10 Genomic Innovation and Virulence Evolution in the Emerging Human Fungal Pathogen *Candida auris*

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Abstract

Candida auris is an emerging human fungal pathogen and global public health threat. Composed of at least five highly clonal clades, major clinical management challenges have included nosocomial outbreaks, difficulties in identification and diagnosis, and increasing multiple drug resistance. With a crude mortality of 45% in invasive candidiasis, virulence is also accompanied by a highly adhesive, stress tolerant, and environmentally persistent phenotype. This review covers examples of the genomic innovation strategies that may drive evolution of virulence in *C. auris*. Chromosomal architecture varies between genetic clades, with evidence of major rearrangements, particularly in the less virulent clade II. As a normally haploid yeast, adaptive aneuploidy (and diploidy) has also been observed, including under drug stress, which may increase virulence. Several gene family expansions, including of hydrolase, adhesin, and transporter families, indicate diversified and polarised roles in virulence. Important genomic variation linked to differing virulence has even been identified within a single patient, including copy number variation and loss of function of the adhesin gene *ALS4* in two otherwise highly related strains. We conclude our review by suggesting research topics that need greater attention including (1) moving fungal genomic enquiry closer to patients in clinical settings, (2) targeting ecological drivers of evolution and escalating environmental discovery as a priority, and (3) interdisciplinary investigation across microbiology and immunology to leverage comparative, molecular, and population genomics to understand and control invasive candidiasis caused by *C. auris*.

Keywords

Candida auris · Fungal pathogen genomics · Population genetics · Comparative genomics · Microbial evolution · Infectious disease emergence

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10.1 Introduction

10.1.1 Emergence Detection

The emerging human fungal pathogen *Candida auris* is a global public health threat (Meis and Chowdhary 2018), an urgent antimicrobial resistance threat (CDC 2019), and a World Health Organization critical priority pathogen (WHO 2022). The earliest known isolate dates from 11th December 1996, when a challenging case of invasive candidaemia was misidentified as *Candida haemulonii* in a one-year-old girl receiving mechanical ventilation in South Korea (Lee et al. 2011). Retrospective internal transcribed spacer (ITS) and D1/D2 26S ribosomal RNA sequencing were used for species confirmation 12 years later, when a novel ascomycetous yeast was recognised and described in 2008 after isolation from the ear canal of a 70-year-old woman with otomycosis in Japan (Satoh et al. 2009). This original species description noted that *C. auris* could tolerate high salinity (up to 10% NaCl) and temperatures (up to 42 °C), with optimum growth at mammalian temperatures (37–40 °C) and phylogenetic placement close to *C. haemulonii* in the Metschnikowiaceae clade. These "first" cases illustrate the two typical diseases associated with *C. auris* in humans: (secondary) invasive candidiasis in an already critically unwell patient and (primary) epithelial infection in an otherwise healthy individual. This review will focus on the life-threatening condition of invasive candidiasis and candidaemia caused by *C. auris*.

10.1.2 Global Spread

Within 15 years of its initial discovery, *C. auris* rapidly transitioned from emergence to prominence—and in some settings, dominance. In the Deenanath Mangeshkar Hospital, Western India, *C. auris* was the number one cause of invasive candidiasis between December 2019 and July 2021, accounting for 43% of cases (Prayag et al. 2022). In the Chris Hani Baragwanath Hospital, Johannesburg, South Africa, *C. auris* was recently found to infect or colonise 33% of infants in the neonatal unit (Shuping et al. 2023). Across low- and middle-income countries (LMICs), *C. auris* was the third most common cause of neonatal candidiasis in the recent NeoOBS study (14% of cases) (Cook et al. 2023). A massive ongoing outbreak in the State of New York has now affected 1343 patients across 151 healthcare facilities, including 59 hospitals and 92 nursing homes (Zhu et al. 2020). Clinical cases and colonisation cases increased across the USA by 44% and over 80%, respectively, in 2019 and a further 95% and over 200%, respectively, in 2021 (Lyman et al. 2023). The rise of *C. auris* has now affected at least 42 countries across every major continent (Ragusa et al. 2023) and shows no sign of abating.

Whole-genome sequencing (WGS) was used to uncover the primary puzzle of *C. auris* emergence: its underlying population structure. After performing WGS on 47 isolates from India/Pakistan (n = 31), Japan (the first identified isolate), South Africa (n = 10), and Venezuela (n = 5), four clades separated by at least 10,000 single nucleotide polymorphisms (SNPs) were identified and given clade assignments I–IV that appeared to correlate with geographic location (Lockhart et al. 2017). These multiple, distinct, and highly clonal clades appeared to indicate multiple simultaneous emergences of distantly related strains—or a long history of hidden transmission before detection. At this point, the apparent geographic structure led to each clade being named accordingly: South Asian (I), East Asian (II), South African (III), and South American (IV). A further keystone population genetic study then expanded WGS analyses to 304 isolates, observing a "weaker phylogeographic substructure" thought to relate to ongoing transmission of *C. auris* around the globe since its first detection in each global area (Chow et al. 2020).

Further clades have been confirmed or proposed. Isolates belonging to the more recently discovered Clade V were identified as the cause of otomycosis in four cases in Iran; Clade V has over 200,000 SNPs compared with any other clade and occupies a basal position in the phylogeny (Abastabar et al. 2019; Chow et al. 2019; Muñoz et al. 2021; Spruijtenburg et al. 2022). A sixth clade has been proposed and is currently

under review, having caused three cases in Singapore and one in Bangladesh (Suphavilai et al. 2023). These isolates are over 36,000 SNPs in distance from clade IV, the "South American" clade, highlighting how phylogeographical nomenclature is misleading. Given recent findings, it is likely that additional clades will be discovered. Indeed, the authors of the clade VI paper propose a machine-learning based genomic surveillance system that could be used to detect further clade emergences (Suphavilai et al. 2023).

10.1.3 Clinical Management

Clinical management challenges in dealing with *C. auris* outbreaks have been overwhelming, with requirements for high technology diagnostics for identification and limited treatment options due to multiple drug resistance (MDR). Only molecular genomic techniques and mass spectrometry systems can reliably identify *C. auris*, due to the limitations of morphological and biochemical laboratory testing. Therefore, identification requires ideally WGS or partial genome sequencing such as ITS sequencing and, for routine clinical identification, Matrix-assisted laser desorption/ionisation-time-of-flight (MALDITOF), or VITEK-2 species determination with up-to-date software systems (Lone and Ahmad 2019). Concerns around high rates of resistance (fluconazole 80%, amphotericin B 27%, micafungin 7%) and MDR in 23% of cases (Chow et al. 2020) have now escalated with increasing reports of pan-resistance to all three major classes of antifungal in several clades of *C. auris* (Ostrowsky et al. 2020; O'Brien et al. 2020; Lyman 2021; Maphanga et al. 2021). Even more alarming is the ability for *C. auris* to continue to adapt to antifungal exposure. In a notable recent example, a single critically unwell patient suffered from pan-resistant invasive infection with *C. auris*, including resistance to a fourth class of antifungal, 5-flucytosine (5-FC), alongside acquired SNPs in phosphoribosyltransferase gene *FUR1* (Jacobs et al. 2022), which has been linked to 5-FC resistance in *C. auris* (Rhodes et al. 2018).

Challenges in infection control have also been extreme. Early high-profile nosocomial outbreaks in Intensive Care Units (ICUs) (Schelenz et al. 2016; Eyre et al. 2018) garnered prominent media attention with accompanying metaphors such as *C. auris* being a "creature from the black lagoon" that "bubbled up and now is everywhere" (Richtel 2019; Richtel and Jacobs 2019). In fact, drastic control measures beyond disinfection have been attempted, including hydrogen peroxide fumigation, and hospital wall and ceiling tiles being removed (Richtel 2019; Richtel and Jacobs 2019). In a "Gordian Knot" for public health services (Rapti et al. 2023), initial infection control costs have exceeded £1 million for a single facility outbreak, excluding ongoing monthly expenditure in the aftermath (Taori et al. 2019; Thoma et al. 2022). Despite clinical management challenges in diagnosis, resistance, and control, the core problem is its impact on human morbidity and mortality.

