient 1- d'identifier les mécanismes impliqués dans l'évolution des pathogènes des systèmes forestiers et agricoles et de les comparer, 2a- d'utiliser des approches génomiques pour décrire la structure génétique des populations des champignons responsables de la MHO, 2b- de caractériser l'effet de l'hybridation et de l'introgression sur la diversité génétique et sur le phénotype chez ces champignons, et enfin 3- d'explorer la diversité génétique pour identifier des variants associés avec la variation de virulence et croissance.

Dans un premier chapitre, nous avons comparé les caractéristiques des pathogènes des systèmes agricoles et forestiers. Nous avons remarqué que, malgré des différences très importantes, comme le degré d'intervention humaine sur ces milieux (de la forêt naturelle à champ cultivé) ou bien le temps de génération des plantes hôtes, les grands mécanismes moteurs de l'évolution de ces pathogènes étaient similaires. Nous listons des techniques variées pouvant permettre de lutter contre les futures menaces imposées par les changements climatiques et les potentielles nouvelles épidémies de pathogènes. Dans cette « boîte à outils » se trouvent les programmes de développement des plantes comme l'amélioration génétique et l'édition génétique ou encore des approches de terrain comme l'agroforesterie ou la migration assistée. Nous pensons qu'il n'existe pas de technique meilleure qu'une autre, mais que seule une approche intégrative, combinant le meilleur des connaissances en génomique, en pathologie mais également en écologie et en climatologie permettra à la fois de supporter une production alimentaire pour la population future mais également à la protection des milieux naturels.

L'utilisation de données génomiques et phénotypiques permet de jeter une lumière nouvelle sur les mécanismes impliqués dans l'adaptation des champignons pathogènes responsables de la MHO. Bien que la structure des populations de la MHO ait déjà fait l'objet de nombreuses études au cours des dernières décennies, nous avons choisi d'explorer notre jeu

de données génomiques de manière naïve afin d'éviter tout biais. Nous retrouvons bien deux espèces génétiquement divergentes, et présentant des différences phénotypiques. Nous mettons en évidence une structure des populations plus complexe que décrite précédemment, avec deux lignées distinctes au sein de *O. novo-ulmi americana*. Celles-ci se diffèrentent en termes de taux de croissance et pourraient correspondre aux deux types d'incompatibilité végétative présents en Amérique du Nord. Bien que nous possédions les génomes complets de ces isolats, pour confirmer cette hypothèse il serait nécessaire de caractériser le type végétatif phénotypique de chacun de ses isolats en réalisant des tests de compatibilité avec des isolats dont le type est connu. D'autre part, nous pensons donc que le terme « lignée génétique » plutôt que « sous-espèce » convient mieux pour désigner NOV, AME1 et AME2. Cette idée peut se justifier par le faible taux de différenciation entre les lignées, la possibilité de découvrir d'autres lignées cryptiques au sein d'*O. novo-ulmi americana* en utilisant un plus grand échantillonnage, ainsi que la forte perméabilité de barrière reproductive entre les lignées. Nous montrons que l'introgression de portions de génomes de *O. ulmi* dans celui de *O. novo-ulmi* est associé à une augmentation de diversité génétique dans cette espèce montrant plutôt des signatures de reproduction clonale. Des gènes possédant d'importantes fonctions comme le locus reproducteur MAT-1, ainsi que des gènes de détoxification et de transport se trouvent dans les régions introgressées. Enfin, nous montrons que les isolats de *O. novo-ulmi* possédant une introgression de *O. ulmi* présentaient un taux de croissance plus élevé à 30°C ainsi qu'une taille de nécrose sur pomme plus ou moins grande en fonction du chromosome concerné. Cependant, les variations nucléotidiques ne sont pas les seules sources de variations importantes dans l'évolution des champignons pathogènes. Les variations structurelles comme les réarrangements chromosomiques ou les duplications de gènes peuvent avoir un impact très fort sur le phénotype et la fitness de ces pathogènes. Produire des génomes *de novo* en utilisant une technique de séquençage de longs fragments permettrait de découvrir et d'étudier ces variations structurelles.

Dans le troisième et dernier chapitre, nous utilisons une approche GWA pour identifier de nouveaux gènes candidats associés à la variation de virulence sur pommes et de croissance dans deux milieux différents à trois températures différentes. Le petit nombre d'échantillons et le fort déséquilibre de liaison présent dans les génomes des champignons de la MHO limitent la puissance et la résolution de notre analyse. Cependant, nous sommes parvenus à proposer de nouveaux gènes candidats parmi lesquels se trouvent des transporteurs transmembranaires

impliqués dans la détoxification comme les transporteurs ABC, des cytochromes, enzymes impliquées dans la production de métabolites primaires et secondaires chez les champignons, ou bien encore des protéines de choc thermique, produites en réponse à des conditions de stress et assurant la stabilisation d'autres protéines. Cette approche génomique exploratoire nous a donc permis de dresser une liste de candidats à l'édition de génome. En effet, des techniques moléculaires de pointe ont récemment été développées chez *O. novo-ulmi* comme la technique OSCAR, ou la technique CRISPR-cas9 dont nous avons discuté dans l'introduction. Pour faire suite au présent travail, nous planifions de produire des mutants pour les gènes les plus prometteurs. Le phénotypage *in vitro*, ou *in planta* si possible, permettra de mesurer l'effet des gènes candidats sur la croissance et sur la virulence sur l'arbre hôte, et potentiellement identifier le ou les gènes spécifiques à la virulence chez cette espèce. D'autre part, les données de séquençage obtenues lors de ce projet peuvent également être utilisées en conjugaison avec des données environnementales pour conduire une étude génotype-environnement en Amérique du Nord. Les points forts de notre jeu de données sont la faible structuration génétique et géographique de la population nord-américaine d'*O. novo-ulmi* ainsi que la diversité des climats couverts. Cette approche complémentaire au travail présenté dans cette thèse permettrait de renforcer les gènes candidats déjà découverts ou bien d'en découvrir de nouveaux.

L'origine précise de la MHO reste encore inconnue après ce travail. Cependant, la quantité de données produites nous permettra d'utiliser des approches de d'inférence démographique, et de tester différents scénarios pour en déterminer le plus probable. Nous serons également capables de calculer les temps de divergence entre les champignons de la MHO et les autres espèces d'*Ophiostoma* qui sont eux non pathogènes. De plus, le séquençage d'espèce proches a été réalisé et une étude de génomique comparative est cours pour déterminer les gènes spécifiques aux champignons de la MHO.

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