

Evolutionary genomics reveals variation in structure and genetic content implicated in virulence and lifestyle in the genus *Gaeumannomyces*

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1 ABSTRACT

2 *Gaeumannomyces tritici* is responsible for take-all disease, one of the most important wheat
3 root threats worldwide. High-quality annotated genome resources are sorely lacking for this
4 pathogen, as well as for the closely related antagonist and potential wheat take-all biocontrol
5 agent, *G. hyphopodioides*. As such, we know very little about the genetic basis of the
6 interactions in this host-pathogen-antagonist system. Using PacBio HiFi sequencing
7 technology we have generated nine near-complete assemblies, including two different
8 virulence lineages for *G. tritici* and the first assemblies for *G. hyphopodioides* and *G. avenae*
9 (oat take-all). Genomic signatures support the presence of two distinct virulence lineages in
10 *G. tritici* (types A and B), with A strains potentially employing a mechanism to prevent gene
11 copy-number expansions. The CAZyme repertoire was highly conserved across
12 *Gaeumannomyces*, while candidate secreted effector proteins and biosynthetic gene clusters
13 showed more variability and may distinguish pathogenic and non-pathogenic lineages. A
14 transition from self-sterility (heterothallism) to self-fertility (homothallism) may also be a key
15 innovation implicated in lifestyle. We did not find evidence for transposable element and
16 effector gene compartmentalisation in the genus, however the presence of *Starship* giant
17 transposable elements likely contributes to genomic plasticity in the genus. Our results depict
18 *Gaeumannomyces* as an ideal system to explore interactions within the rhizosphere, the
19 nuances of intraspecific virulence, interspecific antagonism, and fungal lifestyle evolution. The
20 foundational genomic resources provided here will enable the development of diagnostics and
21 surveillance of understudied but agriculturally important fungal pathogens.

22 INTRODUCTION

23 *Gaeumannomyces* is a broadly distributed genus of *Poaceae* grass-associated root-fungi
24 (Hernández-Restrepo et al. 2016), best known for the species *Gaeumannomyces tritici* (Gt)
25 which causes take-all disease, the most serious root disease of wheat (Palma-Guerrero et al.
26 2021). *Gaeumannomyces* is a comparatively understudied genus despite belonging to the
27 *Magnaporthales*, an economically important order of pathogens including the rice and wheat

28 blast fungus *Pyricularia oryzae* (syn. *Magnaporthe oryzae* (Zhang et al. 2016)). This is perhaps
29 due to a historical research bias towards above-ground pathogens, in part simply due to the
30 fact that characteristic symptoms of root pathogen diseases are hidden from view
31 (Raaijmakers et al. 2009; Balmer and Mauch-Mani 2013). Recently the rhizosphere has
32 received more research attention as its key role in plant health and productivity has become
33 apparent (van der Heijden et al. 2008). There have also been considerable difficulties in
34 producing a reliable transformation system for *Gt*, preventing gene disruption experiments to
35 elucidate function (Freeman and Ward 2004).

36 Although genetic studies of *Gt* have been limited, single-locus phylogenetic analyses of *Gt*
37 have consistently recovered two distinct lineages within the species (Daval et al. 2010), which
38 we will refer to using the 'A/B' characterisation established by Freeman et al. (2005) based on
39 *ITS2* polymorphism. Although very little is known about the dynamics of these two lineages,
40 each is found across the world and both lineages persistently co-occur in the same field,
41 prompting the suggestion that the two lineages may actually be cryptic species (Daval et al.
42 2010; Palma-Guerrero et al. 2021). Although variation within lineages is high, there is also
43 some evidence that type A strains are more virulent (Bateman et al. 1997; Lebreton et al.
44 2004, 2007), which is a major impetus for improving our understanding of these two lineages.
45 The sister species to *Gt*, *G. avenae* (*Ga*), can also infect wheat, but is not the predominant
46 agent of wheat take-all, and is distinguished by the fact that production of avenacinase enables
47 *Ga* to additionally infect oat roots (Osbourne et al. 1991; Bowyer et al. 1995).

48 *Magnaporthales* are also home to several commensal and/or mutualistic fungi (Xu et al. 2014),
49 including those with the potential to inhibit take-all (Chancellor 2022). For instance, *G.*
50 *hyphopodiooides* (*Gh*) — a species closely related to *Gt* that also grows on wheat roots— is
51 not only non-pathogenic, but actually capable of suppressing take-all to varying degrees
52 (Osborne et al. 2018). It is now apparent that prior *Gh* colonisation primes the host plant's
53 immune response (Chancellor et al. 2023), a mechanism that has been reported in various
54 other plant–microbe interactions associated with disease prevention (Van Wees et al. 2008;

55 Zamioudis and Pieterse 2012). This has prompted interest in *Gh* as a potential biocontrol
56 agent, for instance by adding *Gh* inoculant to wheat seedstock via seed coating (Accinelli et
57 al. 2016) and/or selecting for wheat cultivars that support enhanced levels of *Gh* root system
58 colonisation (Osborne et al. 2018). Novel disease prevention approaches for take-all are
59 especially desirable as up to 30% of *Gt* strains are found to be naturally resistant to the seed-
60 dressing fungicide routinely used to treat take-all, silthiofam (Freeman et al. 2005).

61 Understanding the genetic machinery underpinning virulence and lifestyle in
62 *Gaeumannomyces* has previously been hampered by a lack of genomic data. Prior to the
63 present study, a single annotated *Gt* assembly (strain R3-111a-1), sequenced using the 454
64 platform, was available on NCBI (accession GCF_000145635.1) (Okagaki et al. 2015) – one
65 other more recent PacBio assembly has been released for the same strain, but remains
66 unannotated (GCA_016080095.1). This scarcity of genomic resources has not only limited our
67 understanding of the genetics of the system, but also accounts for a lack of molecular
68 diagnostics for take-all. Given the increase in research activities since 2005 following the
69 production of genomic resources for *P. oryzae* (Sperr 2023; Dean et al. 2005), we are
70 optimistic that providing similar high-quality assemblies for *Gaeumannomyces* species will
71 bolster research efforts in the global take-all community.

72 Here, we have addressed the gap in genomic resources for *Gaeumannomyces* by generating
73 near-complete assemblies for nine strains, including both type A and B *Gt* lineages and the
74 first assemblies for *Gh* and *Ga*. Using an evolutionary genomics approach, we identified
75 variation in structure as well as gene features known to be involved in plant-fungal interactions
76 — candidate secreted effector proteins (CSEPs), carbohydrate-active enzymes (CAZymes)
77 and biosynthetic gene clusters (BGCs) — to address the questions: (1) Are there genomic
78 signatures distinguishing *Gt* A/B virulence lineages? (2) How do gene repertoires differ
79 between pathogenic *Gt* and non-pathogenic *Gh*? and (3) Is there evidence of genome
80 compartmentalisation in *Gaeumannomyces*? In the process of doing so, we also identified

81 giant cargo-carrying transposable elements belonging to the recently established *Starship*
82 superfamily (Gluck-Thaler et al. 2022).

83 **RESULTS**

84 **Evidence of greater take-all severity caused by *G. tritici* type A strains**

85 As the five *Gt* strains sequenced in this study included representatives of both the type A and
86 B lineages, we performed a season long inoculation experiment to determine the relative
87 capacity for each strain to cause take-all disease symptoms. From general visual inspection,
88 inoculation of *GtA* strains into the highly susceptible winter wheat cultivar Hereward resulted
89 in notably depleted roots compared to a control and, to a lesser extent, *GtB* strains (Fig. 1a).
90 Inoculation with *GtA* strains also resulted in a visible reduction of overall plant size compared
91 to the control, while *GtB*-inoculated plants were less easily distinguished from the control (Fig.
92 1b). Although above- and below-ground characteristics of wheat varied depending on *Gt*
93 strain, our statistical analysis showed that the *GtA* strains had a greater capacity to reduce
94 plant height and reduce root length, and both *GtA* strains consistently produced the greatest
95 root disease symptoms, i.e. highest Take-all Index (TAI) scores (Bateman et al. 2004) (Fig.
96 1c). Furthermore, five out of six wheat plants that died during the experiment were inoculated
97 with *GtA* strains. Several characteristics were inconsistently affected by *Gt* inoculation,
98 including mean floral spike (ear) length; dried root biomass; number of roots; and number of
99 roots per tiller.