10.1.4 *C. auris* Pathogenicity

Current clinical observational data are limited in their ability to quantify mortality that can be attributed to *C. auris* bloodstream infection (BSI). A meta-analysis of 15 studies describing *C. auris* BSI has reported a 45% associated crude mortality (Chen et al. 2020). Invasive candidiasis tends to occur in critically unwell patients as a secondary phenomenon, e.g. developing weeks into the course of an illness such as severe community-acquired pneumonia requiring mechanical ventilation and multiple invasive procedures. Identifying the relative contribution of one species in already unwell patients suffering from multiple conditions and co-infections is complex. For *Candida* infections in general, propensity matching has been used to compare 90-day crude mortality. For example, in 269 patients with invasive candidiasis compared to 1083 matched controls, the attributable mortality was 28.4%, accounting for a 2.1-fold increase in risk of death (Mazi et al. 2022). The crude mortality rates were 42.4% (cases) and 17.1% (controls), and antifungal treatment was associated with lower mortality (35 vs. 69%, $p < 0.001$).

There are only a handful of studies comparing the mortality associated with *C. auris* BSI to

other *Candida* spp., limiting generalisation across different settings. In 269 critically unwell adults across three New York hospitals with *Candida* BSI, there was no significant difference in crude mortality with *C. auris* infection ($n = 83$) compared to other *Candida* spp. at 90 days ($n = 113$, 44.6 vs. 46.9%, $p = 0.75$); the odds ratio of recurrence was higher with *C. auris* (11.9 vs. 4.0%, $p = 0.04$) (Simon et al. 2023). *C. auris* BSI cases were more likely to come from a nursing home, with a longer length of stay preceding their infection, and higher frequencies of previous infection with gram-negative and MDR bacteria (60.2 vs. 11.5%, $p < 0.001$), as well as higher rates of cerebrovascular disease, hemiplegia, chronic pulmonary disease, and use of bladder catheterisation. Rates of preceding GI surgery or parenteral nutrition use were lower in *C. auris* BSI. Propensity matching indicated an adjusted odds ratio for 90-day mortality in *C. auris* infection versus other *Candida* spp. of 0.863 (95% confidence interval 0.563–1.828), indicating no significant difference.

However, a larger propensity matched study across seven Colombian hospitals found a lower mortality associated with *C. auris* (38.1% of $n = 134$) compared to other *Candida* spp. (51.1% of $n = 378$, $p = 0.013$) (Ortiz-Roa et al. 2023). A hazard ratio (rate of mortality with *C. auris* BSI vs. with other *Candida* spp.) of 0.69 (95% CI 0.53–0.90) was calculated by propensity matching, indicating a lower risk of dying with *C. auris* for individuals with sets of matched characteristics. However, the comparator group for of non-*auris Candida* spp. was quite different between the first and second studies (26.5 vs. 49.4% *C. albicans* and 28.3 vs. 7.1% *C. glabrata*), so these results reflect different population comparisons and cannot be seen as fully representative without expanding study to many more centres.

Aside from these two studies in adult populations, this comparison was also made in newborn infants, who experience a range of different host physiologies and pathologies. The recent NeoOBS study, which collected data on neonatal candidaemia in LMICs, recorded a 22% mortality from *Candida* spp. infection overall and a hazard ratio that was not significantly different between *C. auris* and other *Candida* spp. (which was 22% overall; *C. auris* mortality univariable hazard ratio was (1.28, 95% CI 0.44–3.70) (Cook et al. 2023)). Understanding the relative contribution of *C. auris* (and other *Candida* spp.) to human morbidity and mortality will require both much broader and more granular clinical investigation, including information about both patient and causative agent(s) of invasive candidiasis, including when there is mixed infection. Overall, the mortality of *C. auris* BSI appears similar to the mortality in BSI caused by other *Candida* spp. The baseline resulting assumption is that invasive candidiasis is associated with a massive attributable mortality, including double the risk of dying in infected persons.

10.1.5 *C. auris* Virulence

Fundamentally, pathogenicity is an either-or characteristic describing whether an organism can cause disease (Shapiro-Ilan et al. 2005), and virulence is "the relative capacity of a microorganism to cause damage in a host" (Casadevall and Pirofski 1999; Casadevall and Pirofski 2001, 2003). In evolutionary terms, virulence can also be defined as "disease severity as assessed by reductions in host fitness following infection" (Read 1994; Cressler et al. 2016). *C. auris* has been isolated in humans from head to toe, including (i) bodily fluids and epithelial surfaces, such as skin, sputum, urinary tract, ear discharge, vagina, and stool; (ii) abiotic implants such as arterial catheters, central venous catheters, extraventricular drains, intercostal drains; and (iii) normally sterile bodily sites indicative of invasion, such as pleural fluid, cerebrospinal fluid, and blood—though each of these sterile areas can be instrumented, e.g., with intercostal drainage, extraventricular drainage, or venepuncture (Osei Sekyere 2018). The huge range of human tissues that can be colonised by *C. auris* indicates an ability to penetrate or bypass endothelial and other internal defensive barriers to enter organs and digest host macromolecules to survive, which is necessary for invasive human fungal pathogens (Köhler et al. 2017).

C. auris possesses a unique array of virulence factors, including adhesion, aggregation, biofilm

formation, stress tolerance, secreted hydrolase production, and host immune evasion (Spivak and Hanson 2018; de Cássia Orlandi Sardi et al. 2018; Rossato and Colombo 2018; Nett 2019; de Jong and Hagen 2019; Du et al. 2020; Desoubeaux et al. 2022; Egger et al. 2022; Watkins et al. 2022; Gómez-Gaviria et al. 2023; Horton et al. 2023; Rapti et al. 2023). Genomic investigation has massive potential to explain what underlies these virulence factors (Fig. 10.1), including by uncovering the fundamental structure and regulation of the *C. auris* genome, the history of its evolution, emergence and epidemiology, the genomic drivers and signatures of virulence, drug resistance,

