CHAPTER EIGHT

The Natural Histories of Species and Their Genomes: Asymbiotic and Ectomycorrhizal *Amanita* Fungi

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Abstract

Genome architectures are likely shaped by species' ecologies, but genomes are rarely discussed in ecological contexts. A major force in evolution is symbiosis, and a symbiotic niche may shape a genome's size, gene order and gene content. The mycorrhizal symbiosis is ubiquitous and critical to the function of diverse ecosystems. Although there are tens of thousands of mycorrhizal fungi, there are no general principles defining the genetic architectures of these fungi. General principles may not exist, perhaps because of the multiple, independent origins of the symbiosis. But research with pathogenic

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fungi suggests general principles at work in the evolution of pathogen genomes, and to enable a more holistic understanding of the forces shaping genomes of mutualists, we focus on the genus *Amanita* and the role of ecology in genome evolution. *Amanita* is an emerging model for the ecology and evolution of symbiosis, and to date, our laboratory has sequenced the genomes of six species with diverse niches. We describe the natural histories of these species and current research on genomics. We offer novel analyses targeting two questions: did the evolution of the ectomycorrhizal symbiosis facilitate an adaptive radiation of symbiotic *Amanita* and how are the genomes of asymbiotic fungi different from the derived genomes of ectomycorrhizal fungi? We also discuss the role transposable elements may have had in generating genomic variation and a potential link between transposable element proliferation and patterns of speciation. Our descriptions of the genus identify as yet unexplored questions connecting genomics to the ecology of species' ranges and range expansions.

1. INTRODUCTION

How does symbiosis shape genetic architectures? In this chapter, we consider symbiosis as a close physical association between two individuals of different species and mutualism as any interaction benefitting both individuals. Symbioses may also be parasitisms and mutualisms may or may not be symbioses.

Mutualisms are often asymbiotic, for example, plants and pollinators or insects associated in Müllerian mimicry. Asymbiotic mutualisms are rarely considered as an explicit force shaping the genetic architectures of mutualists. However, floral morphology is critical to pollination and clearly evolves in response to pollinator choice (Venail, Dell'Olivo, & Kuhlemeier, 2010), and Müllerian mimics evolve to look like each other. It seems likely asymbiotic mutualisms will influence the spectrum of genes and their arrangement in a genome (Heliconius Genome Consortium, 2012). Fungi seem more often involved in symbiotic mutualisms, and we will not consider asymbiotic associations further.

Research on the genetic architectures of symbionts has focused on bacteria (McCutcheon & Moran, 2012; Moran, 1996; Moran, McLaughlin, & Sorek, 2009; Moran & Wernegreen, 2000), but how these data translate to fungi is unclear. Bacteria enabled paradigms defining the evolution of endosymbiosis (Martin & Schnarrenberger, 1997; Tamas et al., 2002), for example, endosymbiotic bacteria lose many of the genes found in closely related asymbiotic lineages and have reduced genome sizes (Mira, Ochman, & Moran, 2001; van Ham et al., 2003). However, fungal symbioses often

involve individuals that live both inside and outside of organisms. In mycorrhizal symbioses, some parts of the fungus are physically associated with a plant, while others are in soil and exposed. The evolutionary trajectory of an organism that maintains itself outside of a host may be very different from the trajectory of an endosymbiont (Law & Lewis, 1983; Zoller & Lutzoni, 2003), and in contrast to an endosymbiotic bacterium, an ectosymbiotic fungus may maintain the genes necessary for independent growth. The dynamics of genome size evolution may also be quite different, because mycorrhizal symbioses are not vertically transmitted (Smith & Read, 2010).

The ectomycorrhizal (ECM) symbiosis has multiple, independent origins (James et al., 2006), and because the ancestor of these symbionts was a saprotroph, extant ECM species represent different gains of the symbiotic niche (James et al., 2006; Matheny et al., 2006). Comparative genomics of published ECM genomes identifies coarse genomic differences between saprotrophic and biotrophic fungi (Martin et al., 2008, 2010; Martin & Selosse, 2008; Nagendran, Hallen-Adams, Paper, Aslam, & Walton, 2009; Plett & Martin, 2011), and intriguing patterns of evolution are suggested from the two genomes published to date: *Laccaria bicolor* and *Tuber melanosporum*. Both genomes are characterized by elevated transposable element (TE) content (Martin et al., 2008, 2010). The basidiomycete *L. bicolor* has a total genome size of 61 MB and an estimated TE content around 20–25%, while the ascomycete *T. melanosporum* genome is of 125 MB and reaches to as much as 60% TEs. However, the gene content in these two species is radically different. While *L. bicolor* encodes around 23,000 genes, many more than most free-living basidiomycetes, *T. melanosporum* only harbours about 7500 genes, which only barely exceeds the numbers found in a larger yeast genome (Jones et al., 2004). Only 19% of *T. melanosporum* genes are part of larger gene families, compared to 55% of the genes found in *L. bicolor* (Martin et al., 2010), revealing very different genome architectures that both enable the same kind of symbiosis. But there are also commonalities, including the loss of plant cell wall degrading enzymes (PCWDEs) and expansions in gene families involved in signalling (Veneault-Fourrey & Martin, 2011).

Parasitisms also shape the genetic architectures of fungi; although in ecological contexts disease may seem very different from mutualism, the interactions may share genetic pathways, for example, oomycete pathogens and arbuscular mycorrhizal fungi use a signal expressed from the same plant gene to colonize plants (Wang et al., 2012). Parasitisms and mutualisms may impose common selective forces on genomes, perhaps including changes

in genome size, but even when selective forces diverge the mechanisms enabling change may be similar, for example, changes in gene family copy number or the proportions of TEs.

