CURRENT REVIEW

Accessories Make the Outfit: Accessory Chromosomes and Other Dispensable DNA Regions in Plant-Pathogenic Fungi

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Fungal pathogen genomes can often be divided into core and accessory regions. Accessory regions (ARs) may be comprised of either ARs (within core chromosomes (CCs) or wholly dispensable (accessory) chromosomes (ACs). Fungal ACs and ARs typically accumulate mutations and structural rearrangements more rapidly over time than CCs and many harbor genes relevant to host-pathogen interactions. These regions are of particular interest in plant pathology and include host-specific virulence factors and secondary metabolite synthesis gene clusters. This review outlines known ACs and ARs in fungal genomes, methods used for their detection, their common properties that differentiate them from the core genome, and what is currently known of their various roles in pathogenicity. Reports on the evolutionary processes generating and shaping AC and AR compartments are discussed, including repeat induced point mutation and breakage fusion bridge cycles. Previously ACs have been studied extensively within key genera, including *Fusarium*, *Zygomycetia*, and *Alternaria*, but are growing in frequency of observation and perceived importance across a wider range of fungal species. Recent advances in sequencing technologies permit affordable genome assembly and resequencing of populations that will facilitate further discovery and routine screening of ACs.

All organisms require the maintenance of a stable genome that encodes essential functions for life. In some cases, such as for several fungal pathogen species, certain conditions such as environmental stress, continuously evolving host defenses, or adoption of resistant cultivars may also promote genome plasticity. The concept of a ‘core’ genome that is common to all isolates encoding all genes necessary for survival under normal growth conditions has emerged and is distinguished from the ‘accessory’ genome (Ma et al. 2010; Raffaele and Kamoun 2012; Syme et al. 2013; Vanheule et al. 2016). Accessory genetic elements are well-studied in bacterial genomes, which may contain plasmids that harbor pathogenicity islands (Jackson et al.

2011), and in plant genomes, which may contain supernumerary ‘B’ chromosomes (Jones et al. 2008; Makunin et al. 2014). The accessory genomes of fungi may be comprised of either segments of core chromosomes (CCs) that may be partially lost (accessory regions [Ars]) or wholly dispensable (accessory) chromosomes (ACs). ACs have been described by several terms (Covert 1998), (conditionally) dispensable chromosomes, lineage-specific chromosomes, supernumerary chromosomes, and B chromosomes. The term ‘minichromosome’ also describes a short chromosome (i.e., <2 Mbp) that may (or may not) be an AC.

Known fungal ACs exhibit one or more common properties that differentiate them from CCs. An AC may confer an advantage under certain conditions, for example, ACs of plant pathogens may encode genes that determine an advantageous outcome during interaction with a host, including effectors and metabolic gene clusters (MGCs) (Table 1). Fungal ACs are often meiotically unstable and may be inherited in a non-mendelian manner (Covert 1998). Some ACs have also been observed to transfer from a competent to a deficient strain of the same species (Akagi et al. 2009; He et al. 1998; Ma et al. 2010; Manners and He 2011; Vlaardingerbroek et al. 2016b). Compared with CCs, ACs typically have lower gene density and are enriched in transposable elements (TEs) and other repeats (de Jonge et al. 2013; Galazka and Freitag 2014; Hatta et al. 2002; Klosterman et al. 2011; Ma et al. 2010; Vanheule et al. 2016; Williams et al. 2016). They are typically small (<2 Mbp), low in number, make up a small proportion of the total genome content, and encode fewer ‘housekeeping’ genes than CCs (Chuma et al. 2011; Goodwin et al. 2011; Jones et al. 1998; Luo et al. 2007; O’Connell et al. 2012; Talbot et al. 1993; Zolan 1995). Their reduced gene content suggests that there may be lowered selection pressure on ACs, which is supported by increased observations of transposon and other repetitive sequence content, single-nucleotide polymorphisms and structural mutations in ACs compared with CCs (Hane et al. 2011; Tsuge et al. 2016; Williams et al. 2016). The activity of fungal-specific genome mutagenesis mechanisms such as repeat induced point mutation (RIP) (Hane et al. 2015) and increased rates of intra-chromosomal recombination (resulting in mesosynteny) (Hane et al. 2011) are likely to have heavily influenced the evolution of repeat-rich ACs. Large sections of chromosomes may be partially lost (ARs) and may have similar properties to ACs.

Supporting evidence for the presence of ARs and ACs varies widely in type and reliability. Early studies used karyotype profiling—sometimes assigned to known genetic linkage

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groups, genetic maps, or physical maps—which can indicate chromosome length polymorphism (CLP), copy number variation, and presence/absence variation (PAV) (Zolan 1995). Whole-genome sequencing has been complementary to karyotyping (Vlaardingerbroek et al. 2016a and b) but has also been used independently to infer ACs. For species in which reference chromosomes have been established, resequencing

of multiple isolates has been used to determine PAV versus reference chromosomes (McDonald et al. 2016). Purely bioinformatic methods can also infer ACs or ARs in even highly fragmented genome assemblies, either by subtraction of highly conserved core genome matches (Williams et al. 2016), by prediction of AC-like properties (Ohm et al. 2012), or through observation of both macro- and mesosynteny between