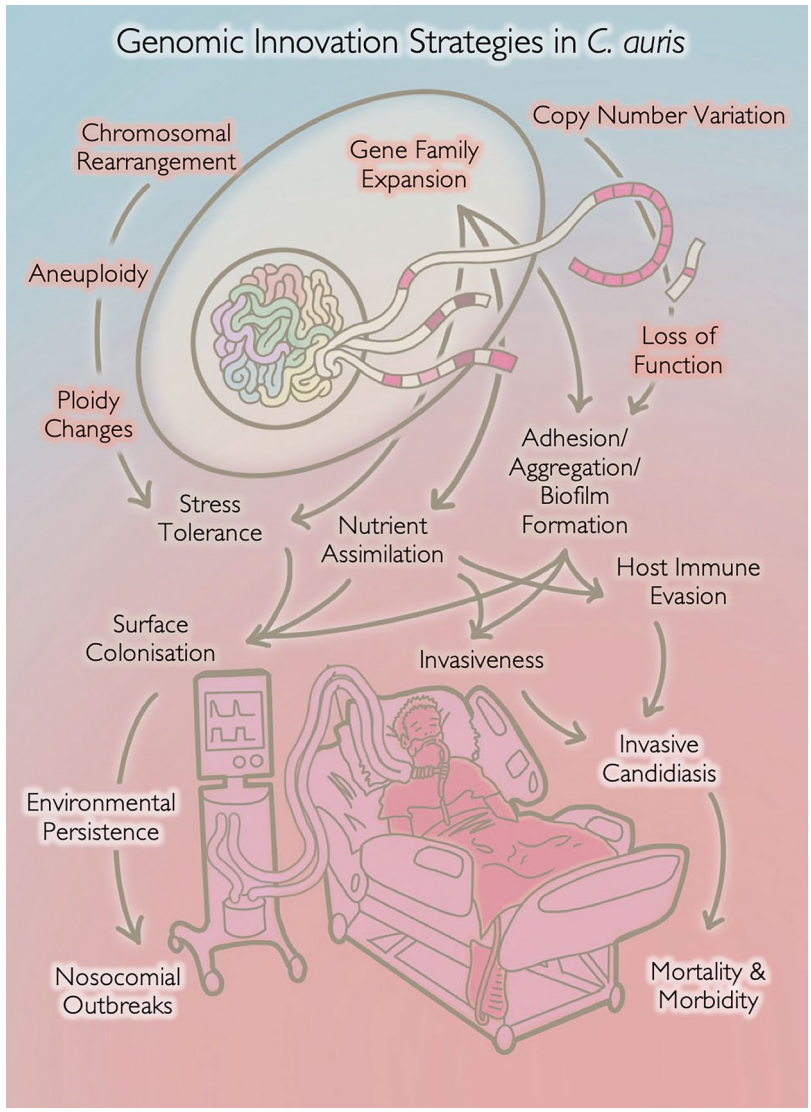

Fig. 10.1 Genomic innovation strategies in *C. auris*. The emerging human fungal pathogen's seven-chromosome karyotype has undergone structural rearrangements between five clades linked to changes in virulence. Reversible aneuploidy under drug pressure has also been linked to resistance and stress tolerance. Diploid strains may be more virulence than haploid strains in an animal model. Gene family expansions, including of transporters, hydrolases, and adhesins, have allowed diversification and polarised expression patterns in nutrient assimilation, stress tolerance, and adhesion/aggregation/biofilm formation. These virulence factors are understood to be pivotal in host evasion and human tissue invasion, leading to the major clinical challenges of environmental persistence and nosocomial outbreaks, and invasive candidiasis leading to morbidity and mortality. Illustration by and © the authors

and targets for potential control (Chybowska et al. 2020).

10.2 Genomic Innovation Strategies

10.2.1 Structural Variation

The haploid genome of *C. auris* is 12.2–12.7 Mb consisting of seven 0.7–3.25 Mb chromosomes, together encoding 5294–5506 predicted protein-coding genes (Bravo Ruiz et al. 2019; Muñoz et al. 2021). *C. auris* belongs to the haploid Metschnikowiaceae clade of *Candida* spp. together with *C. haemulonii* and *C. lusitaniae*. In contrast, other *Candida* spp. that have received greater attention, including *C. albicans*, *C. parapsilosis*, and *C. tropicalis*, belong to the diploid Debaryomycetaceae subclade. Pulsed field gel electrophoresis has been used to identify karyotype variation in *C. auris* under thermal, osmotic, and genotoxic stress (Bravo Ruiz et al. 2019). Small segmental gains and losses occurred up to an estimated 1.7 Mb in length over five passages, and chromosomal losses were also identified. For example, some clade II isolates reduced to only five chromosomes and some clade I isolates to six. This reduction in chromosome number also manifested in a genome length as low as 10 Mb in some isolates (Bravo Ruiz et al. 2019).

Major chromosomal rearrangements have occurred between related strains within each clade, according to 12 long read assemblies from clades I–V (Muñoz et al. 2021). Given the variation in virulence between *C. auris* clades, the major structural variants in the karyotype of *C. auris* are thought to account for lower virulence for clade II, with the most genomic instability relative to the B8441 clade I reference strain. Eleven large deletion sites between 5 and 10 kb were observed, concentrated at sub-telomeric regions, and including 14 candidate adhesins (*Hyr/Iff*, *ALS* and other GPI-anchored proteins). Clade II isolates also demonstrated two to three inversions and four to eight translocations concentrated around centromeres, and even a low GC content candidate second centromere in chromosome six. One cluster of genes required for L-rhamnose assimilation is present only in clades III and V, suggesting deletion from other clades. Some chromosomal rearrangements are associated with retroelements and may be evidence for recent ectopic recombination. A further study made use of chromatin immunoprecipitation sequencing (ChIP-seq) to confirm gene sparse centromere regions with few repeats across clades I–IV and extensive chromosomal rearrangement in clade II (Narayanan et al. 2021). Such instability indicates a high likelihood of ongoing genomic innovation, supporting the ongoing use of WGS and analysis of structural variation in understanding virulence evolution.

Given the predominance of structural variants in clade II, it has been hypothesised that DNA damage response (DDR) genes may play a mechanistic role in variance generation. As many as 37 clade II-specific genes with loss-of-function mutations were identified, including the DNA Damage Checkpoint gene *DDC1* (Muñoz et al. 2021). *DDC1* was first described in *S. cerevisiae* as a critical DDR component (Longhese et al. 1997). Most DDR genes remain uncharacterised in *Candida* spp. in general (Yao et al. 2021) and are likely to be mechanistically important in generating structural variation resulting in different virulence profiles. Further genomic and molecular characterisation of DDR genes in *C. auris* could explain other forms of genomic variation, as follows, making systematic and comprehensive DDR description an important area of future research—not only for *C. auris* but for *Candida* spp. in general.

10.2.2 Aneuploidy

Aneuploidy is a well described mechanism of reversible stress adaptation across the fungal kingdom, and *C. auris* is no exception (Vande Zande et al. 2023). Microevolution experiments have explored genomic innovation under antifun-

gal pressure and identified aneuploidy as a common feature (Narayanan et al. 2022–11/2022–12; Bing et al. 2020; Carolus et al. 2021; Li et al. 2022; Burrack et al. 2022). In one study, an originally susceptible clade I strain developed fluconazole resistance over multiple passages, associated with an extra copy of chromosome 5 containing the transcriptional regulator *TAC1B* (Bing et al. 2020). An additional chromosome 5 was associated with increased expression of the *ERG11* gene, which codes the target of azole antifungals, lanosterol 14, α-demethylase (Karst and Lacroute 1977). Both these genes (*TAC1*, *ERG11*) have been implicated in *C. albicans* resistance via copy number variation (Selmecki et al. 2008). Another study used WGS and electrophoretic karyotyping to show chromosome 5 aneuploidy developing under fluconazole pressure in a drug-susceptible clade II strain and conferring resistance that was reversible without the additional chromosome (Narayanan et al. 2022–11/2022–12). However, this additional chromosome was partial, including only 638 or 675 kb of the chromosome, which is normally ~1 Mb in length (Narayanan et al. 2022–11/2022–12). Extending the range of strains studied, 17 strains from clades I and IV were exposed to fluconazole and assessed by flow cytometry and contour-clamped homogenous electric field (CHEF) electrophoresis to determine karyotype (Billmyre 2022; Burrack et al. 2022). The latter study also found that a potential chromosome 3 aneuploidy arose under azole pressure (Burrack et al. 2022). As well as an additional chromosome 5, an additional chromosome 6 has been noted during drug exposure, containing Multi-Drug Resistance gene *MDR1* (a known drug efflux pump in other *Candida* spp.), which can contribute to azole resistance independent of *ERG11* (Li et al. 2022). A study of in vitro drug exposure to all three main classes of antifungal also showed a chromosome 5 aneuploidy developed in the face of azole exposure (Carolus et al. 2021), as well as a duplication of a contig containing *ERG11* on chromosome 1. However, it is unknown how these cases of aneuploidy impact virulence.

10.2.3 Hypermutation

Studying genomic responses to drug pressure has demonstrated that aneuploidy and other accompanying forms of variation such as SNPs in genes encoding drug targets can rapidly increase drug resistance. Two examples of genomic change seen in the face of drug pressure with associated development of fluconazole resistance in a clade IV clinical strain include sub-telomeric deletions up to 103.8 kb, as well as a mutator phenotype (Burrack et al. 2022). The mutator phenotype in this study was confirmed by measurement of a mutation rate over ten times that of related parents (Burrack et al. 2022). It was also observed that this high mutation rate was associated with a missense mutation in the DNA mismatch repair MutL Homologue gene orthologue *MLH1* (Burrack et al. 2022). Other missense mutations were observed and included RNA-interference pathway related Dicer RNase III (*DCR1*) and Transformation/Transcription Domain Associated Protein (*TRRAP*) associated histone acetyltransferase subunit, *TRA1* (Grant et al. 1998; Bernstein et al. 2012), both of which play roles in regulation of gene expression. Such a mutator phenotype has not been described elsewhere, to our knowledge. Different mutation rates between strains and clades could be responsible for rapid acquisition of resistance in *C. auris*, but also impact branch length estimation and ancestry in phylogenetic analysis.