Like *L. bicolor* and *T. melanosporum*, many plant pathogens maintain expanded genomes densely populated by TEs (Grandaubert, Balesdent, & Rouxel, 2014). Plant pathogens show differing patterns of expansions and contractions in the gene families involved in pathogenicity, including effectors and PCWDEs (Raffaele & Kamoun, 2012). Although the direct impact of TEs on these genomes is only discussed in a few cases, their effects are striking and include accelerated evolutionary rates of effectors caused by repeat-induced point mutation of nearby TEs (Grandaubert et al., 2014; Rouxel et al., 2011) and a fusion of an effector family with a TE resulting in joint proliferation (Sacristán et al., 2009). Moreover, simulations suggest rearrangements mediated by TEs may contribute towards the compartmentalization of genomes into slower- and faster-evolving regions and so aid the generation of genomic plasticity underpinning adaptation to new environments (Crombach & Hogeweg, 2007).

TEs appear as a common theme in research on the architectures of fungal genomes, but the discovery of TEs and their evolutionary potential is not restricted to the fungi; the impact of TEs on genomes is widespread and their significance as a mechanism generating heritable variation is widely appreciated (Kidwell & Lisch, 2001; Levin & Moran, 2011; Raffaele & Kamoun, 2012; Werren, 2011). TEs can generate large amounts of genetic diversity, for example, by facilitating chromosomal rearrangements, and besides their immediate consequences on gene content and gene order, the rearrangements mediated by TEs may also play a role in reproductive isolation, accelerating the process of speciation (Böhne, Brunet, Galiana-Arnoux, Schultheis, & Volff, 2008; Oliver & Greene, 2009, 2011, 2012; Oliver, McComb, & Greene, 2013; Zeh, Zeh, & Ishida, 2009).

To begin dissecting the variety of mechanisms shaping the genomes of ECM fungi, and maybe identify causal changes, comparisons of more closely related species are necessary. There are great evolutionary distances among species with sequenced genomes, and identifying the causes of genomic differences is difficult: genomic differences may be correlated with differences in ecological niche but may be caused by the unique evolutionary trajectories taken by distantly related species.

The Amanita *are a novel model for understanding the changes in genetic architecture associated with an evolution of a mutualistic symbiosis*: In this genus, the evolution of the ECM symbiosis occurred once, and saprotrophic *Amanita*

form a strongly supported clade basal to a monophyletic clade of ECM species (Wolfe, Tulloss, & Pringle, 2012; Fig. 8.1). *Amanita* houses over 500 described species, and the majority are ECM. Approximately 30 species of *Amanita* appear to be asymbiotic. But the full diversity of asymbiotic *Amanita* may be unknown; at least a handful of species remain undescribed (e.g. "sp-C13", http://www.amanitaceae.org/?Amanita+sp-C13), while others are known only from a single collection. Asymbiotic *Amanita* are often found in Africa, Asia or South America, at sites far away from traditional centres of mycology.

The single origin of symbiosis and its species richness render the genus *Amanita* a great model system to investigate the genomic changes around the evolution of symbiosis and the subsequent evolutionary trajectories of individual ECM species at a finer scale. We have sequenced the genomes of five *Amanita* and an out-group species (Fig. 8.1 and Table 8.1). We chose to sequence three symbiotic *Amanita* (*A. brunnescens*, *A. polypyramis* and *A. muscaria* var. *guessowii*, sampling from each of the major ECM clades) and two asymbiotic *Amanita* (the closely related *A. thiersii* and *A. inopinata*). The saprotrophic fungus *Volvariella volvacea* was sequenced as an out-group.

To facilitate thinking about the *Amanita* as a model, we briefly describe the natural histories of sequenced species and then describe current analyses of genomes, focusing on TEs. Species descriptions focus on ecology; useful information about morphology and taxonomy are provided by both mushroomexpert.com and amanitaceae.org. At least three salient questions emerge from the descriptions of species and their genomes, and we concentrate on two: does symbiosis influence the pace of speciation in ECM *Amanita*, and are TEs causing apparent changes in synteny among the different species? We relate the second question to patterns of speciation. We conclude by briefly discussing the third, unanswered question of whether or how genome evolution may enable range expansions.

2. THE FUNGI AND THEIR GENOMES

2.1. The out-group *Volvariella volvacea*, an edible mushroom and decomposer of agricultural waste

An extensive literature on *V. volvacea* focuses on its use as a crop (Bao et al., 2013; Chang, 1977; Date & Mizuno, 1997). The fungus is cultivated throughout Asia, and especially in China, where it has been grown since the eighteenth century. Protocols to grow the mushroom were developed at least in part by Buddhist monks (Chang, 1977). The mushrooms are