Table 1. Summary of known fungal accessory chromosomes (ACs), accessory regions (ARs), and their properties^a

Taxonomy	Species	Strain/isolate/biotype	ACs (number, length, or sequence IDs)	Function/virulence factors	Pathogenicity phenotype and gene content	Evidence type	References
Dothideomycetes Botryosphaeriales	<i>Macrophomina phaseolina</i>		0 to 6 ACs: (785 kb to 2 Mb)	Unknown	Unknown	Electrophoretic karyotype	Jones et al. 1998
Dothideomycetes Capnodiales	<i>Zymoseptoria ardabiae</i>	ST04IR_1.1.1	4 ACs	Unknown	Unknown	In silico read mapping	Kellner et al. 2014; Stukenbrock et al. 2012
Dothideomycetes Capnodiales	<i>Zymoseptoria psuedotritici</i>	ST04IR_2.2.1	6 ACs	Unknown	Unknown	In silico read mapping	Kellner et al. 2014; Stukenbrock et al. 2012
Dothideomycetes Capnodiales	<i>Zymoseptoria tritici</i> (syn. <i>Mycosphaerella graminicola</i> , <i>Septoria tritici</i>)	IPO323	8 ACs: 14 to 21 (0.4 to 0.8 Mb)	Unknown	Virulence (wheat)	Sequence alignment; electrophoretic karyotype; in silico read mapping	Croll and McDonald 2012; Goodwin et al. 2011; McDonald et al. 2016; Mehrabi et al., 2007; Stewart et al. 2018; Ware 2006; Wittenberg et al., 2009
Dothideomycetes Capnodiales	<i>Pseudocercospora</i> (syn. <i>Mycosphaerella</i>) <i>fijiensis</i>	CIRAD86	Scaffolds 11 to 26	Unknown	Unknown	Prediction—sequence properties; Comparative genomics	Arango Isaza et al. 2016; Ohm et al. 2012
Dothideomycetes Pleosporales	<i>Alternaria alternata</i> f. sp. <i>citri</i> (tangerine pathotype)	SH20	<2 Mb	ACT-toxin biosynthesis cluster	Virulence (tangerine)	Electrophoretic karyotype	Akamatsu et al. 1999; Miyamoto et al. 2008
Dothideomycetes Pleosporales	<i>Alternaria alternata</i> f. sp. <i>citri</i> (rough lemon pathotype)	HC1	<1.5 Mb	ACR-toxin biosynthesis cluster	Virulence (lemon)	Electrophoretic karyotype	Akamatsu et al. 1999; Masunaka et al. 2005
Dothideomycetes Pleosporales	<i>Alternaria alternata</i> f. sp. <i>fragariae</i>	NAF8	1.0 Mb	AF-toxin biosynthesis cluster	Virulence (strawberry)	Electrophoretic karyotype	Akamatsu et al. 1999; Hatta et al. 2002
Dothideomycetes Pleosporales	<i>Alternaria alternata</i> f. sp. <i>mali</i>	IFO8984	<1.8 Mb	AM-toxin biosynthesis cluster	Virulence (apple)	Electrophoretic karyotype	Akagi et al. 2009; Akamatsu et al. 1999; Harimoto et al. 2007; Johnson et al. 2000; Taga et al. 1999
Dothideomycetes Pleosporales	<i>Alternaria arborescens</i> (syn. <i>A. alternata</i> f. sp. <i>lycopersici</i>)	EGS 39-128 As-27	1.0 Mb	AAL-toxin biosynthesis cluster	Virulence (tomato)	Electrophoretic karyotype	Akagi et al. 2009; Akamatsu et al. 1999; Hu et al. 2012
Dothideomycetes Pleosporales	<i>Alternaria gaisen</i>	O-276, ML10E, K-111, 15 A	<2 Mb to 4.1 Mb	AK-toxin biosynthesis cluster	Virulence (japanese pear)	Electrophoretic karyotype	Akamatsu et al. 1999; Tanaka and Tsuge 2000
Dothideomycetes Pleosporales	<i>Cochliobolus carbonum</i>	SB111 (wt), 243-7 (deletion strain)	3.5 Mb AC (1.4 Mb deletion) *	HC-toxin biosynthesis cluster	Virulence (maize, genotype <i>hm1/hm1</i>)	Electrophoretic karyotype	Pitkin et al. 2000
Dothideomycetes Pleosporales	<i>Cochliobolus heterostrophus</i>	Race T isolates (e.g., C5 parental strain: B30.A3.R.45)	~1.2 Mb ARs on Chr 6, 12, and 16	<i>Tox1</i> (T-toxin biosynthesis cluster) on the AR, no known virulence factors map to Chr16	Virulence (corn, Texas male sterile cytoplasm)	Electrophoretic karyotype; RFLP	Condon et al. 2013; Kodama et al. 1999; Ohm et al. 2012; Tzeng et al. 1992
Dothideomycetes Pleosporales	<i>Leptosphaeria maculans</i>	V23.1.3 /JN3	Scaffold SC22 (731.4 kb) ~1 Mb AC	<i>AvrLm11</i> avirulence effector	Virulence (<i>Brassica rapa</i> , cultivars lacking <i>Rlm11</i>)	Prediction—sequence properties	Balesdent et al. 2013; Ohm et al. 2012; Plummer and Howlett 1995
Dothideomycetes Pleosporales	<i>Parastagonospora nodorum</i>	SN15	1 AC: ~ 300 to 700 kb Scaffold 46 ^b	Unknown	Reported AC observed only in wheat or barley adapted strains	Electrophoretic karyotype; prediction—sequence properties	Ohm et al. 2012; Syme et al. 2013; Zolan 1995
Dothideomycetes Pleosporales	<i>Setosphaeria turcica</i>	Et28A	Scaffold 28	Unknown	Unknown	Prediction—bioinformatic survey of sequence properties	Ohm et al. 2012
Leotiomycetes Sclerotiniaceae	<i>Botrytis cinerea</i>	B05.10	2 minichromosomes, 17 and 18 247 and 209 kb Chr 18 lacking in strain 09Bc11	Unknown	Unknown	Southern hybridization	Van Kan et al. 2017
			Chr 17 nonuniform coverage in strain 09Bc11	Unknown	Unknown	Comparative genomics	
Sordariomycetes Glomerellales	<i>Colletotrichum gloeosporioides</i>	Biotype A (common)	1 AC (~ 2 Mb)	Unknown	Virulence (<i>Stylosanthes</i> spp.)	Electrophoretic karyotype; RFLP	He et al. 1998
		Biotype B (rare)	1 AC (~ 1.2 Mb)	Unknown	Virulence (<i>S. guianensis</i>)		
		Bx, UQ396		Unknown	Chlorosis (<i>S. scabra</i>)		