10.2.4 Polyploidy

The frequency and clinical relevance of diploid *C. auris* is currently unknown. Diploid strains have been demonstrated from clinical isolates of clade I and III using phloxine B red dye staining and flow cytometry DNA content analysis, with differing transcriptional profiles to haploid counterparts and higher virulence (measured by brain, kidney, and lung fungal burden) in a mouse model (Fan et al. 2020). It is not clear why other studies have not detected diploidy (Du et al. 2022); previous detection of exclusively haploid

C. auris strains used flow cytometry to compare a range of isolates from clades I–IV to haploid and diploid *S. cerevisiae* and diploid *C. albicans* (Bravo Ruiz et al. 2019). Many bioinformatic investigations do not validate or at least report ploidy and instead may simply use anticipated haploid parameters when running variant calling, missing genetic diversity. Polyploid states are thought to drive accelerated evolution within the fungal kingdom (Selmecki et al. 2015), with accelerated rates of aneuploidy and copy number variation in other species (Vande Zande et al. 2023). Characterisation of *C. auris* isolates in future should check for the possibility of diploidy to appreciate any role in *C. auris* evolution and virulence.

10.2.5 Hybridisation

Currently, it is unclear if *C. auris* has a cryptic sexual cycle (Ross and Lorenz 2020). Each clade appears to contain only one mating type locus (MTL): *MTL***a** in I, IV, and V, and *MTL*$\boldsymbol{\alpha}$ in II and III (Chow et al. 2020; Muñoz et al. 2021). Despite co-existence of clades with opposite MTL (I and III) in a single hospital in Kenya or in China (Chow et al. 2020; Bing et al. 2022) or in multiple individuals ($n = 5$) in Nevada, USA (Massic et al. 2023), there was no evidence of successful recent sexual recombination, suggesting barriers to reproduction and gene flow between clades. Based on variant calling of 1285 *C. auris* strains, several population genetic tests have implied a lack of any recombination in recent millennia (Wang and Xu 2022). Ongoing or recent mating has therefore not yet been detected in *C. auris*.

Several critical components of putative sexual or parasexual cycles appear missing or broken in *C. auris*. The *C. auris* orthologue of pheromone transporter *STE6*—originally described for its mutant conferring sterility in *Schizosaccharomyces pombe* (Girgsdies 1982)—appears dysfunctional in at least the clade I reference strain (B8441), with a two-nucleotide deletion rendering this a pseudogene (Wasi et al. 2019). Clade I isolates from the Nevada study also had a truncation in *STE6* from the 387th amino acid as well as a lack of meiosis-related α2 gene (Massic et al. 2023). At least seven meiosis-specific genes have not been detected in clades I–IV, including *DMC1*, *MEI5*, *SAE3*, *ZIP1*, *HOP1*, *RAD55*, *RAD57*, and *MSH4/5* (Muñoz et al. 2018). However, *C. lusitaniae* also lacks important meiosis-related genes but still appears to undergo meiosis during sexual reproduction (Reedy et al. 2009). Any discovery of parasexual reproduction in *C. auris* would be highly useful in advancing the study of its genomic innovation strategies. Continued asexual reproduction may however indicate a degree of genomic stability and a limitation to the adaptability of *C. auris*.

10.2.6 Gene Family Expansions

Several gene family expansions have been identified in *C. auris*. PFAM domain searches based on transcriptome data have identified various expanded gene families, including secreted aspartyl protease (*SAP*), lipase (*LIP*), oligopeptide transport (*OPT*), major facilitator superfamily (*MFS*), ATP-binding cassette (*ABC*) transport, siderophore iron transporters (*SIT*), and adhesin families (Muñoz et al. 2018). Gene family expansions can occur through duplication, as often as one duplication per gene per 100 million years (Lynch and Conery 2000), a rate comparable to the rate of nucleotide substitutions per site, depending on the organism (Demuth and Hahn 2009). Exact mechanisms of duplication are uncertain (Reams and Roth 2015), but the process allows a divergence of function and even development of a cast of novel proteins to facilitate host microenvironment adaptation (Hughes 1994; Innan and Kondrashov 2010). Most recently, multiple gene duplications have been linked to extremotolerance in the ecdysozoan lineage Tardigrada (Fleming et al. 2024). The following sections describe investigations into prominent gene family expansions.

10.2.6.1 Hydrolases

Hydrolases and related enzymes are critical for host tissue digestion. As with other *Candida* spp.,

C. auris has multi-copy families of secreted hydrolases, such as at least three Secreted Aspartyl Protease (*SAP*) and eight Secreted Lipase (*LIP*) genes (Muñoz et al. 2018). A role for secreted lipases in virulence has been demonstrated using knockout experiments in *C. albicans* (Gácser et al. 2007a) and *C. parapsilosis* (Gácser et al. 2007b) gene deletion mutants. Phylogenetic analysis indicates a clear divergence of *C. auris SAP* genes from *C. albicans* and the most prominent gene identified in deletion testing for virulence and biofilm formation in *C. auris* has tentatively been named *Sapa3* (Kim et al. 2023). A multiomic approach has confirmed at least seven active phospholipases in *C. auris* (Zamith-Miranda et al. 2019). One intriguing observation is that the lipase orthologue *LIP2*, which suppresses the immune signalling molecule interleukin 17 (IL-17) via palmitic acid production and is necessary for deep tissue invasion by *C. albicans*, was expressed during *C. auris* co-incubation with macrophages (Basso et al. 2022; Miramón et al. 2023). Expanded *C. auris* protease and lipase gene families may play roles not only in nutrient assimilation but also in host immune evasion and biofilm formation.

10.2.6.2 Transporters

Following hydrolytic digestion by proteases, resultant peptides must be imported to be used as a nutrient source. The oligopeptide transport (OPT) proteins are typically proton-coupled symporters that enable nitrogen assimilation from one to eight residue peptides through as many as 17 transmembrane domains (Gomolplitinant and Saier 2011). Opt1–5 have also been shown to be necessary for *C. albicans* uptake of oligopeptides when they are the sole nitrogen source (Dunkel et al. 2013). Siderophore transporters are also required for virulence in distinct types of *C. albicans* infection in differing host niches, confirmed by deletion experiments (Heymann et al. 2002). Peptide transporters can also play other roles in yeast, such as pheromone secretion and quorum sensing (Magee et al. 2002; Homer et al. 2016; Abele and Tampé 2018). Investigations into the biological roles for these transporters in other *Candida* spp. suggest that systematic functional assessment in *C. auris* will also be fruitful.

Gene family expansions allow each gene new evolutionary potential, including changes in function and regulation. A polarisation of expression patterns within the same gene family has been observed several times. For example, orthologues of both Siderophore Transporter *SIT1* and amino acid transporter dicarboxylic amino acid Permease *DIP5* had members with both a significantly higher (over four-fold change) and a lower (under four-fold change) differential expression during a macrophage phagocytosis experiment (Miramón et al. 2023).

10.2.6.3 Adhesins

Expanded families of cell wall adhesins can facilitate surface colonisation, biofilm formation, host immune evasion, and tissue adhesion and invasion. The *ALS* (agglutinin-like sequence) adhesin family in *C. albicans* contains nine genes (*ALS1–9*) that play major roles in adhesion, with *ALS3* additionally mediating cellular invasion by induced endocytosis (Phan et al. 2007; Hoyer and Cota 2016); host cell invasion has not been explored or noted in *C. auris*. *C. auris* contains only three *ALS* orthologues that have been referred to inconsistently across different studies as *ALS3–5* (Pelletier et al. 2024). Another adhesin family is the *Hyr/Iff*-like (*Hil*) family, comprising eight genes in *C. auris* that each contains a range of central tandem repeat lengths that may contribute to functional variation (Smoak et al. 2023). The more recently described Surface Colonisation Factor, encoded by the *SCF1* gene, acts via cationic charge in a manner like marine bivalve adhesion, unlike other known fungal adhesins, and mediates virulence in immunocompromised mice (Santana et al. 2023). Expression of *SCF1* correlates with adhesion across various strains and, together with the hydrophobic adhesin *IFF4109*, is required to colonise in vivo murine skin and ex vivo human skin explants. *SCF1* was not seen to be necessary for adhesion in *C. haemulonii* despite the presence of an orthologue (Santana et al. 2023). The discovery of *SCF1* demonstrates the broad and nuanced range of potential adhesion mechanisms for *C. auris*.