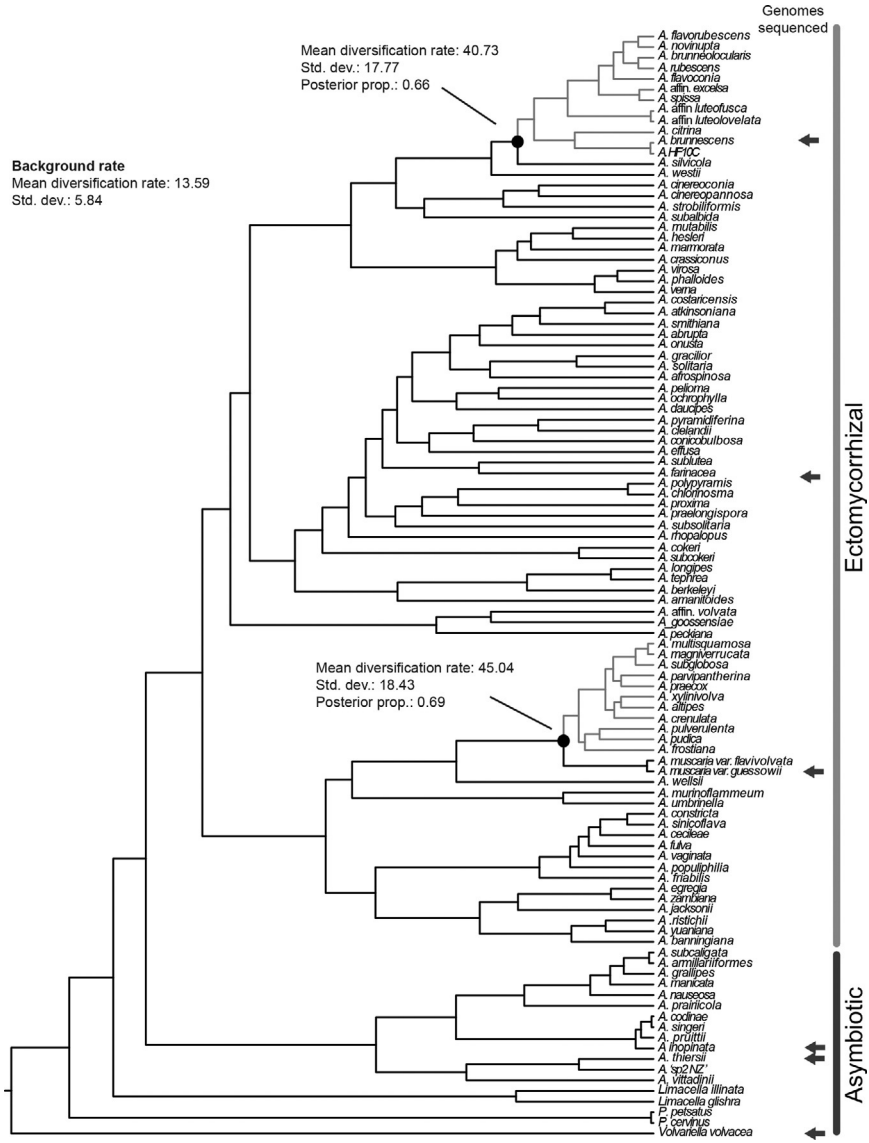

Figure 8.1 Phylogeny of the genus *Amanita*, based on the analysis of Wolfe, Tulloss, et al. (2012). Branch lengths were recalculated using the nucLSU gene and a relaxed molecular clock model in BEAST (Drummond & Rambaut, 2007). Rates of diversification were estimated with MEDUSA (Harmon, Weir, Brock, Glor, & Challenger, 2008) on a sample of 1000 trees from the BEAST posterior distribution, and inferred shifts recovered in >50% of trees are highlighted. Arrows mark the species for which genomic data are available.

Table 8.1 Basic statistics of the assemblies of sequenced Amanita genomes

Species	Sequencing centre	Assembly size (bp)	N50 (kB)	CEGMA genes (%)	CEGMA redundancy	TE (assembled) (%)	TE (coverage corrected) (%)
A. brunnescens	Harvard	57,556,770	11	94.35	1.81	17.9	36.4
A. polypyramis	Harvard	23,557,560	64	95.56	1.28	11.6	59.6
A. muscaria guess.	JGI	40,699,759	17	92.34	1.10	8.9	21.6
A. thiersii	JGI	33,689,220	77	95.97	1.11	26.4	36.6
A. inopinata	Harvard	22,122,871	156	95.97	1.11	4.8	8.9
V. volvacea	Harvard	52,426,718	55	95.56	1.57	4.6	5.2

Percentages of CEGMA (Parra, Bradnam, & Korf, 2007) genes recovered in each assembly were used as estimates of gene space completeness. CEGMA redundancy is the average copy number of single copy CEGMA genes detected in each genome. Assembly size is given as an approximation to genome size but may be a poor estimator. CEGMA redundancy and the proportion of unassembled TEs (as reflected by the difference between corrected and assembled TE content; Hess et al., in review) may give an indication of how assembly size related to true genome size. Higher redundancy values mean the true genome size is smaller than assembly size, while a high unassembled portion of TEs means that true genome size is larger than assembly size.

considered a health food. The species is relatively inefficient at converting substrates to mushrooms, with yields described as between 10% and 13% when the fungus is grown on rice "straw" (plant stalks leftover when rice is harvested) and 30–40% when grown on cotton wastes, for example, old clothes (Date & Mizuno, 1997). The fungus is tropical and requires temperatures greater than 25 °C to fruit but is introduced to North America where it can be found in woodchips, compost piles, greenhouses and gardens (Kuo, 2011). The mycelia of *V. volvacea* have no clamp connections. The species appears to be homothallic and capable of mating with itself (Bao et al., 2013).

2.2. *Amanita thiersii*, a fungus of lawns undergoing a range expansion

An extensive account of the natural history of *A. thiersii* is provided by Wolfe, Kuo, and Pringle (2012). The native range of the fungus may or may not include North America; although it was originally described from Texas in 1952, over the last decades, *A. thiersii* has moved from North to southern Illinois (Kuo, 2013a), and the fungus may be an invasive species originally introduced to Texas from an as yet unidentified home range. Alternatively, the fungus may be native and moving in response to environmental change. Recently, the fungus was found near Baltimore, MD, on the East Coast of the United States (www.mushroomobserver.org; Tulloss personal communication). Mushrooms are generally found in lawns, where the species decomposes grass litter. Although *A. thiersii* is not mycorrhizal, it stimulates plant growth (Wolfe, Kuo, et al., 2012), perhaps because decomposition releases limiting nutrients to soil. Nothing is known about the mating system of the fungus, but the genetic diversity of populations across North America is low.