100 **Nine near-complete *Gaeumannomyces* assemblies, including first genome assemblies
101 for *G. avenae* and *G. hyphopodiooides***

102 We used PacBio HiFi sequencing technology to produce highly contiguous genome
103 assemblies for five *Gt*, two *Gh* and two *Ga* strains. All nine assembled genomes had N50
104 values of more than 4 Mb (Supplemental Table S1), a 100-fold increase on the N50 of the
105 existing annotated *Gt* RefSeq assembly (NCBI accession GCF_000145635.1). In addition,
106 transcriptomes were sequenced for all nine strains to inform gene prediction, and between

107 22–29% of annotated gene models had two or more isoforms across all strains (Supplemental
108 Fig. S1). Contigs corresponding to mitochondrial genomes were identified from all assemblies
109 (Supplemental Table S1), however circularisation was only successfully detected for two
110 strains (Gt-23d and Ga-CB1). For most strains the overall mitogenome size, GC content and
111 number of genes fell within the expected range for ascomycetes (Fonseca et al. 2021),
112 however the mitogenome assembly for Gt-LH10 is likely incomplete, as it was a third of the
113 size of the other *GtB* strains, and only had 23 genes annotated compared to the 38–40 genes
114 found for all other strains (Table S1).

115 Combined GENESPACE (Lovell et al. 2022) and telomere prediction results suggested six
116 chromosomes for *Gaeumannomyces* (Fig. 2), one less than *P. oryzae* (Dean et al. 2005).
117 Telomere-to-telomere sequences were assembled for at least five out of six
118 pseudochromosomes for most strains. By plotting GC content alongside transposable element
119 (TE) and gene density, we also identified AT- and TE-rich but gene-poor regions, which are
120 putative candidates for centromeres (Supplemental Fig. S2). Some of these regions
121 additionally correspond well with points of fragmentation in other strains, presumably due to
122 the difficulties associated with assembly of such highly repetitive regions. Other than these
123 occasional splits into two fragments, in most cases pseudochromosomes were entire, the
124 exception being Gh-1B17 pseudochromosome 2 which was fragmented across five contigs.

125 Both *GtA* and, to a slightly lesser degree, *GtB* were broadly syntenic across whole
126 pseudochromosomes, with the exception of a major chromosomal translocation between
127 pseudochromosomes 2 and 3 in Gt-LH10 (Fig. 2). Visualisation of the spanning reads and
128 coverage across the regions of the apparent translocation suggests the depicted arrangement
129 is correct and not an artefact due to misassembly (Supplemental Fig. S3a), moreover there
130 was no evidence of a block of repeats consistent with a telomere anywhere but at the ends of
131 the pseudochromosomes (Supplemental Fig. S3c). Ga was also largely syntenic with *Gt*,
132 although there were a number of inversions in Ga-CB1 pseudochromosome 3 (Fig. 2). The
133 more distantly related *Gh* showed chromosomal translocations involving

134 pseudochromosomes 1, 2 and 5, which were again supported by spanning reads and the
135 absence of intrachromosomal telomeric repeats (Supplemental Fig. S3b, c).

136 **No evidence for significant colocalisation of transposable elements and effectors**

137 Compartmentalisation of effectors within genomic regions enriched in transposable elements
138 (TEs) has previously been reported for various fungal phytopathogens (Dong et al. 2015). In
139 all the *Gaeumannomyces* strains sequenced here, however, we did not observe that predicted
140 CSEPs were more likely to occur in regions of high TE density (Fig. 3a). We found a weak
141 significant positive correlation between CSEP density and TE density for a minority of strains,
142 however the scatterplot and local polynomial regression lines were unconvincing (Fig. 3b).
143 CSEP density was more frequently found to significantly correlate with gene density, although
144 this was still only a weak association (Fig. 3b). For all but one strain, there was no significant
145 difference in mean distance to closest TE for CSEPs versus other genes (Fig. 3c). For strain
146 Gt-19d1, the mean distance from a CSEP to the closest TE was marginally lower (10,036 bp)
147 than for other genes (12,565 bp), which permutation analysis confirmed was closer than
148 expected based on the overall gene universe ($p=0.03$), although this only remained significant
149 for pseudochromosomes 2 and 6 when testing pseudochromosomes separately
150 (Supplemental Fig. S4a). Individual pseudochromosomes for other strains also had lower than
151 expected CSEP–TE distances, but with low z-scores (a proxy for strength) across the board.
152 Comparing across strains, mean gene–TE distance was significantly different both within and
153 between lineages, and lowest in *GtB* (Fig. 3c). Within *GtB*, *Gt-LH10* had significantly lower
154 mean gene–TE distance, and the same strain has also undergone an apparent expansion in
155 total number of TEs compared to all other strains (Supplemental Fig. S5).

156 Although CSEPs were not broadly colocalised with TEs, we did observe that they appeared
157 to be non-randomly distributed in some pseudochromosomes (Fig. 3a). Permutation analyses
158 confirmed that overall CSEPs were significantly closer to telomeric regions in all strains
159 ($p=<0.008$), although by testing pseudochromosomes separately we found that this pattern
160 varied across the genome (Supplemental Fig. S4b). CSEPs on pseudochromosomes 1, 2 and

161 5 were consistently closer to telomeric regions, whereas for pseudochromosomes 3 and 4
162 CSEPs were no closer than expected based on the gene universe. CSEPs were also closer
163 to telomeres in pseudochromosome 6, but only in *Gt* strains.

164 **Core gene content in *Gaeumannomyces***

165 The total number of genes was relatively similar for all strains, although, as indicated in Fig.
166 2, *GtB* and *Gh* strains had 3–6% more genes than *GtA* or *Ga* (Fig. 4a). *GtA* and *GtB* had a
167 very similar number of CSEPs, CAZymes and BGCs, however, and more CSEPs and BGCs
168 than either *Ga* or *Gh*. Almost all total genes, CSEPs and CAZymes were core or soft-core (i.e.
169 present in all but one strain) in *Gt*, while there was a greater proportion of BGCs that were
170 accessory or strain-specific. From a pangenome perspective, the core gene content for *Gt*
171 from sampling these five strains amounted to ~10,000 genes (Fig. 4b), which equates to ~88%
172 of genes per strain being core, consistent with reports in other fungi (McCarthy and Fitzpatrick
173 2019). The majority of BUSCO genes found to be missing in the assemblies were missing
174 from all strains (Supplemental Fig. S6), suggesting that they are not present in the genus,
175 rather than being missed as a result of sequencing or assembly errors. Three of these 18
176 missing core genes belonged to the *Snf7* family, which is involved in unconventional secretion
177 of virulence factors in fungi (da C. Godinho et al. 2014), and is essential for pathogenicity in
178 *P. oryzae* (Cheng et al. 2018). The next greatest set of missing BUSCOs (8) also seemed to
179 be lineage specific – i.e. missing in *Gh* but present in *Gt/Ga* (Supplemental Fig. S6).

180 The avenacinase gene required for virulence on oat roots (Osbourne et al. 1991; Bowyer et al.
181 1995) was identified in all strains in a conserved position on pseudochromosome 4
182 (Supplemental Fig. S7a). Two mating-type (MAT) loci were identified in *Gt* and *Ga*, with
183 homologues of *Pyricularia grisea* *MAT1-1* and *MAT1-2* idiomorphs located in conserved but
184 unlinked positions on pseudochromosomes 2 and 3, while only one MAT locus and idiomorph,
185 *MAT1-1*, was identified in *Gh* on pseudochromosome 3 (Supplemental Fig. S8).

186 **Differences in effectors and secondary metabolite production potential between**
187 **pathogenic and non-pathogenic *Gaeumannomyces* species**

188 The predicted BGC content ranged from 9 to 17 per strain, which is low compared to many
189 other ascomycete fungi (Gluck-Thaler et al. 2020; Franco et al. 2021; Llewellyn et al. 2023).
190 Using a phylogenetically-informed permutational multivariate analysis of variance
191 (PERMANOVA) method (Mesny and Vannier 2020) to identify associations between gene
192 variance and lifestyle, we also found BGCs to have the lowest level of variance described
193 purely by ancestry, 61% compared to 75%–85% for all genes, CSEPs and CAZymes (Fig.
194 4a). This was coupled with a relatively high proportion of BGC variance described by lifestyle
195 (17%), which was also the case for all genes (17%) and CSEPs (14%), while lifestyle had
196 much less descriptive power for CAZymes (7%). CAZymes that are known to act on plant cell
197 wall substrates were highly conserved across the genus, and there were highly similar
198 numbers of each CAZyme family across all strains (Supplemental Fig. S9a). The only
199 discernible pattern was marginally more copies of GH55 and GH2 (hemicellulose and pectin)
200 in *Gh* versus the other lineages.