Phenotypic diversity of *C. auris* strains has been identified even within a single patient via copy number variation or loss of function of a single gene (Bing et al. 2023). In an 81-year-old woman with a urinary infection caused by clade III *C. auris*, one strain contained a tenfold increase in copy number in a sub-telomeric location of adhesin *ALS4* (B9J08_004112) with flanking polyA/polyT motifs and was associated with aggregation, biofilm formation, and enhanced skin colonisation in a mouse skin model. A further isolate from the same patient, separated by only 38 SNPs and 90 insertions/deletions, contained a truncated *ALS4* lacking its predicted GPI anchor and fewer central repeats (8 rather than 30), and was found to be phenotypically divergent, including exhibiting reduced skin colonisation in the mouse skin model and significantly higher virulence in a *Galleria mellonella* infection model. This intriguing example shows a huge range of genomic plasticity associated with phenotypic variation that relates to both an invasive phenotype and a colonising phenotype. Of note, sub-telomeric locations of these expansions could in theory also be coupled with potential disrupted telomere maintenance, which could enable a rapid accumulation of SNPs, as has been shown in ascomycetous rice blast fungus *Pyricularia oryzae* (Rahnama et al. 2021).

Adhesins can also play a role in cellular aggregation, which has been observed in some *C. auris* isolates as a clumping together of isolates in planktonic cultures. Aggregating strains display increased biofilm formation but decreased virulence in a *G. mellonella* model (Borman et al. 2016; Sherry et al. 2017; Bing et al. 2023), but little difference in virulence in a *Drosophila melanogaster* model (Wurster et al. 2019). The mechanisms of aggregation appear to be both strain and environment dependent. Whether aggregation occurs through a stress-induced cell separation defect, *ALS*-mediated aggregation, or surface amyloid and extracellular matrix formation, aggregating forms have been hypothesised to prevent phagocytosis through physical size (Malavia-Jones et al. 2023; Pelletier et al. 2024). The adhesins thus exemplify genomic innovation through gene family expansion, copy number variation, and loss of function, and interact with a broad range of factors critical to virulence.

10.3 Discussion

Here we have reviewed several examples of genomic innovation strategies used by *C. auris* that are linked to its virulence (Table 10.1). However, many questions relating to the importance of genotype in pathogenicity remain unanswered including (i) a more comprehensive understanding of genomic variation (in contrast to anecdotal evidence) and its link to virulence, (ii) how evolution has generated and shapes extant and ancestral genotypes, and (iii) the molecular mechanisms governing or regulating

Table 10.1 Genomic innovation strategies and drivers of virulence evolution in *C. auris*

Genomic innovation strategy	Virulence attribution	Reference
Aneuploidy (in vitro—Chromosome three, containing *ERG11*)	Stress tolerance and azole drug pressure only; unknown virulence attribution	Burrack et al. (2022)
Aneuploidy (in vitro—Chromosome five, containing TAC1B, which regulates transcription of *ERG11*)	Stress tolerance and azole drug pressure only; unknown virulence attribution	Bing et al. (2020); Carolus et al. (2021); Burrack et al. (2022); Li et al. (2022); Narayanan et al. (2022)
Aneuploidy (in vitro—Chromosome six, containing drug efflux pump gene *MDR1*)	Stress tolerance and azole drug pressure only; unknown virulence attribution	Li et al. (2022)
Chromosomal rearrangement (ancestral—Sub-telomeric deletions, inversions, translocations)	Extensive rearrangement in clade II; lower virulence and tendency to cause otomycosis only	Bravo Ruiz et al. (2019), Muñoz et al. (2021), Narayanan et al. (2021)

(continued)

Table 10.1 (continued)

Genomic innovation strategy	Virulence attribution	Reference
Copy number variation (ancestral—Gene family expansions of secreted aspartyl protease and lipase genes)	Hypothesised to enable nutrient assimilation and possibly host immune evasion via IL-17 suppression	Muñoz et al. (2018), Basso et al. (2022), Miramón et al. (2023)
Copy number variation (ancestral—Gene family expansions of oligopeptide and siderophore transport genes)	Polarised expression pattern between members of *SIT* and *DIP* families during survival in phagocytes	Muñoz et al. (2018), Miramón et al. (2023)
Copy number variation (ancestral—Gene family expansions of adhesion families *ALS* and *Hil*)	Functional differentiation within family members hypothesised to play critical roles in adhesion, aggregation, and biofilm formation	Muñoz et al. (2018); Smoak et al. (2023), Borman et al. (2016), Sherry et al. (2017)
Copy number variation (in vitro—Chromosome one contig including *ERG11*)	Stress tolerance and azole drug pressure only; unknown virulence attribution	Carolus et al. (2021)
Copy number variation (within patient—Tenfold increase of *ALS4* in sub-telomeric region in a clade III isolate)	Demonstrated in urinary infection; increased skin colonisation in a mouse model	Bing et al. (2023)
Diploidy (clinical strains from clade I and III)	Higher virulence in mouse model measured by fungal burden in brain, kidney, and lung	Fan et al. (2020)
Elevated mutation rates (in vitro—via missense mutation in DNA mismatch repair *MLH1*)	Stress tolerance and azole drug pressure only; unknown virulence attribution	Burrack et al. (2022)
Loss of function (within patient—*ALS4* truncation in a clade III isolate)	Demonstrated in urinary infection; increased virulence in *G. mellonella* model	Bing et al. (2023)

each genomic innovation strategy. We therefore recommend three approaches to address *C. auris* genomic innovation. Firstly, we should consider increasing the proximity of genomic research to the clinical patient bedside to maximise the information yield and relevance to human morbidity and mortality. Secondly, we should escalate the awareness of *C. auris* and emerging fungal pathogens into integrated and One Health domains to harness the power of global environmental genomics. Thirdly, we should optimise interdisciplinary collaboration in comparative *Candida* spp. genomics across microbiology and immunology systematically and comprehensively to unveil the underlying mechanisms of genomic innovation in agents of invasive candidiasis. This is especially the case since (mainly) haploid *Candida* spp. related to *C. auris* (such as *C. haemulonii* and *C. lusitaniae*) are quite distantly related to the majority of well-researched (mainly) diploid *Candida* spp. (especially *C. albicans*, but also *C. parapsilosis* and *C. tropicalis*) (Muñoz et al. 2018).

10.3.1 Moving Fungal Genomic Inquiry Closer to the Patient

Identifying within host genomic diversity relevant to human virulence is critical to understanding the evolution of pathogenicity in *C. auris*. In one case previously mentioned, a single patient contained isolates differentiated by tenfold *ALS4* copy number variation, or truncation resulting in loss of function (Bing et al. 2023). Several other case reports, aided by whole-genome sequencing, have identified SNPs that may impact virulence and drug resistance. In one example of pan-resistance, 19 strains were collected and sequenced from a single patient over 72 days, during which the patient had been exposed to eight different antifungal drugs (Jacobs et al.

2022). The fact that many discoveries can be made from just one clinical case hints at a massive scope for similar work in future.

Near-patient testing is essential. It is well accepted that strains sub-cultured in vitro, away from a natural (e.g. clinical) setting, can rapidly lose their genetic and phenotypic profiles (e.g. via relaxed selection, mutations, adaptive aneuploidy, etc.). Therefore, the further cultures travel from their patient, the fewer genuine genomic innovation strategies we are likely to detect. One of the challenges of WGS is that a patient may be infected with billions of yeast cells, but only a single colony forming unit is cultured from their bloodstream, grown on culture media, and then sequenced, resulting in an under-sampling of in-host diversity. This may exaggerate how highly clonal outbreaks appear (Eyre et al. 2018). Strains isolated from non-outbreak settings have indicated a possibility that within host SNP diversity can include as many as 60 SNPs between recent clonal isolates, compared to under three SNPs from within an individual during outbreak settings (Biswas et al. 2020). Within host diversity can enable the development of clinical resistance (Diaz Caballero et al. 2023), including through bet hedging or phenotypic heterogeneity (Morawska et al. 2022). Therefore, it is important that we develop strategies to detect in patient evolution in the future, perhaps through increased partnerships between clinical settings and bioinformatics/genomics expertise.