(Continued on next page)

^a Chr = chromosome; RFLP = restriction fragment length polymorphism; HGT = horizontal gene transfer; PEP = pea pathogenicity; CC = core chromosome; FISH = fluorescence in situ hybridization; PCR = polymerase chain reaction.

^b Prediction based on bioinformatic survey of sequence properties.

chromosomes of closely related genomes (Hane et al. 2011). The chromatin modification profile of a whole chromosome or large region has also been observed to correlate with ACs and may be an effective method of AC prediction (Galazka and Freitag 2014).

KNOWN ACCESSORY CHROMOSOMES AND OTHER DISPENSABLE REGIONS

Two of the best-studied species with ACs are from the *Fusarium* genus, *F. solani* (syn. *Haematonectria hematococca*)

(Coleman et al. 2009; Mehrabi et al. 2011; Miao et al. 1991) and *F. oxysporum* (Ma et al. 2010; Schmidt et al. 2013; Vanheule et al. 2016; Williams et al. 2016). These ACs are often meiotically unstable (Miao et al. 1991), gene sparse, and enriched for TEs and other repeats (Ma et al. 2010). *F. solani* possess at least three ACs (ATCC18098 chromosomes 14, 15, and 17), one of which encodes a pea pathogenicity (PEP) cluster required to detoxify the phytoalexin pisatin and confer virulence on pea (Temporini and VanEtten 2002). *F. oxysporum* is a species with several recognized formae speciales (pathovars) that are defined

Table 1. (Continued from previous page)