201 In total, 9% of CSEP genes could be attributed to a known gene in the Pathogen-Host
202 Interactions database (PHI-base) (Urban et al. 2020), most of which only had one copy in all
203 strains (Supplemental Fig. S9b). Sixteen of the 19 ‘named’ CSEPs have been associated with
204 virulence via reverse genetics experiments, including five from *P. oryzae* infecting *Oryza sativa*
205 (rice) — *MHP1* (ID PHI:458); *MoAAT* (PHI:2144); *MoCDIP4* (PHI:3216); *MoHPX1* (PHI:5188);
206 and *MoMAS3* (PHI:123065). The latter two were assigned to genes that were only present in
207 *Gh*, although a separate gene present in *GtB* was also characterised as *MoHPX1*. Six CSEPs
208 in total were present in all lineages except *Gh* or vice versa. *PBC1*, also a CAZyme, the
209 disruption of which causes complete loss of pathogenicity of *Pyrenopeziza brassicae* in
210 *Brassica napus*, was present in *Gt* and *Ga* but not *Gh*. While *PBC1* was absent in *Gh*, all
211 *Gaeumannomyces* strains did have some genes belonging to the same CAZyme family (CE5;
212 Supplemental Fig. S9a).

213 The BGC families were predominantly classified as terpenes, type 1 polyketide synthases
214 (PKSI) and hybrid polyketide synthase-nonribosomal peptides (PKS-NRP) (Supplemental Fig.
215 S9c). Presence-absence of each BGC was highly variable across strains, most notably
216 amongst PKSI families which also had high copy-number for certain families. There were five
217 BGCs that were present or absent in *Gh* versus other lineages, four of which belonged to the
218 PKS-NRP hybrids (Supplemental Fig. S9c).

219 **Gene copy-number reduction in *G. tritici* type A**

220 *GtB*, *Ga* and *Gh* all had high copy-number (HCN) gene outliers (>10 copies) that were absent
221 in *GtA* (Fig. 4a). These 22 HCN genes were duplicated both within and across
222 pseudochromosomes (Supplemental Fig. S10a). GO term enrichment analyses found various
223 terms to be significantly overrepresented amongst the HCN genes, namely: vacuolar proton-
224 transporting V-type ATPase complex assembly (*Gh*-1B17, Fisher's exact test, $p=0.01$);
225 ubiquinone biosynthetic process (*Gh*-2C17, $p=0.01$); golgi organisation (*Ga*-CB1, $p=0.03$);
226 mRNA cis splicing, via spliceosome (*Gt*-4e, $p=0.03$); mitochondrial respiratory chain complex
227 I assembly (*Gt*-4e, $p=0.05$); proton-transporting ATP synthase complex assembly (*Gt*-LH10,
228 $p=0.03$); and protein localisation to plasma membrane (*Gt*-LH10, $p=0.03$). Visualising the
229 location of the HCN genes across the genomes (Supplemental Fig. S11) showed them to vary
230 in terms of distribution — from relatively localised to broadly expanded — and in terms of multi-
231 lineage versus lineage specific expansions. HCN genes were also significantly closer to TEs
232 compared to other genes (Supplemental Fig. S10b).

233 Interestingly, of the 22 HCN genes, six that were shared among all species were also present
234 in at least one *GtA* strain but at low copy-number, while seven genes were completely absent
235 in *GtA* (Fig. 4c). In total, nine genes that were HCN in at least one other lineage had low-copy
236 orthologues in *GtA*. Moreover, these were mostly present in just one strain within the type A
237 lineage (*Gt*-8d), clustered in a ~1 Mbp region on pseudochromosome 3 (Supplemental Fig.
238 S10c). This region was flanked by repetitive regions that have been subjected to repeat
239 induced point mutation (RIP), as measured by the composite RIP index (CRI) (Lewis et al.

240 2009), although the region had average CRI of -0.3 compared to an average CRI of -0.5 for
241 the whole pseudochromosome. Average genome-wide RIP levels were highest in *GtA* and *Gh*
242 (13.8% and 13.6% of the genome RIP'd, respectively), compared to *GtB* (10.8%) and *Ga*
243 (12.4%).

244 ***Gaeumannomyces* genomes contain *Starship* giant transposable elements**

245 All nine *Gaeumannomyces* strains were found to contain at least one giant TE belonging to
246 the *Starship* superfamily of giant cargo-carrying TEs (Gluck-Thaler et al. 2022), identified using
247 the tool starfish (Gluck-Thaler and Vogan 2023). Currently the most reliable identifying feature
248 of *Starships* is a single 'captain' gene – a tyrosine recombinase gene containing a DUF3435
249 domain which is found in the first position of each *Starship* and directs the mobilisation of the
250 element (Urquhart et al. 2023b). We found that tyrosine recombinase annotation with starfish
251 largely overlapped with results from a separate blast search to identify DUF3435 homologues
252 at the head of insertions. Overall, only a relatively small number of genes were in agreement
253 as full *Starship* captains after downstream automated (starfish) or manual element inference
254 (Fig. 5a). A gene tree of all tyrosine recombinase and putative captain genes showed the
255 presence of two distinct lineages but no consistent clustering of either gene types or method
256 of identifying them. Note the highly divergent nature of the genes and therefore the difficulty
257 of alignment and subsequent poor branch support throughout the tree (Fig. 5a).

258 *Starship* size varied considerably, ranging from 34–688 kbp. *GtB* strains harboured notably
259 more elements, followed by *Ga* strains which included a nested element (Fig. 5b). *GtA* and
260 *Gh* strains each contained a single smaller (<100 kbp) element, which in both cases we predict
261 to have been vertically transmitted based on similar gene content and conserved location
262 within the genome (Fig. 5b, Supplemental Fig. S12). *GtA* elements were exceptional in that
263 each was gene-poor and positive for element-wide RIP (average CRI=0.2-0.3).

264 **DISCUSSION**

265 In this study we have established foundational genome resources for the genus
266 *Gaeumannomyces*. A particular strength of the *Gt* assemblies reported here is the structural
267 annotation methodology, which capitalised on the fact that multiple strains were sequenced,
268 assembled and annotated in the same way, each with its own transcriptome but also
269 employing a novel ‘multiple lift-off’ approach that provided additional evidence for robust gene
270 models. Another benefit of the annotation approach is that the REAT-Mikado-minos pipeline
271 (EI-CoreBioinformatics 2023b) provides models for gene isoforms alongside the primary
272 transcripts. Alternative splicing has been implicated in regulation of virulence in
273 phytopathogens (Fang et al. 2020), for instance by mediating transcriptome remodelling
274 during pathogenesis in *P. oryzae* (Jeon et al. 2022). Alternative splicing has been reported to
275 be more frequent in pathogens than non-pathogens (Grutzmann et al. 2014), however we
276 found a similar overall percentage of genes with multiple isoforms in *Gh* compared to *Gt* and
277 *Ga* (Supplemental Fig. S1). There was perhaps a skew towards a greater proportion of genes
278 with exactly two or three isoforms in *Gt*, particularly *GtA*, raising the question as to whether
279 this somehow relates to their apparent higher virulence in wheat. These rich annotations
280 resources will allow further exploration of the isoform content of *Gaeumannomyces* and its
281 potential role in virulence.

282 A major finding from our synteny analyses was the presence of a large chromosomal
283 translocation in *Gt-LH10* (Fig. 2). Similar largescale translocations have been identified in
284 *Pyricularia* (Bao et al. 2017; Gómez Luciano et al. 2019). It is entirely plausible that we have
285 identified a genuine translocation, however confidence would be increased by obtaining Hi-C
286 evidence and/or by corroborating with population-level data. Such rearrangements are thought
287 to be a route to accessory chromosome formation (Croll et al. 2013; Hartmann 2022), and this
288 has specifically been reported in *P. oryzae* (Langner et al. 2021). Although we did not find any
289 evidence for accessory chromosomes in our *Gaeumannomyces* strains, it is interesting that
290 the *Gt-LH10* translocation resulted in one of the chromosomes being much smaller, size being
291 a hallmark of accessory or ‘mini-chromosomes’. It is also notable that this large translocation

292 occurred in the same strain we found to have an expansion of TEs (Supplemental Fig. S5), as
293 TEs have been found to mediate interchromosomal rearrangements (Bao et al. 2017; Fourie
294 et al. 2020; Langner et al. 2021). Hi-C data would also allow us to robustly locate centromeres
295 (Varoquaux et al. 2015), which are also implicated in chromosomal rearrangements (Yadav et
296 al. 2020; Guin et al. 2020). Here we used a minimal approach to estimate potential centromeric
297 regions, based simply on the fact that AT-rich regions are a common defining feature of
298 centromeres in *P. oryzae* (Yadav et al. 2019), which we also cross-checked with gene sparsity
299 (Supplemental Fig. S2) — however, we were only able to distinguish potential centromeres
300 for a subset of the pseudochromosomes.