As genomic technology continues to advance, the suggestion of moving genomic investigation to the bedside is increasingly possible. Indeed, combining host and pathogen metagenomics and metatranscriptomics at the bedside has been tested and shown to be accurate for sepsis diagnosis (Kalantar et al. 2022). One study of the metatranscriptome in clinical respiratory samples recently noted that over 51% of reads were from *Candida* spp. (Wang et al. 2023a), indicating the wealth of genomic data that could be interrogated in future to understand near-patient host–pathogen interaction. Near-patient genomic testing is at the frontier of tool development and offers the opportunity to demonstrate *C. auris* and other pathogen genomic innovation strategies in situ.

Currently, existing genomic data often lacks clinical relevance and could be a wasted resource without better clinical integration. The technology which has been made use of most extensively near the patient bedside is Illumina short read WGS, growing exponentially at the same time as the spread of *C. auris* and leading to an unprecedented wealth of data. By 1st February 2024, the National Center for Biotechnology Information Short Read Archive (NCBI SRA) contained over 10,000 paired end genomic DNA isolate datasets. Few studies have used over 1000 isolates' data from this massive source. In May 2023, one study leveraged genome-wide association statistical methods to uncover novel SNPs contributing to antifungal resistance (Wang and Xu 2024). However, only 387 of over 4000 strains with WGS data obtained from NCBI SRA in this study contained metadata denoting antifungal susceptibilities. A search for *ALS4* copy number variation indicated 2.3% of 1156 isolates with short read WGS data publicly available had more than two copies and only 0.4% had lost *ALS4* completely (Bing et al. 2023). Such studies could now be repeated or could make use of the increasing amounts of long read data that are available. To ensure public data is valuable however, relevant clinical and phenotypic information should be included. Perhaps publishing policy guidelines with reinforcement by professional societies may help.

We know little about how *C. auris* bloodstream invasion occurs in most persons—whether it is a matter of hygiene around multiple indwelling device insertion, related to translocation of microbes through broken skin and internal gastrointestinal epithelia, or an alternative route of entry (Pappas et al. 2018). A PubMed search for "*Candida auris*" and "autopsy" yields no primary literature results, and we understand that there are little to no histopathological studies in humans that systematically describe invasion. A clinical predictive model based on retrospective epidemiology of colonised individuals determined a hierarchy of risk factors, including total parenteral nutrition (TPN), a multifocal skin surface colonisation, presence of sepsis, advanced chronic kidney disease, arterial catheterisation,

central venous catheterisation, and antifungal exposure (Garcia-Bustos et al. 2020). A clinical study of 27 *C. auris* colonised patients out of 117 patients admitted to an ICU during February–May 2020 (59% of whom had COVID-19) found a 25% cumulative incidence rate of *C. auris* BSI within 60 days of detection of colonisation (Briano et al. 2022). However, relating putative virulence strategies to human pathological processes is awaited, and there is a need to use genomic techniques alongside clinical pathology research to understand the underlying genomic innovation strategies that drive invasion.

Describing the host response to *C. auris* in existing clinical populations is also lacking. Pathogenesis of invasive infectious disease in general is thought to result from the interaction of host and pathogen (Casadevall and Pirofski 2003). The phenomenon of disseminated infection associated with multiple organ failure in humans has led to a recent definition of sepsis (for any pathogen) as a dysregulated host response to infection (Singer et al. 2016), and septic shock as a resulting failure of tissue perfusion in the context of circulatory failure leading to death. The relevance of host response in invasive candidiasis is illustrated by the demonstration of a high hazard ratio for mortality of 1.73 (95% CI 1.41–2.13) when the patient's presentation included septic shock in one study of *C. auris* BSI (Ortiz-Roa et al. 2023). Additionally, invasive candidiasis tends to take place in a later stage of illness defined by relative immunosuppression (Patricio et al. 2019). Therefore, dynamic host–pathogen assessment will also form a part of understanding the virulence of *C. auris*.

Studies examining the host response to *C. auris* are underway, and sorely needed. One transcriptome study found that 50% of differentially expressed genes (DEGs) in *C. auris* during macrophage phagocytosis were unique, with no orthologues in *C. albicans*, *C. tropicalis*, *C. parapsilosis*, and *C. lusitaniae* (Miramón et al. 2023). Metabolic, transport, lysis, and oxidative stress transcriptional responses to macrophage phagocytosis otherwise appeared highly conserved across these CTG clade members (Miramón et al. 2023). Given the features of *C. auris* BSI as occurring in already critically unwell patients who frequently have pre-existing medical conditions with exposure to other multi-drug resistant pathogens, considering host–pathogen interaction and the wealth of available genomic information (such as the metatranscriptome) is likely to be both a complex and profitable frontier.

10.3.2 Escalate Environmental Fungal Pathogen Genomics Towards Global Priority

Pathogen emergence can be defined in epidemiological terms, framed in evolutionary theory, and studied using genomic tools. A definition of emergence is "an infectious disease whose incidence is increasing following its first introduction into a new host population or…increasing in an existing host population as a result of long-term changes in its underlying epidemiology" (Woolhouse and Dye 2001). Emergence has been described as "an inevitable consequence of changes in the abundance of host populations and the contact networks that exist between them" (Cleaveland et al. 2007). An organism's genomic background enables its "jump" into new niches and its genomic innovation allows further adaptation (Woolhouse 2002; Woolhouse et al. 2005). For example, consideration of the soil-based origins of *Cryptococcus neoformans* played a role in hypothesising the similarities between phagocytosis evasion both in human hosts and when confronted with soil amoeba *Acanthamoeba castellanii* (Steenbergen et al. 2001). The discovery of an "original" source environment for *C. auris* could enable an understanding of the selection pressures that have enabled virulence to develop.

It remains unknown where *C. auris* has been hiding prior to the twenty-first century. It is also not known what the "founder" event was that caused a spill over into the global human population (Fourcade et al. 2014; Bonneaud and Longdon 2020; Snedden et al. 2021). This enigma has been reviewed in some detail (Jackson et al.

2019; Chakrabarti and Sood 2021; Zhai et al. 2021; Sharma and Kadosh 2023; Akinbobola et al. 2023; Garcia-Bustos et al. 2023; Chowdhary et al. 2023). Environmental isolates have so far included coastal wetlands of the Andaman Islands (Arora et al. 2021), the coastal waters of Columbia (Escandón 2022), and swimming pools in the Netherlands (Ekowati et al. 2018) as well as wastewater in both Eastern and Western USA (Barber et al. 2023; Rossi et al. 2023; Babler et al. 2023). *C. auris* has been isolated from picked and stored apples (Yadav et al. 2022) as well as stray dogs' ears in Delhi, India (Yadav et al. 2023) and a pet dog's mouth in Kansas, USA (White et al. 2024). None of these isolates have been proven to be so phylogenetically distant from outbreak strains as to conclusively prove an environmental "origin", but the presence of drug-susceptible isolates in coastal wetlands of the Andaman Islands is consistent with a hypothesis of a marine yeast becoming drug resistant after human exposure (Arora et al. 2021).

Ideally, searches for *C. auris* would make use of existing environmental metagenomic and metabarcoding studies. The "One Health" concept is entirely interdisciplinary, relating human health to the health of animals and ecosystems, an essential framing of the *C. auris* origins search (Garcia-Bustos et al. 2023). At least one group has begun efforts to scan public repository data for *C. auris* (Mario-Vasquez et al. 2024). *C. auris* reads were detected in an iron-reducing medium growth experiment of cultures originally taken from "contaminated soil at a former coal gasification site in Gliwice, Poland" and could be due to human contamination (Marozava et al. 2018). *Candida* spp. have been isolated from soil, e.g. from the Global Soil Mycobiome Consortium's extensive sampling of over 4000 topsoil samples with full length ITS and V9 region sequencing, but not yet explored further at a taxonomic level (Mikryukov et al. 2023). Promoting the importance of emerging fungal diseases in environmental microbial diversity may help to ensure the methods and attention required to consider and collaborate are not overlooked.