Taxonomy	Species	Strain/isolate/ biotype	ACs (number, length, or sequence IDs)	Function/virulence factors	Pathogenicity phenotype and gene content	Evidence type	References
Sordariomycetes Glomerellales	<i>Colletotrichum graminicola</i>	M1.001 (also known as M2)	Chr 11-13 (<1 Mb)	Unknown	Unknown	Prediction—comparative genomics	O’Connell et al. 2012
Sordariomycetes Glomerellales	<i>Colletotrichum higginsianum</i>	IMI 349063	Chr 11-12 (<1 Mb)	Unknown	Unknown	Prediction—comparative genomics	O’Connell et al. 2012
Sordariomycetes Glomerellales	<i>Colletotrichum lindemuthianum</i>	Race 0, multiple isolates	2 to 6 ACs (<2.5 Mb)	Unknown	Unknown	Electrophoretic karyotype; Flow cytometry; Southern hybridization	O’Sullivan et al. 1998
Sordariomycetes Hypocreales	<i>Fusarium asiaticum</i>	Not stated	Chr V (0.45 Mb)	Unknown	Unknown	Prediction—epigenetic profiling	Galazka and Freitag 2014
Sordariomycetes Hypocreales	<i>Fusarium fujikuroi</i>	Not stated	Chr XII (0.69 Mb)	Unknown	Unknown	Prediction—epigenetic profiling	Galazka and Freitag 2014
Sordariomycetes Hypocreales	<i>Fusarium oxysporum</i> f. sp. <i>ciceris</i> #	<i>Foc</i> -38-1	Multiple scaffolds	Unknown	Unknown	Prediction—bioinformatic subtraction of core-chromosome matches	Williams et al. 2016
Sordariomycetes Hypocreales	<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>	Fol-4287	ACs: Chr 3, 6, 14, 15 (0.2 to 1.3 Mb) ARs: Chr 1 and 2	Secreted-in-xylem pathogenicity genes Transcription factors regulating effector gene expression	Virulence (tomato) CCA gene cluster: possible HGT from <i>Cochliobolus lunatus</i> or <i>Oidiodendron maius</i>	Electrophoretic karyotype; AC-specific PCR; Southern hybridization; flow cytometry	Elmore et al. 2015; Ma et al. 2010; Schmidt et al. 2013; van der Does et al. 2016; Vlaardingerbroek et al. 2016a; Williams et al. 2016
				CCA gene cluster (three copies)	Enriched for predicted pathogenicity loci		
Sordariomycetes Hypocreales	<i>Fusarium oxysporum</i> f. sp. <i>medicaginis</i> #	Fom-5190a	Multiple scaffolds	Unknown	Enriched for predicted pathogenicity loci	Prediction—bioinformatic subtraction of core-chromosome matches	Williams et al. 2016
Sordariomycetes Hypocreales	<i>Fusarium oxysporum</i> f. sp. <i>melonis</i>	NRRL 26406	Multiple scaffolds	CCA gene cluster	CCA gene cluster: possible HGT from <i>Cochliobolus lunatus</i> or <i>Oidiodendron maius</i>	Prediction—bioinformatic subtraction of core-chromosome matches	Elmore et al. 2015; Williams et al. 2016
Sordariomycetes Hypocreales	<i>Fusarium oxysporum</i> f. sp. <i>pisi</i>	Fop-37622	Multiple scaffolds	PEP cluster defines host range allowing pisatin detoxification CCA gene cluster	CCA gene cluster: possible HGT from <i>Cochliobolus lunatus</i> or <i>Oidiodendron maius</i>	Prediction—bioinformatic subtraction of CC matches	Elmore et al. 2015; Williams et al. 2016
Sordariomycetes Hypocreales	<i>Fusarium poae</i>	<i>F. poae</i> 2516	172 contigs (total ~ 8.18 Mb)	Unknown	ZIT1 transposon (marker for ACs)	Prediction—bioinformatic subtraction of CC matches	Coleman et al. 2009; Mahmoud and Taga 2012; Mehrabi et al. 2011; Miao et al. 1991; Temporini and VanEtten 2002; Vanheule et al. 2016; Wasmann and VanEtten 1996
Sordariomycetes Hypocreales	<i>Fusarium solani</i> (syn. <i>Haematonectria hematococca</i>)	ATCC18098	ACs: Chr 14, 15, 17 Minichromosome (410 kb) (mendelian)	PEP cluster, <i>Pda6</i> cytochrome p450	Virulence (pea, via pisatin demethylation)	Electrophoretic karyotype; FISH; AC-specific PCR	Coleman et al. 2009; Mahmoud and Taga 2012; Mehrabi et al. 2011; Miao et al. 1991; Temporini and VanEtten 2002; Wasmann and VanEtten 1996
Sordariomycetes Hypocreales	<i>Fusarium verticillioides</i>	FGSC 7983, FGSC 8067, FGSC 8070	Chromosome 12	Unknown	Unknown	Genetic map	Jurgenson et al. 2002
Sordariomycetes Hypocreales	<i>Metarhizium anisopliae</i>	Strain V275	AC (1.05 Mb)	Destruxin biosynthesis	Virulence (insects)	Electrophoretic karyotype	Wang et al. 2003
Sordariomycetes Hypocreales	<i>Verticillium dahliae</i>	JR2	4 ARs (~ 2 Mb) (prediction) AR: Chr 5 (race 2 strains)	<i>Ave1</i> biosynthesis <i>Ave1</i> biosynthesis	Virulence (via <i>Ave1</i>) Enriched for in planta-expressed effectors	In silico read mapping	de Jonge et al. 2013; Faino et al. 2016; Klosterman et al. 2011
Sordariomycetes Magnaporthales	<i>Magnaporthe oryzae</i>	Reference strain 70–15 (no ACs) with ACs: 4224-7-8, P131, Y34, FJ81278, 84R-62B, GFS11-7-2, and HN19311	1 to 4 minichromosomes (0.4 to 2.2 Mb) 2 ACs: strains 84R-62B (1.6 Mb, mendelian) and GFS11-7-2 (1.2 Mb, nonmendelian)	<i>AvrPik</i> avirulence effector	1.6 Mb AC: Virulence (rice) and duplications from Chr 1 and 17	Electrophoretic karyotype; Southern hybridization; comparative genomics	Chen et al. 2013; Chuma et al. 2011; Luo et al. 2007; Talbot et al. 1993; Xue et al. 2012

by their host specificity, itself largely determined by their AC content (Ma et al. 2010; van Dam et al. 2016; Williams et al. 2016). Across *F. oxysporum* formae speciales, the size of the core genome is relatively conserved, while the AC and AR content is variable (van Dam et al. 2016; Williams et al. 2016). All known pathogenic isolates possess an AC, homologous to the tomato-infecting isolate 4287 chromosome 14, encoding several ‘secreted-in-xylem’ proteins that confer pathogenicity, and their presence can be used to predict host specificity (van Dam et al. 2016). Recombination between upstream Helitron TEs has been proposed to have driven the evolution of separate races in this species (Schmidt et al. 2013; Vlaardingerbroek et al. 2016a). Genetic maps have also identified a 0.7-Mb AC of *Fusarium verticillioides* (Jurgenson et al. 2002). *Fusarium* spp. have also been shown to have distinct epigenetic profiles within ACs compared with CCs, which has facilitated further predictions of ACs based solely on epigenetic profiling in *F. asiaticum* and *F. fujikuroi* (Table 1) (Galazka and Freitag 2014).