301 In addition to the chromosomal translocation, Gt-LH10 also stood out from other strains in
302 terms of TE content, with an expansion in total number of TEs (Supplemental Fig. S5) and
303 smaller gene–TE distances (Fig. 3). Aside from the atypical features of the Gt-LH10 genome,
304 there was additional intraspecific variability within the Gt A/B lineages in terms of both genome
305 structure and gene content. For instance, there were strain-specific inversions (Fig. 2) and
306 many of the HCN genes were present in low copy-number in one GtA strain, but completely
307 absent in the other (Fig. 4c). These findings emphasise the need for pangenome references,
308 as an individual strain alone cannot sufficiently represent the variability across the whole
309 species (Golicz et al. 2020; Badet and Croll 2020). Pangenomics is still relatively young and
310 the practicalities of how to define, store and use pangenomes and the tools necessary to do
311 so are continuously evolving (Eizenga et al. 2020). There is also the outstanding question of
312 how best to coordinate pangenome initiatives to ensure high-quality results without duplicating
313 efforts — at least three different pangenomes have been reported for the wheat pathogen
314 *Zymoseptoria tritici* to date (Plissonneau et al. 2018; Badet et al. 2020; Chen et al. 2023). The
315 five Gt strains reported here can act as a UK pangenome, but future research must work
316 towards building a global pangenome so that we can provide a reference for Gt which captures
317 a fuller representation of the species.

318 Another structural feature that these high-quality assemblies allowed us to explore in
319 *Gaeumannomyces* was genome compartmentalisation. A number of fungal phytopathogens
320 exhibit TE- and effector-rich compartments that enable rapid evolution in the plant–fungal arms
321 race, dubbed the ‘two-speed’ genome model (Dong et al. 2015), which has since been
322 extended to ‘multi-speed’ models (Frantzeskakis et al. 2019). Accordingly, we hypothesised
323 that we would find CSEPs and TEs to colocalise across our assemblies, however we did not
324 find consistent evidence for such compartments in *Gaeumannomyces* (Fig. 3). Our results are
325 not altogether surprising as a previous study of selection signatures in *Gt* and two other
326 *Magnaportheales* taxa also found no evidence for multi-speed genomes (Okagaki et al. 2016).
327 We therefore consider *Gaeumannomyces* taxa to have ‘one-compartment’ genomes in
328 relation to TE/effector content – a term that was introduced by Frantzeskakis et al. (2019) for
329 genomes that do not conform to the two- or multi-speed models, and with ‘compartment’
330 suggested as an alternative to ‘speed’ as the defining features of these compartments does
331 not necessarily equate to them being fast-evolving (Torres et al. 2020). With the rising number
332 of high-quality genome resources, more examples are emerging that contradict the suggestion
333 that phytopathogenicity is routinely accompanied by TE/effector compartmentalisation
334 (Frantzeskakis et al. 2019). In fact, TE/effector compartmentalisation has been found in the
335 non-pathogenic arbuscular mycorrhizal fungus *Rhizophagus irregularis* (Yildirir et al. 2022),
336 and TE/virulence factor compartmentalisation has also been found in chytrid animal
337 pathogens (Wacker et al. 2023), demonstrating that it is not necessarily central to
338 phytopathogenicity, but may instead be a mechanism driving genome plasticity in fungi of
339 various lifestyles (Torres et al. 2020). While we did not find compelling evidence for TE/effector
340 compartmentalisation in *Gaeumannomyces*, we did observe non-random patterns in the
341 distribution of CSEPs (Fig. 3a), which permutation analyses found to be closer to telomeric
342 regions in a pseudochromosome-dependent manner (Supplemental Fig. S4b). This could
343 suggest that alternative mechanisms of effector compartmentalisation may be at play.

344 Our results indicate conserved genetic machinery for plant cell wall deconstruction/
345 modification across both pathogenic and non-pathogenic *Gaeumannomyces* (Fig. 4a, S11a),
346 suggesting that the mechanism(s) by which species first colonise roots may be similar, if not
347 the final outcome of the plant-fungal interaction (Chancellor et al. 2023). Using spatial
348 transcriptomics to visualise not only how *Gt* and *Gh* individually colonise wheat roots, but also
349 how they interact with each other in the plant and the gene expression associated with this
350 process, would undoubtedly shed light on this host-pathogen-antagonist system. Two putative
351 orthologues of CSEP genes that have previously been implicated in pathogenicity were
352 present in *Gt* and *Ga* pathogenic taxa but missing in non-pathogenic *Gh*, making them
353 promising targets for future experiments to determine if either is important for *Gt* pathogenicity
354 in wheat. *UvHrip1* (from *Ustilaginoidea virens*) is thought to be involved in suppressing host
355 immunity and has already been reported in *Gt* (Wang et al. 2020), while *PBC1* (from
356 *Pyrenopeziza brassicae*) is a cutinase implicated in host penetration (Li et al. 2003). It was
357 intriguing that none of the CSeps assigned to PHI-base genes were unique to *Gt*, perhaps
358 suggesting that there is relatively high overlap in effector-mediated virulence mechanisms in
359 *Gt* and *Ga*. In a similar pattern to the CSeps, only one BGC (a PKS-NRP hybrid) was found
360 to be specific to *Gt*, with most otherwise scattered across the genus (Supplemental Fig. S9c).
361 There were more differences between *Gh* and the other lineages, and indeed the relative
362 descriptive power of relatedness versus lifestyle on BGC variance (Fig. 4a) suggests that the
363 production of secreted metabolites may be a key factor distinguishing the outcome of plant–
364 fungal interactions in this genus. A single BGC has been shown to determine whether there is
365 a mutualistic or pathogenic outcome of the interaction between root fungus *Colletotrichum*
366 *tofieldiae* and *Arabidopsis thaliana* (Hiruma et al. 2023), demonstrating that minimal
367 differences in metabolite repertoire can have considerable impacts on fungal lifestyle. In terms
368 of host range, *Gt* has been shown to have low avenacinase activity relative to *Ga* (Osbourne et
369 al. 1991), which is understood to be the reason *Gt* is incapable of also infecting oat roots
370 (Osbourne et al. 1994). The avenacinase gene was nonetheless present in all strains across
371 the genus; whether sequence polymorphism (Supplemental Fig. S7c) or differences in

372 regulatory machinery are responsible for the variation in avenacinase activity remains to be
373 determined. It is notable that *Gh* has also been found to be capable of colonising oat roots
374 (Osborne et al. 2018) despite greater divergence of the *Gh* avenacinase protein sequence
375 from *Ga* when compared to *Gt* (Supplemental Fig. S7b).

376 In line with the common understanding that *Gt* is self-fertile or homothallic (Palma-Guerrero et
377 al. 2021), we found both *MAT1-1* and *MAT1-2* idiomorphs to be present in the *GtA* and *GtB*
378 strains. These idiomorphs were located on two unlinked MAT loci, an atypical but occasionally
379 observed homothallic MAT locus architecture in ascomycetes (Wilken et al. 2017; Dyer et al.
380 2016; Thynne et al. 2017). Although it is homothallic, *Gt* is also capable of outcrossing
381 (Pilgeram and Henson 1992; Blanch et al. 1981), the rates of which may be underestimated
382 in many other homothallic fungi (Billiard et al. 2012; Attanayake et al. 2014). Similarly to *Gt*,
383 for *Ga* both MAT loci were identified. To our knowledge, the sex determination system of *Gh*
384 has not previously been reported, but our results indicate only one idiomorph at a single MAT
385 locus suggesting this species is self-sterile, or heterothallic. Evolutionary transitions between
386 heterothallism and homothallism are common in ascomycetes (Thynne et al. 2017; Sun et al.
387 2019; Gioti et al. 2012; Ene and Bennett 2014), but the implications on fitness are not fully
388 understood. In the scenario of a fungus infecting a crop monoculture, it may be advantageous
389 for the fungus to be homothallic when rapidly expanding across the niche, as it will not be
390 delayed by a reliance on the presence of compatible mating types. A higher rate of outcrossing
391 due to heterothallism could be unfavourable, as it could break up combinations that are
392 already well adapted to the genetically uniform host (Hill and McMullan 2023). Together, this
393 could suggest a selective pressure towards homothallism for crop fungal pathogens, and a
394 switch from heterothallism to homothallism may, therefore, have been a key innovation
395 underlying lifestyle divergence between non-pathogenic *Gh* and pathogenic *Gt* and *Ga*.