Ecological change has long been understood to explain emergence, rather than sudden genotypic or phenotypic leaps (Schrag and Wiener 1995). Anthropogenic environmental interaction, including human contact with previously unexplored natural environments leading to major disturbances, is a prime general suspect for driving emergences, such as the novel Severe Acute Respiratory Syndrome Coronavirus SARS-CoV-2 (Wilcox and Gubler 2005; Lindahl and Grace 2015; Platto et al. 2021). Several ecological drivers have been hypothesised for *C. auris* emergence, including (i) global warming as a driver of thermotolerance, (ii) widespread agricultural azole use as a driver of drug resistance, and (iii) migratory birds as a vector for transmission (Casadevall et al. 2021; Aguirre-Liguori et al. 2021). However, none of these hypotheses for *C. auris* emergence have been proven, and there is plenty of room for speculation, conjecture, and doubt. For example, one author recently argued that global warming of 1–1.5 °C may not be responsible for overcoming a thermotolerance-related limiting factor for fungal virulence, as there are tens of thousands of non-pathogenic fungal species that can already grow at mammalian temperatures (Money 2024).

Several further hypotheses exist that have not been fully explored but could be targeted via temporal analyses making use of genomic data. These include marine plastic waste as a vector of *C. auris* global transmission (Akinbobola et al. 2024), and aquaculture via attempted *C. haemulonii* seeding to promote shrimp immunity and reduce infection (Chakrabarti and Sood 2021). Bayesian analysis has been used to estimate the time to most recent common ancestor (TMRCA) of individual clades and emergent isolates in general from WGS data (Chow et al. 2020). Overall, the TMRCA for all emerging *C. auris* isolates fell around the approximate year 25,000 BCE. Excluding isolates with high drug susceptibility, the TMRCA for individual clades I and III was estimated in circa 1983 and 1984, respectively. Including all isolates, the four major clades' TMRCA dated back to circa 1869, 1833, 1658, and 1982. These global genomic epidemiological studies have provided a critical outline for the investigation of the evolution and emergence of *C. auris*. However, model param-

eters such as clock rate and gene choice are likely to have major influences on these results and could be analysed in future (Koch and Carmona 2024). Further tests, such as calculation of the effective population number (N_e) can also be performed using Bayesian phylogenomic analysis and could enrich and inform searches for *C. auris* origins. Calculation of N_e has been used to link the massive increase in prevalence of Hepatitis C virus in Egypt to the period of shared needle use by misinformed public health programmes in the first half of the nineteenth century (Charlesworth 2009).

The isolation of *C. auris* from the Andaman Islands has not been accompanied by a consideration of local indigenous people groups. Andaman Islanders are made up of distinct tribes including Sentinelese, Jarawa, Great Andamanese, and Onge, who may have been isolated from other populations around the world (and even to an extent each other) for tens of thousands of years, according to genetic, historical, and linguistic features (Thangaraj et al. 2003; Thangaraj et al. 2006). A timeline for *C. auris* emergence, including a TMRCA around 25,000 BCE, falls within human prehistory and early migrations (Nielsen et al. 2017). As indigenous people groups emerge from isolation (Pringle 2014)—a phenomenon which may soon cease (Walker et al. 2016)—a prime concern has been the potential for disease introduction (such as measles and influenza) into vulnerable populations with devastating effects (Ferreira and Castro 2015). However, it is possible that *C. auris* may be an example of the opposite process, whereby *C. auris* has been present in isolated human populations until an introduction into the global population in the last 50–500 years. The sudden emergence of each clade suggested by Bayesian analysis (Chow et al. 2020) is consistent with epochs of human movement (Baker et al. 2022), including European colonisation and trans-Atlantic slave trade over the last 500 years, followed by the advent of global air travel in the last 50 years in our modern "age of emerging diseases" (Greger 2021). Sampling of indigenous populations needs extreme care (Bader et al. 2023), so the inclusion of fungal investigation in any research that combines genomics and anthropology should be considered to reduce the burden of sampling through potential repeated studies.

Despite not knowing the origins of *C. auris*, there are many human environments forming ecological niches that now influence its ongoing evolution. The reservoir of colonised and infective individuals and abiotic surfaces, particularly in hospitals, continues to rise (Lyman et al. 2023). *C. auris* appears to thrive in what has been termed "the built environment" (Ciric 2022), especially in urban areas such as, most recently, in a prison (McDougal et al. 2023). In a clinical setting, methods of decolonisation are not fully effective either in infectious patients (Elbahr et al. 2024) or infectious fomites (Nwachukwu et al. 2023), rendering robust infection control strategies critical (Ahmad and Asadzadeh 2023). Outbreaks of *C. auris*, such as during the COVID-19 pandemic, have been partially attributed to deficits in infection control practices (Thoma et al. 2022). When *C. auris* persists for long periods in patients and in healthcare environments, there is an increased potential for further genomic innovation and adaptation (Antia et al. 2003; André and Day 2005). The idea of a virulence "trade off" during evolution leading to lower pathogenicity is complex and should not be assumed (Alizon et al. 2009). The modifiable ecology of *C. auris* in ongoing transmission may be essential in preventing further evolution of virulence (Ewald 2004).

10.3.3 Interdisciplinary Genomics Towards Exhaustive Characterisation

C. auris is only one of multiple emerging infectious diseases from the fungal kingdom with major humanitarian impact (Lockhart et al. 2023). Other examples include *Batrachochytrium dendrobatidis*, a panzootic that has been responsible for massive global amphibian declines (Fisher and Garner 2020), *Magnaporthe oryzae*, the causative agent of rice blast disease threatening food security with catastrophic harvest losses (Mutiga et al. 2021), and *Trichophyton indotineae*, a zoophilic and potentially sexually

transmitted drug-resistant dermatophyte (Caplan 2023). The ability to cause disease in humans has emerged in at least 16 major clades across the fungal tree of life, with no single factor obviously responsible for pathogenicity (Rokas 2022). Diversification from *Candida* clade—whether between strains and clades of *C. auris* or between other *Candida* spp.—gives an opportunity to pick apart the genomic drivers and signatures of virulence.

Comparative studies across *Candida* spp. have identified signatures of selection across multiple genes, with a focus on resistance. A study of over 2000 isolates of *C. glabrata, C. auris, C. albicans, C. tropicalis, C. parapsilosis*, and *C. orthopsilosis* identified genes associated with drug resistance and others associated with selection, but Genome Wide Association Studies (GWAS) for resistance phenotypes were probably limited again by lack of phenotypic data and annotation for *C. auris*; hence, there were no Gene Ontology (GO) biological processes significantly enriched among *C. auris* genes with signatures of selection (Schikora-Tamarit and Gabaldón 2024). The wealth of literature on *C. auris* has a clear focus on antifungal resistance (Wang et al. 2023b; Ragusa et al. 2023; Ettadili and Vural 2024), but comparative genomics will be limited until problems with WGS metadata relating to antifungal susceptibility are improved upon. Virulence-focused investigation has major potential for the discovery of disease-modifying treatment development that could lead to novel antifungal treatments (Roselletti et al. 2023). Understanding the drivers of virulence and pathogenicity in *C. auris* across different populations and compared to other *Candida* spp. will require integration of such clinical and laboratory data with recent genomic discoveries to understand what drives virulence. Such research should certainly consider prioritising research on closely related *Candida* spp. within the haploid *Candida* clade that includes *C. haemulonii* and *C. lusitaniae*, which have had far less attention than other more distantly related species, which tend to be diploid (Muñoz et al. 2018).

Careful molecular biology work is required to understand the DNA damage response (DDR) and other mechanistic bases of genomic innovation, such as via gene knockouts. Difficulty in genetic tractability has been identified alongside the inherent challenge of working with a novel strain (Bravo Ruiz and Lorenz 2021). Screening of a genome-wide mutagenesis programme in *C. auris* demonstrated a disruption of a long-coding RNA that resulted in constitutive filamentous growth (Gao et al. 2021). With deletion resulting in DNA damage, the RNA was named DNA damage-inducible non-coding RNA (DINOR) and found to be involved in co-ordination of multiple stress responses, including to macrophages, antifungals, and oxidative stress. Several direct gene knockouts have been studied during infection in a murine model, such as the Protein Kinase A regulatory subunit *BCY1* (Bypass of CYclic-AMP requirement) (Matsumoto et al. 1982), resulting in attenuated virulence for unclear reasons likely related to reduced stress tolerance (Kim et al. 2021). DDR genes have also been hypothesised to play a role in resisting immune cell oxidative stress (Yao et al. 2021). Therefore, consideration of the role of DDR genes in genomic innovation could be coupled with immunological investigation, as detailed further below.