2.3. *Amanita inopinata*, an *Amanita* known only from introduced ranges

The "unexpected" *Amanita* is an enigmatic fungus originally described from scattered localities in the southeast corner of England (Reid, 1987). After the description was published, a New Zealand mycologist recognized it as a rarely collected species found in both the North and South Islands (Ridley, 2000). The fungus is considered an introduction to both England and New Zealand and has also appeared in the Netherlands (Bas, 2001). Perhaps because the fungus is an *Amanita* and *Amanita* is typically an ECM genus, careful notes of the trees around collections are available (FRDBI, 2013; Ridley, 2000). Many are not hosts of ECM fungi, for example,

Chamaecyparis lawsoniana and *Taxus baccata*, and the fungus is currently assumed to be asymbiotic (Kibby, 2005; Wolfe, Tulloss, et al., 2012). However, molecular probes testing for a specific cellulose decomposition pathway found no evidence of these genes in *A. inopinata*, and its ecological niche remains unknown (Wolfe, Tulloss, et al., 2012).

2.4. *Amanita muscaria*, a species complex of ECM fungi with different ecologies

A. muscaria is the charismatic, widely recognized red-and-white-spotted mushroom of fairy tales and video games. However, it is very clearly a species complex of cryptic genetic species (Geml, Laursen, O'Neill, Nusbaum, & Taylor, 2006; Geml, Tulloss, Laursen, Sazanova, & Taylor, 2008), and these species look different, have different ranges and associate with different hosts. For example, the European mushroom (which keeps the name *A. muscaria*) is red with white spots and is often associated with oak, while the eastern North American mushroom (*A. muscaria* var. *guessowii*) is yellow with white spots and is primarily associated with conifers. A more southern North American mushroom (*A. muscaria* var. *persicina*) has a peach coloured cap and associates with both oak and pine. The genome sequenced to date is an isolate of *A. muscaria* var. *guessowii* collected in Pennsylvania; however, as additional genomes are sequenced, the complex will provide an opportunity to compare the genomes of very closely related genetic species with different habitats. For simplicity sake, we discuss the sequenced genome as "*A. muscaria*" and not "*A. muscaria* var. *guessowii*".

2.5. *Amanita polypyramis*, an ECM fungus

Relatively little is known about the natural history of *A. polypyramis*. The species is found in the United States from New Jersey south to Texas and Florida and in Mexico and Central America (Kuo, 2013b), including in the Guanacaste Conservation Area of Costa Rica (Tulloss, 2013). In contrast to *A. muscaria* but like *V. volvacea*, *A. polypyramis* grows in the tropics. The fungus associates with oaks and perhaps pines as well. Mushrooms are very large, with caps reaching to 20 cm across.

2.6. *Amanita brunnescens*, another ECM fungus about which relatively little is known

The species is found in eastern North America and associates with various hardwoods and conifers. The mushrooms are very common.

2.7. Genomics to date, and comparisons to *L. bicolor* and *T. melanosporum*

In the *Amanita*, decomposition pathways are lost by ECM species (Nagendran et al., 2009; Wolfe, Tulloss, et al., 2012). Preliminary analyses of the *A. muscaria* and *A. thiersii* genomes show a large reduction in many carbohydrate active enzyme (CAZyme) families in *A. muscaria*. The genome of *A. muscaria* encodes 279 CAZymes, while *A. thiersii* encodes 370. Losses are generally concentrated in the families involved in the degradation of plant cell wall material (Chaib de Mares, 2013). This pattern seems to be a common feature of crown group ECM species and a basic strategy used by biotrophic fungi to escape detection by the plant immune system (Veneault-Fourrey & Martin, 2011; MGI http://mycor.nancy.inra.fr/IMGC/MycoGenomes/).

Like *L. bicolor*, *A. muscaria* has an amplified genome encoding 18,153 genes, almost twice as many genes as *A. thiersii*, which houses 10,354 (http://genome.jgi-psf.org/Amamu1/Amamu1.info.html; http://genome.jgi.doe.gov/Amath1/Amath1.info.html). The types of amplified gene families show close similarities to gene families amplified in *L. bicolor*: Among the five largest gene clusters in *A. muscaria*, two contain protein–protein interaction domains (e.g. NACHT and WD40), while another two appear to be tyrosine kinases (Martin et al., 2008; http://genome.jgi-psf.org/clustering/pages/cluster/clusters.jsf?runId=1898&organism=Amamu1). Analysis of the secretome reveals an overall decrease in numbers of secreted proteins in *A. muscaria* compared to *A. thiersii*, but with a clear shift towards a higher percentage of small secreted proteins in *A. muscaria* (Chaib de Mares, 2013).