The causal agent of septoria tritici blotch of wheat, *Zymoseptoria tritici* (syn. *Mycosphaerella graminicola*), is another well-studied species with confirmed ACs. Karyotyping detected isolates with up to 21 chromosomes (Goodwin et al. 2011; Zolan 1995). The ‘finished’ genome of the reference isolate IPO323 contains eight ACs ranging from 409 to 773 kb—the highest number observed so far. An additional finished genome of isolate 3D7 contains four of the ACs present in IPO323 (Plissonneau et al. 2016). The origin of the *Z. tritici* ACs was proposed to have occurred via lateral transfer followed by sequence mutation and recombination events (Goodwin et al. 2011). Recently, they were proposed to have stemmed from ancient CC duplications (non-disjunction) followed by degradation via breakage fusion bridge (BFB) cycles and RIP on duplicated sequences (Croll et al. 2013). Approximately half of *Z. tritici* ACs are paralogs of CC genes (Croll and McDonald 2012; Goodwin et al. 2011). In contrast to ACs in *Fusarium* spp., although *Zymoseptoria* ACs exhibit many typical features, they are not enriched in predicted secreted genes and encode no characterized pathogenicity loci (Ware 2006). Nevertheless, recent genetic mapping studies report a small but significant association with virulence for four ACs (IPO323 chromosomes 14, 15, 18, and 21) (Stewart et al. 2018) (Table 1). *Z. pseudotritici* and *Z. ardabiliae* also possess at least six and four ACs, respectively, which are homologous to ACs of *Z. tritici* (Kellner et al. 2014; Stukenbrock et al. 2012) (Table 1).

Several other species have confirmed ACs with direct roles in plant pathogenicity (Table 1). *Alternaria* spp. are predominantly saprophytic, yet four species have acquired plant pathogenicity via ACs of 1 to 2 Mb (Akamatsu et al. 1999) that encode TOX genes involved in the synthesis of necrotrophic effectors (host-specific toxins) (Akamatsu et al. 1999; Tsuge et al. 2013, 2016). These effector biosynthesis genes have been extensively duplicated and their copy number is quantitatively correlated with virulence (Akagi et al. 2009; Harimoto et al. 2007; Hatta et al. 2002; Johnson et al. 2000; Masunaka et al. 2005; Miyamoto et al. 2008; Ruswandi et al. 2005; Tanaka and Tsuge 2000). In *Colletotrichum gloeosporioides*, vegetatively incompatible biotypes A and B infect tropical legumes of *Stylosanthes* spp. causing either type A or type B anthracnose, respectively. Biotype A isolates commonly carry a 2-Mb AC, while only biotype B isolates belonging to race 3 are observed to carry a 1.2-Mb homologous AC (He et al. 1998). Transfer of the 2-Mb AC from biotype A to B can confer type A anthracnose symptoms to the type B recipient (He et al. 1998). However, ACs in other *Colletotrichum* spp. are less well-defined. *C. graminicola* has three minichromosomes less than 1 Mb in size and *C. higginsianum* has two minichromosomes (O’Connell et al. 2012); all five ACs are repeat-rich and lack homology to chromosomes of other *Colletotrichum* spp. Chromosomes of

C. lindemuthianum show CLP and PAV, which may indicate ACs in this species as well (O’Sullivan et al. 1998). In *Lep-tosphaeria maculans* (causal agent of black leg or phoma canker of canola) there is an approximately 1-Mb AC encoding the avirulence effector gene *AvrRml1* conferring virulence on *Brassica rapa* or avirulence in the presence of the *Rml1* resistance gene (Balesdent et al. 2013; Cozijnsen et al. 2000; Leclair et al. 1996; Plummer and Howlett 1995). Approximately one third of the *L. maculans* genome is also compartmentalized into interspersed transposon- and AT-rich regions, which also frequently harbor avirulence effectors (Rouxel et al. 2011; Testa et al. 2016; Van de Wouw et al. 2010) and have AC- and AR-like properties (Balesdent et al. 2013). *Magnaporthe oryzae*, causal agent of rice blast, has several minichromosomes ranging from 470 kb to 2.2 Mb (Talbot et al. 1993) and two confirmed ACs, a 1.2-Mb AC (Chuma et al. 2011) and a 1.6-Mb AC containing the avirulence gene *AvrPik* (Luo et al. 2007). Independent comparative genomics studies between different pairs of field isolates have also reported ARs (totaling approximately 1.75 Mb) with AC- and AR-like properties (Chen et al. 2013; Xue et al. 2012). *Cochliobolus heterostrophus* (southern corn leaf blight) has one AC (chromosome 16), present in race T isolates and absent from race O, as well as an estimated 1.2-Mb AR (Condon et al. 2013; Tzeng et al. 1992). This AR contains *Tox1*, which encodes proteins that synthesize the polyketide T-toxin conferring virulence on Texas male sterile cytoplasm corn (Kodama et al. 1999) (Table 1).