396 An unanticipated result was the absence of HCN genes in the *GtA* lineage (Fig. 4a), despite
397 all other strains in the genus, including earlier diverging *Gh*, having genes which had
398 undergone copy-number expansions (Supplemental Fig. S11). These HCN genes were on

399 average significantly closer to TEs than other genes (Fig. 5c), which aligns with the fact that
400 TEs are known to play a role in gene duplication (Cerbin and Jiang 2018). GO enrichment
401 analysis identified a variety of fundamental biological processes to be significantly
402 overrepresented amongst HCN genes in the other lineages: regulation of cellular pH and
403 respiratory activity in non-pathogenic strains; and golgi organisation, protein localisation,
404 mRNA cis-splicing and respiratory activity in pathogenic strains. As previously mentioned,
405 alternative splicing has previously been linked to pathogenicity; respiratory activity has been
406 shown to induce a developmental switch to symbiosis in an arbuscular mycorrhizal fungus
407 (Tamasloukht et al. 2003); and mediation of cellular pH by V-ATPase has specifically been
408 linked to pathogenesis in *P. oryzae* (Chen et al. 2013), although here it was implicated in a
409 non-pathogenic *Gh* strain. Further investigation into the specific function of these genes is
410 required to determine whether any of these processes are essential to lifestyle or virulence in
411 *Gaeumannomyces*.

412 Gene duplicates are generally understood to be readily removed unless they serve to improve
413 host fitness, for instance by favourably modifying expression levels or rendering a completely
414 new function (Lynch and Conery 2000; Wapinski et al. 2007). RIP is a genome defence
415 response against unchecked proliferation of duplicated sequences (Hane et al. 2015). In
416 *Gaeumannomyces* we found 10–14% of the genome contained signatures of RIP, which is a
417 moderate level relative to other ascomycetes, e.g. *Pyronema confluens* (0.5%) (Traeger et al.
418 2013), *Fusarium* spp. (<1–6%) (Van Wyk et al. 2019), *Neurospora* spp. (8–23%) (Gioti et al.
419 2013), *Zymoseptoria tritici* (14–35%) (Lorrain et al. 2021) and *Hymenoscyphus* spp. (24–41%)
420 (Elfstrand et al. 2021). Genome-wide RIP was highest in *GtA*, which was consistent with its
421 low level of gene duplication, but not fully explanatory as *Gh* had only marginally lower levels
422 of RIP while still maintaining HCN outliers. We can only presume that *GtA* strains have been
423 under stronger selective pressures to remove duplicates, although the evolutionary
424 mechanisms driving this requires further investigation.

425 There was a similar pattern when exploring the RIP patterns across giant transposable
426 *Starship* elements. We found only a single *Starship* in *GtA* strains, which was gene-poor and
427 had undergone extensive RIP (Fig. 5b), supporting the idea that this lineage employs stringent
428 genome defence measures. By contrast, *GtB* strains contained a proliferation of *Starships*,
429 including one closely approaching the largest size reported thus far (Urquhart et al. 2023a).
430 We expect that the increased availability of highly contiguous, long-read assemblies such as
431 we report here will make the upper size extremes of such giant TEs more feasible to detect
432 (Arkhipova and Yushenova 2019). Giant cargo-carrying TEs that can be both vertically and
433 horizontally transmitted were first identified in bacteria (Johnson and Grossman 2015).
434 Recently the *Starship* superfamily was identified as specific to and widespread in ascomycetes
435 and, aside from the characteristic ‘captain’ tyrosine recombinase gene, each *Starship* contains
436 a highly variable cargo (Gluck-Thaler et al. 2022). Mobilisation of cargo genes by *Starships*
437 has been linked to the acquisition of various adaptive traits in fungal species, such as metal
438 resistance (Urquhart et al. 2022), formaldehyde resistance (Urquhart et al. 2023a), virulence
439 (McDonald et al. 2019), climatic adaptation (Tralamazza et al. 2023) and lifestyle switching
440 (Gluck-Thaler et al. 2022). However, *Starships* are not inherently beneficial to the fungal host.
441 One of the earliest groups of genes associated with the cargo of certain *Starships* was spore-
442 killer or Spok genes, which bias their own transmission via the process of meiotic drive (i.e.
443 by killing spores that do not inherit them) (Vogan et al. 2019). By incorporating Spok genes, a
444 *Starship* element also biases its transmission, leading to it being referred to as a ‘genomic
445 hyperparasite’ (Vogan et al. 2021). This corresponds to the concept of TEs as selfish genetic
446 elements, which can prevail in the genome despite being neutral or deleterious to the overall
447 fitness of the host. Whether mobilisation of an element and associated cargo is beneficial or
448 detrimental to the host, TEs such as *Starships* are nonetheless drivers of genome evolution.
449 Further detailed investigation of the specific cargo in the elements we have identified in
450 *Gaeumannomyces* is a priority to explore how these giant TEs may be contributing to lifestyle
451 and virulence.

452 While the differences in the overall appearance of the wheat plants and their root systems
453 when infected with *GtA* versus *GtB* were visually compelling (Fig. 1A), our sample size was
454 extremely limited and the quantitative data did not show such a strong distinction (Fig. 1C). A
455 study by Lebreton et al. (2004) with a much larger sample size found *Gt* type A strains to be
456 significantly more aggressive *in vitro* despite high intraspecific variability in take-all severity
457 (type A=G2 in their study (Daval et al. 2010)). The dominance of type A strains in a site has
458 also been reported to positively correlate with disease severity (Lebreton et al. 2007). It is also
459 notable that five out of six wheat plants which died were inoculated with *GtA* strains. Our
460 phylogenomic analysis confirmed with significant branch support that the two lineages are
461 indeed monophyletic (Supplemental Fig. S13b) and, together with our comparative genomics
462 results, the question naturally arises as to whether *GtA* and *GtB* are in fact distinct species.
463 We did not find evidence that genetic divergence between *Ga* and *Gt* species was more
464 pronounced than between the *GtA* and *GtB* lineages, and host alone is not a sufficient
465 distinction since, despite being a separate species, *Ga* is also able to infect wheat (Freeman
466 and Ward 2004). Lebreton et al. (2004) suggested that 'genetic exchanges between [A and B]
467 groups are rare events or even do not exist', but this was based on analysis of a limited number
468 of genetic markers. Much broader whole-genome sequencing efforts are required to assess
469 gene flow between lineages at the population-level, as well as the level of recombination.
470 Understanding population dynamics could also shed light on the observed changes in ratio of
471 *GtA* and *GtB* across wheat cropping years (Lebreton et al. 2004), which has implications for
472 strategic crop protection measures.

473 **Conclusions**

474 We have generated near-complete assemblies with robust annotations for under-explored but
475 agriculturally important wheat-associated *Gaeumannomyces* species. In doing so we
476 confirmed that *Gaeumannomyces* taxa have one-compartment genomes in the context of
477 TE/effectector colocalisation, however the presence of giant cargo-carrying *Starship* TEs likely
478 contributes to genomic plasticity. Genomic signatures support the separation of *Gt* into two

479 distinct lineages, with copy-number as a potential mechanism underlying differences in
480 virulence. We also found evidence that variation amongst the relatively low number of BGCs
481 may be a key factor contributing to lifestyle differences in *Gt* and *Gh*. In addition to providing
482 foundational data to better understand this host–pathogen–antagonist system, these new
483 resources are also an important step towards developing much-needed molecular diagnostics
484 for take-all, whether conventional amplicon sequencing, rapid *in situ* assays (Hariharan and
485 Prasannath 2021) or whole-genome/metagenomic sequencing approaches (Weisberg et al.
486 2021). Future research will require whole-genome sequencing of taxa from a broader
487 geographical range to produce a global pangenome, which will provide a comprehensive
488 reference for expression analyses to explore the role of virulence in *Gt* lineages, as well as
489 population genomics to shed light on their evolution and distribution.

490 **METHODS**

491 **Samples**

492 Nine *Gaeumannomyces* strains were selected from the Rothamsted Research culture
493 collections, including five *Gt* strains (two type A and three type B), two *Ga* strains and two *Gh*
494 strains (Supplemental Table S2). All were collected from various experimental fields at
495 Rothamsted Farm (Macdonald et al. 2018) between 2014 and 2018.