TE distributions across the six sequenced genomes show no simple pattern with respect to ecological niche, although we find evidence for changes in TE dynamics following the evolution of the ECM symbiosis (Hess et al., in review). Abundant numbers of TEs are found in two of the three ECM species (36% genomic content in *A. brunnescens* and 59% in *A. polypyramis*), as well as the asymbiotic species *A. thiersii* (37%). The third mycorrhizal species, *A. muscaria*, houses a moderate proportion of TEs (21%), while both *A. inopinata* and *V. volvacea* possess few TEs (less than 10% in both cases). TE repertoires across the *Amanita* are dominated by RNA-based elements from the Gypsy, Copia and LINE superfamilies, and together, these make up over 80% of TE diversity (Hess et al., in review). Phylogenetic analysis of these three most abundant retrotransposon superfamilies mirrors the patterns found in assemblies and reveals large numbers of recently diverged elements in the three TE-rich species. While *A. thiersii* houses amplifications of

all three superfamilies, amplifications in *A. brunnescens* and *A. polypyramis* are concentrated in the LINE and Gypsy superfamilies but are especially prominent among LINE elements where 84% of TEs are from either *A. brunnescens* or *A. polypyramis*. Although *A. muscaria* amplifications are smaller than those found in *A. brunnescens*, *A. polypyramis* or *A. thiersii*, they outnumber those found in *A. inopinata* and *V. volvacea*.

Despite the lack of a simple pattern of elevated TE content in ECM genomes as compared to asymbiotic genomes, the presence of TE amplifications among ECM lineages suggests the evolution of the ECM lineages was accompanied by a period of either increased rates of TE proliferation or lower rates of TE removal, arguably with the same potential for TE-mediated chromosomal rearrangements, duplications and deletions. The patterns of TE content evolution in *A. thiersii* appear different to those in the ECM species. Individual families are amplified among the different ECM species, but all three types of retrotransposons are amplified within *A. thiersii*, suggesting that different mechanisms are at work to elevate TE content.

3. ECOLOGICAL GENOMICS OF ASYMBIOTIC AND ECM *AMANITA* SPECIES

3.1. Does symbiosis influence the pace of speciation in ECM *Amanita*?

Symbioses may facilitate evolutionary radiations by enabling new ecological opportunities, and the literature offers many examples from different domains and kingdoms. Often, mutualisms are also correlated with increased rates of speciation, for example, pollinators appear to drive plant diversification (Johnson, 2010), a mutualism with anemones may have triggered the adaptive radiation of clownfishes (Litsios et al., 2012), and microbes may mediate the diversification of phytophagous insects (Janson, Stireman, Singer, & Abbot, 2008).

However, modern theory offers conflicting opinions on the role of mutualism in speciation. Early theory suggested stochasticity in geographically isolated populations of mutualists would spur diversification, as forces like genetic drift caused isolated populations to diverge (Kiester, Lande, & Schemske, 1984). The geographic mosaic theory of coevolution (Thompson, 1999) also suggests that mutualisms can promote speciation when interacting species are divided into metapopulations. In contrast, a more recent model of two coevolving species suggests that mutualisms can slow diversification when phenotypes "match", for example, when

an aspect of a pollinator's shape matches the shape of a pollinated flower (Yoder & Nuismer, 2010). While plants and ECM fungi often grow as metapopulations, it is not clear what phenotype matching would mean in these symbioses.

It seems logical to ask whether the transition to symbiosis enabled an adaptive radiation of ECM *Amanita* species. Ryberg and Matheny (2012) compared diversification rates and times among genera of ECM in the Agaricales and found constant but different rates of diversification across all clades: the *Amanita* possessed intermediate rates of diversification. However, their analysis did not include asymbiotic species of the genus and does not explicitly test the hypothesis of differences in the rates of diversification between asymbiotic and symbiotic *Amanita*.

To test for changes in rates of diversification after the evolution of the ECM niche in *Amanita*, we used phylogenetic methods to infer a linearized phylogenetic tree and models of rate diversification. We collected nuclear large subunit ribosomal DNA (nucLSU) sequences from the 108 species analysed in Wolfe, Tulloss, et al. (2012), realigned them using PRANK (Löytynoja & Goldman, 2008) and manually removed regions of uncertainty, resulting in a 1598 bp alignment. We then ran BEAST v.1.75 (Drummond & Rambaut, 2007) under the GTR + Γ model with four discrete rate categories and a lognormal uncorrelated relaxed molecular clock to estimate rates of evolution for the nucLSU alignment on the fixed species tree from Wolfe, Tulloss, et al. (2012). The results of three parallel runs were combined after discarding 10% of the estimates as burn-in. A random sample of 1000 trees from the combined posterior set of trees served as the input data for MEDUSA (Harmon et al., 2008). MEDUSA fits a series of birth–death processes, modelling species diversification by using an increasing number of distinct rate partitions until the model improvement becomes insignificant. A summary of the MEDUSA results is shown in Fig. 8.1.

We recover two rate shifts that are well represented among the posterior trees; both are within ECM clades, one in subgenus Lepidella and the other in subgenus Amanita. In both cases, the estimated mean diversification rates are three times as fast as the background rate, although the estimates also have large standard deviations. Because we did not calibrate the molecular clock analysis, the units of the diversification rates are arbitrary, but rates can be compared to each other. The respective rate shifts are found in 66% and 69% of the posterior sample.

The interpretation of our results is complicated by the large confidence intervals on estimated node heights in the backbone of the tree. Large confidence intervals will affect diversification rate estimates in deeper clades and

our ability to test for a shift in diversification rate directly after the evolution of the ECM niche. Increasing the size of our dataset (adding additional loci) might narrow confidence intervals by improving branch length estimates, although rates on long branches are generally difficult to estimate (Schwartz & Mueller, 2010).

The accurate estimation of diversification rates also depends on taxon sampling; a general assumption of most methods developed for this purpose is a completely sampled phylogeny (e.g. Heath, Hedtke, & Hillis, 2008; Pybus & Harvey, 2000). If species sampling is even and so, by corollary, all deep lineages have been sampled, any new species added will likely be near the tips of the tree. The underrepresentation of nodes with shallow branch lengths in "evenly incompletely" sampled trees may lead to underestimation of diversification rates near the tips of the tree (Heath et al., 2008; Pybus & Harvey, 2000). Biases in unevenly sampled phylogenies are obviously more severe and would incorrectly increase or decrease rates in subsets of the tree.