In other fungal species, preliminary data are available supporting the presence of ACs (Table 1). In the broad-host range pathogen *Macrophomina phaseolina*, from zero to six ACs were observed in isolates ranging from 785 Kb to approximately 2 Mb (Jones et al. 1998). PAV patterns between isolates are highly variable and there is not a clear association between AC content and host range. *Verticillium* spp. include several pathogens causing vascular wilt diseases with wide host ranges and are asexual despite rare recombination events occurring as a result of parasexual processes (Milgroom et al. 2014; O’Garro and Clarkson 1992). For *V. dahliae* and *V. albo-atrum*, karyotyping has indicated CLPs but not ACs (Pantou and Typas 2005). Subsequent comparative genomics revealed four ARs (approximately 300 Kb) with AC- and AR-like properties present in *V. dahliae* and absent in *V. albo-atrum* (Klosterman et al. 2011). Further comparison of eleven *V. dahliae* isolates revealed interspersed ARs totalling approximately 4 Mb, with one isolate containing race 1-specific effector gene *Ave1* (de Jonge et al. 2013; Faino et al. 2016). TEs have been proposed to drive diversification within *V. dahliae* ARs by triggering large-scale rearrangements, duplication, and loss (Faino et al. 2016). The recently finished genomic sequence of *Botrytis cinerea* isolate B05.10 identified two small ACs (<250 kb), with chromosome 18 wholly absent and chromosome 17 partially absent from isolate 09Bc11 (Van Kan et al. 2017). Karyotyping of the necrotroph *Parastagonospora nodorum* (septoria nodorum blotch of wheat) identified an AC of 300 to 700 kb that was only reported for strains isolated from wheat or barley (Caten and Newton 2000; Cooley and Caten 1991; Zolan 1995). Bioinformatic prediction of altered gene and repeat density also predicted a *P. nodorum* scaffold as a putative AR (Ohm et al. 2012), however, it does not contain any of its known necrotrophic effector loci (Crook et al. 2012; Tan et al. 2014), which all reside on large sequences (Syme et al. 2013).

MECHANISMS OF GAIN, LOSS, AND MUTAGENESIS IN ACS

ACs can potentially be lost or transferred between isolates, although both are likely to be rare events. The rate of loss of

chromosome 14 of *F. oxysporum* f. sp. *lycopersici* was measured at 1 in 35,000 spores (Vlaardingerbroek et al. 2016b). The mechanism of BFB cycles (McClintock 1941) combined with large sequence insertions have been proposed as a putative cause of AC formation and loss (Croll et al. 2013). BFB cycles begin with loss of telomeres causing instability at the distal regions of chromosomes and potential fusion of sister chromatids. Products of BFB cycles depend on the site of breakage during the separation of erroneously fused sister chromatids, which may lead to sequence duplication, deletion, rearrangement, or the creation of new minichromosomes. In support of this model, telomeric regions and ACs share many similar sequence properties (Galazka and Freitag 2014). Notably, the ARs on partially dispensable chromosomes of *F. oxysporum* f. sp. *lycopersici* are located near the telomeres (Ma et al. 2010), suggesting that these may be BFB-affected regions that have not become separate chromosomes. In the genome of a closely related species, *F. graminearum*, there are hypervariable ‘hot-spot’ regions that also present AC-like characteristics and appear to be fused subtelomeric regions of homologous CCs that are conserved across *Fusarium* spp. (King et al. 2015; Ma et al. 2010). A 1.6-Mb AC of *Magnaporthe oryzae* contains a duplicated distal region of CC 1 (Luo et al. 2007). MGCs, which are frequently laterally transferred between distant fungal species, are also biased toward subtelomeric regions (Galazka and Freitag 2014; Wisecaver and Rokas 2015). Surprisingly, the

formation of *Fusarium* ACs appears to be reversible, as comparison of multiple isolates showed some AC regions had translocated back into the core genome, with a location bias toward subtelomeric regions (Vanheule et al. 2016). This may indicate that following BFB-mediated formation, it is possible for an AC to be reintegrated into a CC (Fig. 1).

ACs may be gained by deficient isolates via the fusion of conidial anastomosis tubes (CATs) between hyphal cells (Roca et al. 2003). The formation of CATs, specialized hyphae that mediate cell fusion uniquely during colony initiation, results in a state of heterokaryosis (Galazka and Freitag 2014; Strom and Bushley 2016; Wisecaver and Rokas 2015), in which two nuclei are present within a single cell. Heterokaryosis may facilitate nonmeiotic recombination between homologous chromosomes, and slow-growing heterokaryons have been hypothesized to be the intermediate step for AC transfer (Manners and He 2011). The fusion of CATs is usually limited (or entirely prevented) by vegetative incompatibility (*vic*) genes (Paoletti 2016) and the continued viability of successful fusions is then determined by heterokaryotic incompatibility (*het*) genes (Strom and Bushley 2016). Incompatible *vic* interactions are unable to undergo CAT fusion, and incompatible *het* interactions trigger local and surrounding cell death (Strom and Bushley 2016). The presence of some ACs may influence compatibility, as predicted ACs of *F. oxysporum* have been observed to be enriched in *het* genes (Schmidt et al. 2013; Williams et al. 2016). CATs themselves