The stress resistance profile of *C. auris* appears to be unique compared to other *Candida* spp., with a sensitivity to organic oxidative stress and an inability to grow in some anaerobic conditions or acidic conditions (Day et al. 2018). A degree of sensitivity to stress in vivo has been implied by how *Hog1* stress-activated protein kinase (SAPK) knockouts led to a reduced virulence in *C. elegans* infection alongside reduced tolerance to various stressors in vitro, including oxidative, ionic, and osmotic stress (Day et al. 2018). One recent review considered the idea of phenotypic switching (which has been clearly shown in *C. albicans*) in *C. auris* as playing a role in a switch from *C. auris* colonisation to invasion (Proctor et al. 2023). However, *C. auris* has only been reported as forming filamentous forms in rare cases, such as after passage through a mammalian body (Yue et al. 2018) or under genotoxic stress (Bravo Ruiz et al. 2020).

We suggest considering in vivo microevolution experiments as a method for detecting genomic innovation strategies relevant to virulence. In vitro microevolution experiments have

demonstrated genomic diversification under drug pressure such as ploidy changes and SNPs (Carolus et al. 2021; Burrack et al. 2022). Serial passage experiments, such as with *C. neoformans* in mice, have been used for other pathogens (Hu et al. 2014) and could be replicated in *C. auris* via a range of different model organisms. An alternative would be to use host-relevant cell lines to explore microevolution in host-relevant conditions, as has been explored with *C. glabrata* during long-term co-incubation with a macrophage cell line (Brunke et al. 2014). To our knowledge, there are no microevolution studies of repeated passage in infection to examine virulence in *C. auris*, despite the potential utility (Schikora-Tamarit and Gabaldón 2022). Such experiments could unveil genomic innovation strategies that have not been previously described, such as SNPs or epigenetic regulatory mechanisms. Virulence and antifungal resistance may be co-evolving together (Pais et al. 2019), especially in human hosts, and it would be especially interesting to consider shared genomic changes in directed evolution experiments under differential and combined selection pressures towards virulence and resistance.

Integration of immunology as a discipline is also critical to understanding virulence factors in *C. auris* related to other *Candida* spp. An ex vivo whole blood model enabled transcriptional identification of host responses to *C. auris* infection (included expected neutrophil killing), with subtle differences compared to that of other *Candida* spp. including *C. albicans, C. glabrata, C. tropicalis*, and *C. parapsilosis* (Allert et al. 2022). Upregulated host genes in the whole blood model included *TLR2*, C-type lectin receptors *CLEC5a*, *MRC1*, and complement receptor *CR3*. In the whole blood model, *C. auris* differentially expressed several sets of genes related to stress tolerance, such as *HSP90*. Global transcriptional programmes need to be examined in vivo, as changes in cis- and trans-gene expression regulators can drive evolution (Hill et al. 2021). A recent hypothesis is that although genes and regulators may be conserved they can undergo rapid switching (or transcriptional rewiring) that results in accelerated evolution in *Candida* spp. (Fowler et al. 2023).

C. auris can survive, replicate within, and lyse human macrophages, with evidence that conserved *Candida* spp. transcriptional networks play a role. Isolates from clades I–IV can escape bone-marrow-derived macrophage (BMDM) phagocytosis within 10 h and cause lysis around 24 h without morphological change or NLRP3 inflammasome activation (Weerasinghe et al. 2023), unlike the dramatic hypha-associated lysis and candidalysin-dependent immune stimulation seen in *C. albicans* (Lewis et al. 2012; Rogiers et al. 2019). Instead, macrophages suffered glucose-starvation metabolic stress, which could be rescued by glycolytic substrate (glucose and pyruvate) provision.

Evasion of neutrophils also appears to be both highly effective and uniquely evolved in *C. auris*. Divergence of pathways relating to cell surface β-glucan masking may represent a highly effective immune evasion strategy compared to other *Candida* spp. Human neutrophil engagement with *C. albicans* (Johnson et al. 2018) and *C. glabrata* (Horton et al. 2021) is three times higher in vitro than with *C. auris* (clade I). Unlike with *C. albicans*, antimicrobial neutrophil extracellular traps (NETs) fail to be produced for *C. auris* both in vitro and in zebrafish hind brain injection (Johnson et al. 2018). Deletion of the orthologue of *C. albicans* cell wall mannosylation gene *PMR1* (Bates et al. 2005) increased neutrophil engagement and yeast killing in *C. auris* by over three times, with no detectable change in engagement for *C. glabrata* and *C. albicans*, in zebrafish hind brain injection (Horton et al. 2021). Despite higher lethality, fungal burden, and early pro-inflammatory cytokine release in a zebrafish swim-bladder experiment, *C. auris* infection was associated with lower expression levels of genes associated with immune recruitment, such as *MPX* and *FOXP3A/B* (Pharkjaksu et al. 2021). However, the question remains as to why *C. auris* may effectively evade neutrophil engagement, and what genomic mechanisms explain differences to other *Candida* spp.

Population genetic studies are another suggestion for genomic searches to understand virulence. Increasing the scale of phylogenetic studies could help resolve the emergence of pathogenicity and population structure of *C.*

auris as it continues to spread. One phylogenetic study of environmental and pathogenic *Candida* strains related to *C. auris* indicated no cladistic basis for a single emergence of pathogenicity as environmental and infecting strains were interspersed across the phylogeny (Schutz et al. 2023). However, it has not yet been possible to pick apart the roles of selection, drift, and other forces acting on the *C. auris* genome (Pouyet and Gilbert 2021). Such molecular genomic approaches for *C. auris* offer an opportunity to enrich and refine its evolutionary history. Systematic and exhaustive genomic studies of, for example, mobile genetic elements are lacking. The comparison of two differentially virulent strains in China demonstrated a large difference in numbers of the Zorro3 retrotransposon, which has unclear roles in DNA/RNA binding, with 64 copies in the more virulent (but fully drug sensitive) clade I strain compared to three copies in the less virulent (but multi-drug resistant) clade III strain (Fan et al. 2021). Zorro3 was also observed in differing numbers across strains in another study (Burrack et al. 2022), but any contribution to virulence is unknown. Large-scale genomics can help identify and quantify genomic innovations that have not been noted at a population level previously and indicate their role in virulence evolution.

10.4 Conclusions

We have focused our review on the genomic innovation strategies underlying virulence evolution in *C. auris*. There is evidence of structural rearrangement across seven chromosomes, particularly in the less virulent clade II. Almost all strains appear haploid, but diploid forms may increase virulence. Aneuploidy is a well described response to stress, including to drug pressures. From available strains and analyses to date, no hybridisation representative of recent mating has been identified, though further efforts are required. Gene family expansions unique to *C. auris* include transporter, hydrolase, and adhesin families. These gene families have been associated with diversification and polarised patterns of expression in virulence studies. Microevolution is an important source of rapid genomic variation. In one clinical case, an isolate underwent rapid adaptation in multiple trajectories, with tenfold copy number variation of the *ALS4* adhesin in a sub-telomeric location in one strain (associated with better skin colonisation) or a truncation and loss of function of *ALS4* in a second strain (associated with higher virulence in an experimental model).

Controlling the global public health threat of *C. auris* requires interdisciplinary integration. Clinically, understanding pathogenesis in humans requires bedside proximal research and immunological investigation. Considering *C. auris* and other fungal pathogens in global metagenomic, anthropological, and one health investigations may also help understand original niches associated with pathogenicity. Phylogenetic structures indicate a diversion of clades within human prehistory, and ancient ecological drivers of evolution and diversification are unknown. In the context of ongoing transmission in healthcare environments, the ecological factors influencing ongoing evolution in *C. auris* are modifiable by infection control practices. Comparative genomic and phenotypic studies within *C. auris* and across the *Candida* clade could explain how pathogenicity emerges in the fungal kingdom and help to reduce the global morbidity and mortality associated with invasive fungal infection.

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