Given the relative overrepresentation of asymbiotic *Amanita* in our phylogeny (slightly less than half of the described species, compared to about a fifth of the ECM species), we may be underestimating relative rates of diversification for the ECM species. We are also likely to be underestimating diversification rates near the tips of the tree, due to the relatively sparse sample of our dataset. A more comprehensive analysis including additional species and a better understanding of biogeography and natural history of the genus will be key to detangling the biases that may complicate our analysis. Nevertheless, there is at least some support for an increase in diversification rates following the evolution of the ECM symbiosis, and the large number of extant ECM *Amanita* proves that these species have been very successful.

The genus *Amanita* is not the only ECM clade proposed to have undergone an adaptive radiation, and multiple other clades also contain specious ECM lineages (Ryberg & Matheny, 2012; Smith & Read, 2010). Two competing hypotheses are proposed to explain potential radiations: the "dual origins" hypothesis, in which ECM species are proposed to have radiated at the same time as the diversifications of major ECM plant hosts (with the Pinaceae during the Jurassic and the Angiosperms during the Cretaceous (Halling, 2001)), and the "convergent radiation" hypothesis (Bruns, Szaro, Gardes, & Cullings, 1998), in which ECM lineages are proposed to have radiated more recently, as ECM plant communities expanded ranges into cooling climates. Ryberg and Matheny's (2012) study offers support for the second hypothesis but with the caveat that radiations may not be specific to ECM clades and may therefore be unrelated to ECM niche (Bruns et al.,

1998; Ryberg & Matheny, 2012). Our results suggest that ECM clades of *Amanita* have higher rates of diversification than asymbiotic clades of *Amanita*, and give some weight to the idea that radiations of ECM lineages were indeed adaptive and facilitated by symbiosis.

3.2. Does symbiosis reshape the ECM genome?

A variety of mutational mechanisms shape genes and genomes. In addition to single nucleotide substitutions and short insertions or deletions in protein-coding genes and regulatory regions, larger-scale events may involve duplications, losses or rearrangements. The size of the genomic regions involved in these events will vary widely and may encompass anything from segments of a single gene up to an entire genome but in all cases will provide evolutionary novelty (Kondrashov, 2012; Ramos & Ferrier, 2012; Zhang, 2003).

The mechanisms generating duplications, deletions and chromosomal rearrangements include nonallelic homologous recombination, involving either neighbouring stretches of DNA on the same chromosome or dispersed genomic regions, and nonhomologous end joining of double-strand breaks (Lynch, 2007; Ramos & Ferrier, 2012). TEs play a critical role in facilitating nonhomologous recombination events, by providing dispersed stretches of identical sequence that can act as seeds for recombination (Fiston-Lavier, Anxolabéhère, & Quesneville, 2007; Ponce, Martinsen, Vicente, & Hartl, 2012; Ramos & Ferrier, 2012); Small sequence repeats have been shown to be sites of chromosomal rearrangement in fungi (Ohm et al., 2012). In theory, the younger the TE family and the more abundant it is across the genome, the higher the probability it will facilitate a recombination event. An analysis of TEs found within a genome and the extent of gene order conservation, or "synteny", among related genomes may suggest (i) the extent to which chromosomal rearrangements mediated by TEs have influenced the evolution of gene content within the clade and (ii) whether and when TE dispersal within the genome has shaped synteny; if TEs influence chromosomal rearrangements, they may colocalize with synteny breakpoints.

Because gene content is very different between *A. thiersii and A. muscaria* (Chaib de Mares, 2013; J. Hess et al., unpublished; Wolfe, Tulloss, et al., 2012) and because TEs are found in abundance in two of the three ECM genomes (Table 8.1; Hess et al., in review), we designed an analysis to explore synteny conservation between our canonical saprotroph *A. thiersii*, the closely related *A. inopinata* and the ECM *Amanita* genomes.

We first identified *A. thiersii* scaffolds containing the key extracellular cellulases of CAZy families GH6 and GH7 (www.cazy.org), because the loss of PCWDEs appears to have been an important event in the early evolution of the *Amanita* ECM symbiosis (Wolfe, Tulloss, et al., 2012). Draft genome assemblies were aligned using PROmer from the MUMmer package (Kurtz et al., 2004) and filtered for matches to the three *A. thiersii* target scaffolds. Matching segments shorter than 1000 bp were removed, because we are primarily interested in visualizing long-range synteny of gene-sized segments. Adjacent regions were combined if there was no intervening segment from a different scaffold, internal duplication or change in directionality of the match.

Figure 8.2 illustrates the conservation of synteny between the *A. thiersii* scaffolds 2, 4 and 18, containing the predicted GH6 (scaffold 2) and the two predicted GH7 (scaffolds 4 and 18) genes, and homologous scaffolds in other *Amanita* genomes. Comparisons reveal different amounts of synteny conservation. As expected, *A. inopinata*, the closest relative (Fig. 8.1), shows the strongest amount of synteny conservation. All three *A. thiersii* scaffolds house long segments in synteny with scaffolds in *A. inopinata*, and two of these segments span cellulase loci (see radial bars, Fig. 8.2). Nevertheless, we find evidence for chromosomal rearrangements: The third cellulase locus, on scaffold 2 (the first *A. thiersii* scaffold, as you move in a clockwise direction), appears to be adjacent to a large chromosomal inversion and missing from *A. inopinata*, and the locus on scaffold 4 (the second *A. thiersii* scaffold), which contains a fragmented GH7 gene, consists of a complex segmental duplication. The different parts of scaffold 4 are superimposed onto the same scaffold in *A. inopinata*. Despite the conserved syntenic segments spanning two of the three target cellulases, all three enzymes are absent from *A. inopinata* and the scaffolds are not alignable in these regions, suggesting gene loss was independent of chromosomal rearrangements. As discussed previously, the ecological niche of *A. inopinata* remains undefined, and although it appears asymbiotic, all of the key PCWDEs are missing (Wolfe, Tulloss, et al., 2012).