Fig. 1. Summary of potential genome evolutionary processes involving fungal accessory chromosome gain and loss, formation, and modification. **A**, Accessory chromosomes may be lost due to linkage disequilibrium or gained through lateral transfer. **B**, New accessory chromosomes may be formed through duplication via nondisjunction. The sequence of duplicated chromosomes may be mutated by repeat induced point mutation (RIP), and larger duplicated core chromosomes may become degraded into shorter chromosomes over time. **C**, The structure of accessory chromosomes may be modified by breakage-fusion bridge cycles resulting in shortening or addition of duplicated sequence or fusion with another chromosome.

may have a suppressive effect on incompatibility relative to other types of hyphal fusions, as observed in *C. lindemuthianum* (Ishikawa et al. 2012; Ma et al. 2010). In addition to the exchange of genetic material between individual isolates of the same species, CATs may also facilitate lateral gene transfer (LGT) (Coleman et al. 2009; Ma et al. 2010; Mehrabi et al. 2011; Hu et al. 2012) between different species. A relatively distant LGT from *Aspergillus* spp. has been proposed as the origin of *F. solani* ACs (Coleman et al. 2009); however, they may also have emerged prior to the divergence of these two genera (Croll and McDonald 2012). Transfers of ACs conferring increased virulence have been observed under laboratory conditions for *F. oxysporum* (Ma et al. 2010), *A. alternata* (Vlaardingerbroek et al. 2016a), and *C. gloeosporioides* (Manners and He 2011). Isolates of *F. oxysporum* have also been observed to exhibit different rates of lateral transfer between similarly sized ACs, and CCs were also rarely observed to be partially transferred when there was also cotransfer of an AC (Vlaardingerbroek et al. 2016a).

Many ACs and ARs exhibit high levels of sequence polymorphism or disruption of chromosomal colinearity. The majority of the lengths of ACs and ARs do not appear to be under strong selection pressures and ACs are more likely to retain mutations than CCs, likely due to their gene scarcity. Therefore, over time, sequence and structural variations may accumulate within ACs. Their high repetitive DNA contents also make them prone to the activity of RIP (Hane et al. 2015). RIP is a fungal-specific mechanism of mutagenesis that targets repetitive DNA or duplicated elements in the genome, causing C to T single nucleotide polymorphism (SNP) transitions. The accumulation of RIP can also influence the epigenetic state of sequences (Lewis et al. 2009), altering its physical accessibility and, thus, its availability for mRNA transcription (Taga et al. 1999). Chromosome instability also affects the internal structure of ACs and ARs (Hane et al. 2011) and there is evidence for locational biases of structural rearrangements. *F. oxysporum* f. sp. *lycopersici* ACs possess hotspots in which sequences are commonly deleted between isolates that are nonrandomly distributed and do not share conserved sequence motifs (Vlaardingerbroek et al. 2016a and b). Similar locational biases were observed across multiple isolates of the closely related *F. poae* (Vanheule et al. 2016). Moreover, synteny comparisons between *F. oxysporum* f. sp. *lycopersici* and *F. solani* show a distinct variation in the accumulation of intrachromosomal rearrangements between CCs and ACs, in which ACs either lacked homologs or presented mesosyntenic patterns (Hane et al. 2011). The process of intrachromosomal rearrangement resulting in patterns of mesosynteny (Hane et al. 2011; Ohm et al. 2012) is likely a result of accumulating numerous inversions, which are associated with low-complexity DNA (i.e., simple repeat) regions (Ellison et al. 2011; Ohm et al. 2012) or repetitive DNA (including transposons) (Faino et al. 2016). The dual association with RIP and structural rearrangements make repetitive DNA a major driver of genome evolution in ACs and ARs.

EVOLUTION OF ACCESSORY SEQUENCES AND PATHOGENICITY

Accessory chromosomes and genomic regions are, by definition, not required for survival yet, in some cases, harbor genes that confer a major competitive advantage under specific conditions. Many fungal ACs and ARs harbor pathogenicity genes (Table 1), which include effectors, secondary metabolite biosynthesis clusters or other MGCs. There is a common theme among plant-pathogenic fungi of ‘two-speed genome evolution’, or the separation of their genomes into compartments comprised of core conserved regions and highly plastic regions.

Plastic compartments, including ACs, ARs and other AT-rich regions (Testa et al. 2016), which have been described as “the cradle for adaptive evolution” (Croll and McDonald 2012), are highly variable in sequence and structure and, typically, accumulate RIP and other SNP mutations (Oliver 2012; Raffaele and Kamoun 2012; Rouxel et al. 2011; Vanheule et al. 2016; Williams et al. 2016) and mesosyntenic rearrangements (Hane et al. 2011; Ohm et al. 2012) more rapidly than the core genome. The location of pathogenicity loci within these plastic compartments often results in their rapid diversification, which can be advantageous if their gene products are recognized by host defenses (Jones and Dangl 2006). The extent of compartmentalization across fungal species appears to vary considerably according to mode of infection and evolutionary history, with evidence that at least AT-rich regions are more strongly associated with pathogens that reside asymptotically on the host for longer periods (Testa et al. 2016) (Table 1). Compartmentalization may allow the diversification of non-essential genes to be accelerated within ACs and ARs, while genes encoding essential functions on CCs can remain relatively static over time, either through targeted mutations or compartment-based selection. Thus, differentiation of genes into core and accessory regions likely permits normal growth and metabolism while also allowing rapid adaptation to periodically challenging stimuli (e.g., environmental stress, nutrient deficiency, and disease resistance).