496 ***G. tritici* virulence test in adult wheat plants**

497 To test the virulence of the five *Gt* strains, we performed inoculations of each strain (six
498 replicates) into the highly susceptible winter wheat cultivar Hereward. First the roots of
499 seedling plants were inoculated with the fungus by using plastic drinking cups (7.5 cm wide x
500 11 cm tall) as pots, ensuring that all seedlings were well colonised before transferring to a
501 larger pot. Pots were drilled with four drainage holes 3 mm in diameter. A 50 cm³ layer of damp
502 sand was added to each pot, followed by a 275 g layer of naïve soil collected from a field at
503 Rothamsted Farm after a non-legume break crop. Inoculum was prepared by taking a 9 mm
504 fungal plug with a cork borer number 6 from the outer part of a fungal colony grown on a potato

505 dextrose agar (PDA) plate and mixing with sand to make up a 25 g inoculum layer. A final 150
506 g layer of naïve soil was added on top of the inoculum layer. One wheat seed was sown on
507 the surface of the soil and covered with a 50 cm³ layer of grit to aid germination and create a
508 humid environment for fungal colonisation. Pots were watered well and placed in a controlled
509 environment room (16 hr day, light intensity 250 µmols, 15°C day, 10°C night, watered twice
510 a week from above). A randomised block design was generated in Genstat 20th Edition to
511 take potential environmental differences across the growth room into account.

512 After two weeks of growth, each wheat seedling in a small pot was transferred by removing
513 the plastic cup and placing the entire contents undisturbed into a larger 20 cm diameter pot
514 containing a 2 cm layer of clay drainage pebbles. Three small pots were transferred to each
515 large pot and filled in with more soil, resulting in three plants per pot. There were 6 replicates
516 for each treatment, and a control pot with no fungus was also set up in the same manner, but
517 a PDA plate without fungus was used for preparing the inoculum layer. The pots were
518 transferred to a screenhouse and arranged randomly within blocks containing one pot per
519 treatment. The pots were established in September and remained outside in the screenhouse
520 to ensure exposure to winter conditions and therefore allow plant vernalisation to take place.

521 Measurements of the above-ground characteristics were first undertaken to note the severity
522 of any take-all symptoms once the floral spike (ear) was fully emerged. The height of each
523 labelled plant was measured from the stem base to the tip of the ear to the nearest 0.5 cm to
524 identify whether there was stunted growth. Additionally, the length of the ear and flag leaf were
525 recorded, again to the nearest 0.5 cm. The number of ears per plant was also recorded.

526 For below-ground measurements, the pots were washed out post full plant senescence and
527 the plants were well rinsed to remove the soil while minimising damage to the roots. Any roots
528 that broke off were collected and put into the cup with the main plants to maintain accuracy of
529 the biomass measurements. The stems were then cut about 10 cm from the base. The plants
530 were placed in a white tray filled with water to enable clear observation of the roots. The
531 number of tillers for each plant was counted. The severity of take-all infection was then

532 estimated by using the Take-All Index (TAI), classified through the following categories: Slight
533 1 (0–10% of roots infected), slight 2 (11–25%), moderate 1 (25–50%), moderate 2 (51–75%)
534 and severe (76–100%). This was then input into the following formula: $TAI = ((1 \times \% \text{ plants})$
535 $slight \ 1) + (2 \times \% \text{ plants slight 2}) + (3 \times \% \text{ plants moderate 1}) + (4 \times \% \text{ plants moderate 2}) + (5$
536 $\times \% \text{ plants severe}) / 5$ (Bateman et al. 2004). Following this, the length of the roots was
537 measured to the nearest 0.5 cm. By cutting off one root at a time, the number of roots for each
538 plant was counted and the roots transferred into cardboard trays, one per pot. These were
539 then dried at 80°C on metal trays for 16 hours. One tray at a time was removed from the oven
540 to reduce any moisture gain before weighing. The dried root biomass per pot was then
541 recorded.

542 To statistically test for mean differences in the various characteristics between strains, we first
543 made Q-Q plots using the `ggqqplot` function from `ggpubr v0.6.0` (Kassambara 2020) to confirm
544 approximate data normality. We then used the `levene_test` function from the package `rstatix`
545 `v0.7.2` (Kassambara 2021) to assess the assumption of homogeneity of variance, where a
546 significant p value ($p < 0.05$) means that the assumption is violated. If we could ascertain
547 homogeneity of variance, a multiple comparison test between strains was performed with the
548 `tukey_hsd` `rstatix` function. Where homogeneity of variance was violated, the
549 `games_howell_test` `rstatix` function was instead used for multiple comparison testing (Sauder
550 and DeMars 2019).

551 **Genome sequencing**

552 For DNA and RNA extractions of all nine *Gaeumannomyces* taxa, a 4 mm plug of mycelium
553 from axenic cultures was transferred to 500 ml of potato dextrose broth treated with
554 penicillium/streptomycin (10,000 U/mL) using a sterile 4 mm corer. Cultures were grown at
555 20°C in dark conditions on an orbital shaker at 140 rpm for ~ 7–14 days. Mycelia were
556 collected via vacuum filtration and flash frozen using liquid nitrogen and stored at -80°C, before
557 grinding with a sterilised mortar and pestle until a fine powder was created.

558 DNA was extracted using one of two kits: the Phytopure Nucleon Genomic DNA kit (Cytiva,
559 MA, USA) eluted in 50 μ l low-pH TE buffer; and the NucleoBond HMW DNA kit (Macherey-
560 Nagel, North Rhine- Westphalia, Germany) eluted in 100 μ l–200 μ l low-pH TE buffer. The
561 manufacturer's protocols were modified to optimise for high molecular weight (M. Grey,
562 personal communication). Sufficient DNA concentration (50 ng/ μ l DNA) was confirmed by
563 Qubit fluorometer (Invitrogen, MA, USA) and purity (260/280 absorbance ratio of
564 approximately 1.6–2.0 and 260/230 absorbance ratio of approximately 1.8–2.4) confirmed with
565 a NanoDrop spectrophotometer (Thermo Fisher Scientific, MA, USA). Sufficient strand lengths
566 (80% > 40 Kbp length) were confirmed using the Femto Pulse System (Agilent Technologies,
567 Inc, CA, USA).

568 RNA from the same sample material was extracted using the Quick-RNA Fungal/Bacterial
569 miniprep kit (Zymo Research, CA, USA) using the manufacturer's protocol and eluted in 25 μ l
570 of DNase/RNase free water. Sufficient RNA concentration (71 ng/ μ l RNA) was confirmed by
571 Qubit fluorometer (Invitrogen, MA, USA) and purity (260/280 absorbance ratio of
572 approximately 1.8–2.1 and 260/230 absorbance ratio of > 2.0) confirmed with a NanoDrop
573 spectrophotometer (Thermo Fisher Scientific, MA, USA). An RNA integrity number > 8 was
574 confirmed by Bioanalyzer RNA analysis (Agilent Technologies, Inc, CA, USA).

575 DNA and RNA extractions were sent to the Genomics Pipelines Group (Earlham Institute,
576 Norwich, UK) for library preparation and sequencing. 2–5.5 μ g of each sample was sheared
577 using the Megaruptor 3 instrument (Diagenode, Liege, Belgium) at 18-20ng/ μ l and speed
578 setting 31. Each sample underwent AMPure PB bead (PacBio, CA, USA) purification and
579 concentration before undergoing library preparation using the SMRTbell Express Template
580 Prep Kit 2.0 (PacBio) and barcoded using barcoded overhang adapters 8A/B (PacBio) and
581 nuclease treated with SMRTbell enzyme cleanup kit 1.0 (PacBio). The resulting libraries were
582 quantified by fluorescence (Invitrogen Qubit 3.0) and library size was estimated from a smear
583 analysis performed on the Femto Pulse System (Agilent). The libraries were equimolar pooled
584 into four multiplex pools and each pool was size fractionated using the SageELF system (Sage

585 Science, MA, USA), 0.75% cassette (Sage Science). The resulting fractions were quantified
586 by fluorescence via Qubit and size estimated from a smear analysis performed on the Femto
587 Pulse System, and 1–2 fractions per pool were selected for sequencing and pooled equimolar
588 to have equal representation of barcodes per pool. The loading calculations for sequencing
589 were completed using the PacBio SMRTLink Binding Calculator v10.1.0.119528 or
590 v10.2.0.133424. Sequencing primer v2 or v5 was annealed to the adapter sequence of the
591 library pools. Binding of the library pools to the sequencing polymerase was completed using
592 Sequel II Binding Kit v2.0 or 2.2 (PacBio). Calculations for primer to template and polymerase
593 to template binding ratios were kept at default values. Sequel II DNA internal control was
594 spiked into the library pool complexes at the standard concentration prior to sequencing. The
595 sequencing chemistry used was Sequel II Sequencing Plate 2.0 (PacBio) and the Instrument
596 Control Software v10.1.0.119549 or 10.1.0.125432. Each pool was sequenced on 1-2 Sequel
597 II SMRTcells 8M (PacBio) on the Sequel IIe instrument. The parameters for sequencing were
598 as follows: CCS sequencing mode; 30-hour movie; 2-hour adaptive loading set to 0.85 or
599 diffusion loading; 2-hour immobilisation time; 2–4-hour pre-extension time; and 70–86pM on
600 plate loading concentration.