The three ECM species display variable amounts of synteny conservation, with *A. muscaria* showing long contiguous matches to *A. thiersii* scaffolds, followed by *A. polypyramis* and finally *A. brunnescens*, which displays the largest breakdown in synteny. None of the cellulase loci in are syntenic. Phylogenetically, all three ECM species are equally distant to *A. thiersii* (cf. Fig. 8.1), raising questions as to what might cause differences in observed patterns of conservation.

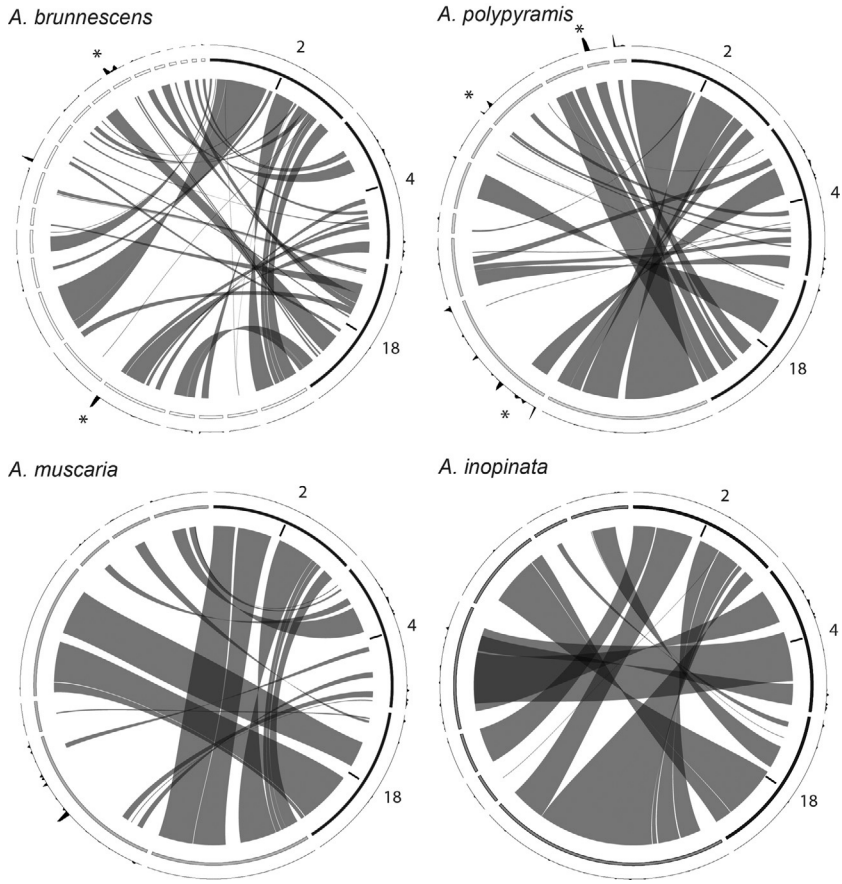

Figure 8.2 Conservation of synteny between the *Amanita thiersii* scaffolds containing key cellulases (GH6 and GH7), one on each scaffold, and matching scaffolds in other *Amanita* species. *A. thiersii* scaffolds are numbered and shown in black, and for the purpose of visualization, they are cropped to a total length of 100 kb surrounding the cellulase genes, the positions of which are indicated by the radial bars. Matching syntenic scaffolds are grey and cropped to the aligned positions plus a buffer of 10 kb on either side, if available. The line graph on the outer ring indicates TE density per 1000 bp window at the equivalent genomic coordinates. Asterisks mark the sites of potential TE-mediated synteny breakpoints.

The full interpretation of our data will first require a brief discussion of technical issues. The *A. muscaria* genome was sequenced by the US DOE Joint Genome Institute (http://genome.jgi-psf.org/Amamu1/Amamu1.home.html) using multiple Illumina libraries, including a 3.5 kb mate pair library, while the *A. brunnescens*, *A. polypyramis* and *A. inopinata* genomes

were assembled from a single 0.3 kb paired end library. We expect the *A. muscaria* assembly to be of higher contiguity than the other assemblies because repeat regions will have been more easily resolved. Nevertheless, the *A. inopinata*, *A. polypyramis* and *A. brunnescens* assemblies are directly comparable and prove that assembly contiguity and synteny conservation are not strictly determined by the sequencing approach.

Relative TE abundance and distribution may also explain the degree of synteny conservation among the different ECM species. Among the ECM species, the *A. muscaria* genome houses the lowest proportion of TEs (Table 8.1). Technically, *A. polypyramis* houses a much larger proportion of TEs than *A. brunnescens*, but the higher assembly contiguity in *A. polypyramis* (N50 of 61 kb, compared to 11 kb in *A. brunnescens*; Table 8.1) suggests TEs are concentrated outside of gene-rich regions and may therefore be less of an influence on our predominantly genic target scaffolds. The *A. brunnescens* assembly is much more fragmented than the *A. polypyramis* assembly; the fragmentation may be caused by a more randomly distributed population of TEs. TEs and other repeated regions frequently form breakpoints in genome assemblies (Alkan, Sajjadian, & Eichler, 2011). The pattern of synteny conservation among ECM species may reflect positive relationships between the abundance of TEs, their distribution in a genome and synteny degradation.