RIP is a major driver of the evolution of pathogenicity loci in repeat-rich ACs and ARs (Hane et al. 2015), which, although targeted to repeats, can also ‘leak’ outwards into single-copy regions (Irelan et al. 1994), rapidly mutating nearby genes (Fudal et al. 2009; Testa et al. 2016; Van de Wouw et al. 2010). RIP leakage has been observed to accelerate one or both the mutation and loss of avirulence effectors that are recognized by corresponding resistance genes of their hosts, e.g., *L. maculans* (Balesdent et al. 2013; Van de Wouw et al. 2010). Homology between ACs and CCs, either via duplication or TE activity would drive RIP-mediated ‘hypermutation’ (and potentially further RIP leakage) targeted to the homologous regions within the otherwise relatively stable CCs (Dhillon et al. 2010). RIP can also be a major factor influencing the conditional expression of pathogenicity genes during infection. When RIP acts upon repeated sequences and depletes GC content, it enhances the accumulation of simple AT-rich sequences (Testa et al. 2016) and promotes the binding of DNA methylation modifier proteins (Singer and Selker 1995; Tamaru and Selker 2003). This, in turn, leads to histone modifications resulting in tightly packed and transcriptionally inactive heterochromatin (Connolly et al. 2013; Galazka and Freitag 2014; Lewis et al. 2009; Taga et al. 1999) and may influence the conditional expression of pathogenicity genes during infection. ACs and ARs of *Z. tritici* (Schotanus et al. 2015; Soyer et al. 2015) and *L. maculans* (Soyer et al. 2014) are enriched for one or both chromatin modifications H3K9me3 and H3K27me3, which suppress gene expression across large regions of DNA but may be lifted in response to stimuli such as host infection (de Jonge et al. 2013; van der Does et al. 2016). Indeed, *L. maculans* effectors residing in AT-rich regions have been observed to be expressed early during infection, in contrast to effectors in GC-equilibrated regions that are expressed later (Gervais et al. 2016). Chromatin modifications in ACs and ARs may also facilitate the coregulation and preserve the structural integrity of MGCs by preventing structural rearrangements (Galazka and Freitag 2014).

CONCLUSIONS AND FUTURE PERSPECTIVES

ACs and ARs are reported across a wide range of fungal taxa but, predominantly, from pathogenic species. Although this may

reflect a bias in the species studied, many reported fungal ACs also have demonstrated roles in plant pathogenicity. Compartmentalization of gene content is an important strategy for genome adaptation and innovation, particularly in the context of pathogenicity. We speculate a possible model for the 'birth and death' of fungal genes, in which loci could conceivably be i) mobilized by BFB-cycles and other structural rearrangements, ii) potentially group into functionally related clusters, iii) become rapidly mutated within AC and AR compartments in which they may undergo selection for a useful phenotype, and iv) potentially reintegrate into stable CCs (Fig. 1).

Continued study of ACs and ARs in pathogen genomes will assist in the identification of novel pathogenicity genes. Much of our understanding of ACs and ARs has been learned through study of species with high-quality genomic resources, i.e., finished genome assemblies, resequencing data for multiple isolates, or both. With advances in genome sequencing now permitting chromosome-scale assembly and affordable resequencing of populations, the discovery and routine screening of ACs and ARs will become an increasingly useful tool in modern molecular plant pathology. Past AC discoveries have revealed key pathogenicity genes, including host-specific virulence factors and phytoalexin degradation enzymes (Table 1). Thus, further detection of ACs are likely to yield more novel disease markers. Trends in genomics toward full or near chromosome-length genome assembly are improving fungal genome resources from fragmented assemblies toward being capable of providing reliable information on the genome landscape and its compartmentalization. Yet accuracy remains an issue for current long-read sequencing platforms, often relying on short reads to improve base call accuracy. While this performs well for the assembly of gene-rich regions and overall chromosome structure, this may be problematic for the resolution of repeat-rich regions. Without overcoming this barrier, the roles of transposon repeats and fungal-specific RIP in AC evolution and the interplay of these factors between ACs and CCs cannot be intensively studied. Accessory chromosomes have been well-studied in both plants and animals, albeit often under the term B chromosomes; however, their further study in Fungi may yet provide unique insight into the relationship between ACs and fungal-specific genome mutation phenomena such as RIP (Hane et al. 2015), elevated rates of intra-chromosomal rearrangement (Hane et al. 2011), and LGT (Akagi et al. 2009; He et al. 1998; Hu et al. 2012; Ma et al. 2010; Mehrabi et al. 2011; Vlaardingerbroek et al. 2016a) observed in the Fungi.

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