601 RNA libraries were constructed using the NEBNext Ultra II RNA Library prep for Illumina kit
602 (New England Biolabs, MA, USA), NEBNext Poly(A) mRNA Magnetic Isolation Module and
603 NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs) at a concentration
604 of 10 μ M. RNA libraries were equimolar pooled, q-PCR was performed, and the pool was
605 sequenced on the Illumina NovaSeq 6000 (Illumina, CA, USA) on one lane of a NVS300S4
606 flowcell with v1.5 chemistry producing a total of 3,370,873,981 reads.

607 **Genome assembly**

608 See Supplemental Fig. S14a for a schematic summarising the bioinformatics analyses. HiFi
609 reads were assembled using hifiasm v0.16.1-r375 (Cheng et al. 2021) with the -l 0 option to
610 disable purging of duplicates in these haploid assemblies. The assemblies were checked for
611 content correctness with respect to the input HiFi reads using the COMP tool from KAT v2.3.4

612 (Mapleson et al. 2017), and QUAST v5.0.2 (Mikheenko et al. 2018) was used to calculate
613 contiguity statistics. BlobTools v1.0.1 (Laetsch and Blaxter 2017) was used to check for
614 contamination (Supplemental Fig. S15) — this required a hits file, which we produced by
615 searching contigs against the nt database (downloaded 21/05/2021) using blastn v2.10, and
616 a BAM file of mapped HiFi reads, which we produced using minimap2 v2.21 (Li 2018) and
617 samtools v1.13 (Li et al. 2009).

618 Gene set completeness was assessed using the ascomycota_odb10.2020-09-10 dataset in
619 BUSCO v5.2.1 (Manni et al. 2021). This revealed some gene duplication due to the presence
620 of small contigs that had exceptionally low coverage (median of 1 across each small
621 sequence) when projecting the kmer spectra of the reads onto them using KAT's SECT tool.
622 This was taken as evidence that the sequences did not belong in the assemblies. A custom
623 script was written to filter out these small, low-coverage sequences, using the output of KAT
624 SECT. KAT COMP, BUSCO and QUAST were re-run for the coverage filtered assemblies to
625 verify that duplicated genes were removed without losing core gene content and produce final
626 assembly contiguity statistics (Supplemental Fig. S16, Supplemental Table S1).

627 **Genome annotation**

628 Repeats were identified and masked using RepeatModeler v1.0.11 (Smit and Hubley 2015)
629 and RepeatMasker v4.0.7 (Smit et al. 2015) via EIRrepeat v1.1.0 (Kaithakottil and Swarbreck
630 2023). Gene models were annotated via the Robust and Extendable Eukaryotic Annotation
631 Toolkit (REAT) v0.6.3 (EI-CoreBioinformatics 2023b) and MINOS v1.9 (EI-CoreBioinformatics
632 2023a). The REAT workflow consists of three submodules: transcriptome, homology, and
633 prediction. The transcriptome module utilised Illumina RNA-Seq data, reads that were mapped
634 to the genome with HISAT2 v2.1.0 (Kim et al. 2019) and high-confidence splice junctions
635 identified by Portcullis v1.2.4 (Mapleson et al. 2018). The aligned reads were assembled for
636 each tissue with StringTie2 v1.3.3 (Kovaka et al. 2019) and Scallop v0.10.2 (Shao and
637 Kingsford 2017). A filtered set of non-redundant gene models were derived from the combined
638 set of RNA-Seq assemblies using Mikado v2.3.4 (Venturini et al. 2018). The REAT homology

639 workflow was used to generate gene models based on alignment of protein sequences from
640 publicly available annotations of 27 related species (Supplemental Table S3) and a set of
641 proteins downloaded from UniProt including all the proteins from the class *Sordariomycetes*
642 (taxid:147550) and excluding all proteins from the publicly available annotation of *Gt* R3-111a-
643 1 (GCF_000145635). The prediction module generated evidence-guided models based on
644 transcriptome and proteins alignments using AUGUSTUS v3.4.0 (Stanke et al. 2006), with
645 four alternative configurations and weightings of evidence, and EVidenceModeler v1.1.1
646 (Haas et al. 2008). In addition, gene models from the *Gt* R3-111a-1 annotation were projected
647 via Liftoff v1.5.1 (Shumate and Salzberg 2021), and filtered via the multicomparescript from
648 the ei-liftover pipeline (Venturini and Yanes 2020), ensuring only models with consistent gene
649 structures between the original and transferred models were retained.

650 The filtered Liftoff, REAT transcriptome, homology and prediction gene models were used in
651 MINOS to generate a consolidated gene set with models selected based on evidence support
652 and their intrinsic features. Confidence and biotype classification was determined for all gene
653 models based on available evidence, such as homology support and expression. TE gene
654 classification was based on overlap with identified repeats (> 40 bp repeat overlap).

655 To make best use of having multiple identically generated annotations for the genus, we opted
656 to additionally repeat a lift-over process projecting the gene models from each MINOS run to
657 all nine assemblies. We then removed gene models overlapping rRNA genes from the
658 multiple-lift-over annotations and the previously consolidated MINOS annotation using
659 RNAmmer v1.2 (Lagesen et al. 2007) and BEDTools v2.28 (Quinlan and Hall 2010). The
660 MINOS consolidation stage was repeated using four files as input: the high-confidence models
661 from the lift-over; the high-confidence genes of the previous MINOS run for the specific
662 assembly; the low-confidence models of the previous MINOS run for the specific assembly;
663 and the low-confidence models of the lift-over of all the closely related species. This multiple-
664 lift-over approach allowed us to cross-check gene sets across strains and determine whether
665 missing genes were truly absent from individual assemblies or had just been missed by the

666 annotation process. Finally, mitochondrial contigs were identified using the MitoHiFi v2.14.2
667 pipeline (Uliano-Silva et al. 2023), with gene annotation using MitoFinder v1.4.1 (Allio et al.
668 2020) and the mitochondrion sequence from *Epichloë novae-zelandiae* AL0725 as a reference
669 (GenBank accession NC_072722.1).

670 Functional annotation of the gene models was performed using AHRD v3.3.3 (Hallab et al.
671 2023), with evidence from blastp v2.6.0 searches against the Swiss-Prot and TrEMBL
672 databases (both downloaded on 19/10/2022), and mapping of domain names using
673 InterProScan v5.22.61 (Jones et al. 2014). Additional annotations were produced using
674 eggNOG-mapper v2.1.9 (Cantalapiedra et al. 2021) with sequence searches against the
675 eggNOG orthology database (Huerta-Cepas et al. 2019) using DIAMOND v2.0.9 (Buchfink et
676 al. 2021). CAZymes were predicted using run_dbcan v3.0.1 (Le and Yohe 2021) from the
677 dbCAN2 CAZyme annotation server (Zhang et al. 2018) this process involved (i) HMMER
678 v3.3.2 (Mistry et al. 2013) search against the dbCAN HMM (hidden Markov model) database;
679 (ii) DIAMOND v2.0.14 search against the CAZy pre-annotated CAZyme sequence database
680 (Drula et al. 2022) and (iii) eCAMI (Xu et al. 2020) search against a CAZyme short peptide
681 library for classification and motif identification. A gene was classified as a CAZyme if all three
682 methods were in agreement.