Additional support for an influence of TEs on the degradation of synteny conservation is seen in the localization of TE-dense regions near synteny breakpoints (asterisks, Fig. 8.2). However, the many small scaffolds in the *A. brunnescens* may also reflect "interruptions" caused by repeats, rather than chromosomal rearrangements.

3.3. Conclusions

Emerging theory explicitly connects the questions we have explored: advances in genome sequencing and comparative genomics are enabling mechanistic frameworks synthesizing molecular patterns, including chromosomal rearrangements, with evolutionary phenomena, for example, adaptive radiations (Böhne et al., 2008; Jurka, Bao, & Kojima, 2011; Oliver et al., 2013; Oliver & Greene, 2009, 2011, 2012; Zeh et al., 2009). TEs are central to the new ideas proposed to explain adaptive radiations.

Adaptive radiations are defined by evolutionary innovation and increased rates of speciation, and TEs may facilitate both processes. Active transposition and ectopic recombination between young TE copies reshuffle

the functional content of a genome. Domestication or "exaptation" of TE sequences and changes in gene regulation in the neighbourhood of TEs may also influence functional variation. An exhaustive list of examples and their evolutionary significance is found in Böhne et al. (2008), Oliver and Greene (2009, 2011, 2012) and Oliver et al. (2013). Moreover, the karyotypic variation resulting from chromosomal rearrangements can create reproductive barriers and cause reduced recombination and gene flow between chromosomal variants, accelerating the path to speciation (Böhne et al., 2008; Rieseberg, 2001). These ideas are encapsulated by the "TE-Thrust" model (Oliver & Greene, 2009, 2011, 2012), which proposes that lineages with large quantities of young TEs may be especially prone to speciation.

Based on our tentative evidence for an adaptive radiation following the evolution of the ECM symbiosis and our knowledge of genome architecture evolution in the *Amanita*, we can begin to ask whether and how TEs shaped the success of the ECM lineage. Two of the three ECM genomes we sequenced, *A. brunnescens* and *A. polypyramis* (Fig. 8.1), are rich in TEs, and the presence of closely related TE families in *A. polypyramis* and *A. muscaria* suggests a period of increased TE activity early in the ECM lineage (Hess et al., in review). Increased TE content, especially in *A. brunnescens*, and less so in *A. polypyramis*, coincides with a breakdown in long-range synteny (Fig. 8.2), suggesting that these ECM genomes may have undergone chromosomal rearrangements. The ECM fungus *A. brunnescens* falls within a lineage of increased speciation rates, as determined by our MEDUSA analysis (Fig. 8.1), while *A. polypyramis* and *A. muscaria* do not. Aggregate evidence strengthens the notion of a link between increased rates of speciation and changes in genome architecture.

The TE-Thrust model is focused on the age and abundance of TE families, but not on their distribution within the genome; on this point, the *Amanita* may provide a novel perspective. Our *A. polypyramis* results suggest that despite a high TE content, genomic rearrangements are less than in a species with lower TE content, *A. brunnescens*, perhaps because TE insertions are concentrated in regions without genes. A more nuanced model of adaptive radiations and TEs might consider patterns of TE distributions within genomes as an additional variable to explain the relationships between TEs and speciation rates.

Nonetheless, whether or not TEs influenced the radiation of ECM *Amanita* remains to be determined. Genomes from additional species, long-range sequencing libraries collected to improve existing assemblies and the sequencing of more individuals from different populations of already sequenced species may distinguish the potential influence of natural

selection from stochasticity and distinguish between TE amplification as an active driver of speciation and patterns of TE content as a by-product of population genetics and demographic histories (Lynch, 2007). The *Amanita* belong to a charismatic genus, but even so, we also have remarkably little knowledge of the mating strategies, life cycles and demographic histories of many species. As we learn more about the genus, disentangling the various forces shaping speciation will be an ever more exciting and fruitful field of inquiry.

4. UNANSWERED QUESTIONS: RANGE EXPANSIONS AND GENOMIC ARCHITECTURES

An obvious feature of the natural histories of *Amanita* is range expansion; *A. thiersii* is moving north from Texas, while *A. inopinata* and *A. muscaria* (Vellinga, Wolfe, et al., 2009) seem to be establishing on several continents at once. In New Zealand, *A. muscaria* is invading in association with invasive pines (Dickie, Bolstridge, Cooper, & Peltzer, 2010). Other unsequenced *Amanita* are also invading novel habitats, for example, the ECM *A. phalloides* in California (Pringle, Adams, Cross, & Bruns, 2009). Although invasions appear as idiosyncratic phenomena, relatively little is known about the genomes of invasive species, and research on the ecological genomics of invasions may provide novel tools and discoveries (Suarez & Tsutsui, 2008). The genus *Amanita* is unique because it encompasses multiple introductions and invasions by species with both asymbiotic and symbiotic niches. Beyond obvious comparisons between decomposer and ECM fungi, salient questions will focus on what features of genomes enable dispersal, establishment or spread (Vellinga et al., 2009). The theory suggests targeting mating systems (is selfing an advantage?), genes involved in enabling associations with novel hosts (are generalists more likely to establish or spread? Pringle, Bever, et al., 2009), and genome plasticity perhaps mediated by the diversity of TEs in introduced populations (what do TE populations look like in native and invasive ranges?). The *Amanita* offer an exciting opportunity to push invasion biology in new directions.

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