683 CSEPs were predicted using a similar approach to Hill et al. (2022), with some
684 additions/substitutions of tools informed by Jones et al. (2021); see Supplemental Fig. S14b
685 for a schematic overview. Briefly, we integrated evidence from SignalP v3.0 (Dyrøv Bendtsen
686 et al. 2004), v4.1g (Petersen et al. 2011), v6.0g (Teufel et al. 2022); TargetP v2.0 (Almagro
687 Armenteros et al. 2019); DeepSig v1.2.5 (Savojardo et al. 2018); Phobius v1.01 (Käll et al.
688 2004); TMHMM v2.0c (Krogh et al. 2001); Deeploc v1.0 (Almagro Armenteros et al. 2017);
689 ps_scan v1.86 (Gattiker et al. 2002); and EffectorP v1.0 (Sperschneider et al. 2016), v2.0
690 (Sperschneider et al. 2018) and v3.0 (Sperschneider and Dodds 2021). CSEPs were then
691 matched to experimentally verified genes in the PHI-base database (Urban et al. 2020)
692 (downloaded 21/07/2023) using a BLAST v2.10 blastp search with an e-value cutoff of 1e-25.

693 In the event of multiple successful hits, the hit with the top bitscore was used. Secondary
694 metabolites were predicted using antiSMASH v6.1.1 (Blin et al. 2021). Reference protein
695 sequences for avenacinase from *Ga* (GenBank accession AAB09777.1) and mating-type
696 locus idiomorphs *MAT1-1* and *MAT1-2* from *Pyricularia grisea* (Latorre et al. 2022) were used
697 to identify their respective genes in each of the nine assemblies using a blastp search (e-value
698 cutoff 1e-25).

699 **Phylogenetic classification of *G. tritici* types**

700 To confirm the classification of *Gt* strains within established genetic groups — *sensu* Daval et
701 al. (2010) and Freeman et al. (Freeman et al. 2005) — gene trees were produced for gentisate
702 1,2-dioxygenase (*gdo*; GenBank accessions FJ717712–FJ717728) and *ITS2*. GenePull (Hill
703 2021) was used to extract the two marker sequences from the new assemblies reported here.
704 *ITS2* could not be found in the existing *Gt* R3-111a-1 assembly (RefSeq accession
705 GCF_000145635.1), so that strain was only included in the *gdo* gene tree. We aligned each
706 marker gene separately using MAFFT v7.271 (Katoh and Standley 2013) and manually
707 checked the gene alignments. The gene trees were estimated using RAxML-NG v1.1.0
708 (Kozlov et al. 2019) and the GTR+G nucleotide substitution model (Supplemental Fig. S13a).
709 Branch support was computed using 1,000 Felsenstein's bootstrap replicates, or until
710 convergence according to the default 3% cutoff for weighted Robinson-Foulds distances
711 (Pattengale et al. 2009), whichever occurred first. An avenacinase gene tree was produced in
712 the same way but using the JTT+G4 amino acid substitution model.

713 **Phylogenomics of *Gaeumannomyces***

714 A genome-scale species tree was produced to provide evolutionary context for comparative
715 analyses. We used OrthoFinder v2.5.4 (Emms and Kelly 2019) to cluster predicted gene
716 models for primary transcripts into orthogroups — in addition to the newly sequenced
717 *Gaeumannomyces* taxa, this also included *Gt* R3-111a-1 and the outgroup *Magnaportheopsis*
718 *poae* ATCC 64411 (GenBank accession GCA_000193285.1). Alongside the coalescent

719 species tree produced within OrthoFinder by STAG (Emms and Kelly 2018), we also used a
720 concatenation-based approach. We used MAFFT to produce gene alignments for 7,029
721 single-copy phylogenetic hierarchical orthogroups or HOGs (hereafter, genes) that were
722 present in all taxa. These were trimmed using v1.4.rev15 (Capella-Gutiérrez et al. 2009),
723 concatenated using AMAS and run in RAxML-NG with genes partitioned and the JTT+G4
724 amino acid substitution model. Branch support was calculated as above.

725 Alongside the species tree we visualised assembly N50; the number of gene models; the
726 proportion of these that were functionally annotated by AHRD; and the number of unassigned
727 gene models from OrthoFinder (Supplemental Fig. S17). Due to concerns regarding the
728 comparability of the existing *Gt* R3-111a-1 annotation to the strains reported in this study, and
729 to avoid introducing computational bias, the existing *Gt* R3-111a-1 annotation was excluded
730 from downstream comparative analyses for the sake of consistency.

731 **Genome structure and synteny**

732 To identify both potential misassemblies and real structural novelty in our strains, we used
733 GENESPACE v1.1.8 (Lovell et al. 2022) to visualise syntenic blocks across the genomes.
734 Fragments were considered to have telomeres at the ends if Tapestry v1.0.0 (Davey et al.
735 2020) identified at least five telomeric repeats (TTAGGG), and this was used together with the
736 GENESPACE results to inform pseudochromosome designation. Telomeric repeats were also
737 cross-checked with results from tidk v0.2.31 (Brown 2023). We calculated GC content across
738 pseudochromosomes in 100,000 bp windows using BEDTools v2.29.2 (Quinlan and Hall
739 2010), and TE, gene and CSEP density were calculated in 100,000 bp windows with a custom
740 script, plot_ideograms.R. The composite RIP index (CRI) (Lewis et al. 2009) was calculated
741 in 500 bp windows using RIP_index_calculation.pl (Stajich 2023).

742 To statistically test for correlations between CSEP density and TE and /or gene density, we
743 again made Q-Q plots using the ggqqplot function to assess approximate data normality. This
744 being violated, we calculated Kendall's tau for each strain (rstatix cor_test function,

745 method="kendall"). The assumption of normality being similarly violated for distances from
746 CSEPs/other genes to the closest TE, we performed a Wilcoxon rank sum test (wilcox_test
747 function) to compare mean distances for CSEPs versus other genes for each strain. To
748 compare the mean gene–TE distance across strains, we used a Games-Howell test
749 (games_howell_test function) for multiple comparison testing. Comparison of distances
750 between HCN genes and TEs versus other genes and TEs was tested in the same way.

751 We also performed permutation tests of CSEP–TE distances using the meanDistance
752 evaluation function from the R package regioneR v1.32.0 (Gel et al. 2016), with the
753 resampleRegions function used for randomisation of the gene universe over 1,000
754 permutations. Permutation tests of CSEP–telomere distances were performed in the same
755 way, having assigned the first and last 10,000 bp of each pseudochromosome as telomeric
756 regions.

757 **Comparative genomics**

758 Functional annotations were mapped to orthogroups using a custom script,
759 orthogroup_assigner.R, adapted from Hill et al. (2022), which also involved retrieval of
760 CAZyme names from the ExplorEnz website (McDonald et al. 2009) using the package rvest
761 v1.0.3 (Wickham 2020). CAZyme families known to act on the major plant cell wall substrates
762 were classified as by Hill et al. (2022) based on the literature (Glass et al. 2013; Levasseur et
763 al. 2013; Zhao et al. 2013; Miyauchi et al. 2020; Hage and Rosso 2021; Mesny et al. 2021).

764 For *Gt*, gene content was categorised as core (present in all strains), soft-core (present in all
765 but one strain), accessory (present in at least two strains) and specific (present in one strain).

766 Broadscale differences in gene repertoires due to lifestyle (pathogenic *Gt* and *Ga* and non-
767 pathogenic *Gh*) were statistically tested using a permutational analysis of variance
768 (PERMANOVA) approach to estimate residual variance of gene content after accounting for
769 variance explained by phylogenetic distance (Mesny and Vannier 2020). To analyse the
770 potential for secondary metabolite production with this PERMANOVA approach, a presence-

771 absence matrix for biosynthetic gene cluster families was produced from the antiSMASH
772 results using BiG-SCAPE v1.1.5 (Navarro-Muñoz et al. 2020).

773 Gene duplicates were categorised as intrachromosomal (on the same pseudochromosome)
774 or interchromosomal (on a different pseudochromosome) using the pangenomes output files from
775 GENESPACE. We conducted gene ontology (GO) enrichment analysis for high copy-number
776 (HCN) genes using the R package topGO v2.50.0 (Alexa and Rahnenfuhrer 2022) with
777 Fisher's exact test and the weight01 algorithm.

778 **Starship element identification**

779 Giant transposable *Starship* elements were identified in our assemblies after noting dense
780 blocks of transposons forming gaps between annotated genes. Manual inspection of these
781 regions via synteny plots built with OMA v2.5.0 (Altenhoff et al. 2019) and Circos v0.69
782 (Krzywinski et al. 2009) revealed *Starship*-sized insertions (Gluck-Thaler et al. 2022), and an
783 NCBI blastp search of the first gene in one such insertion in strain Gt-8d (Gt-
784 8d_Elv1_0041140) returned 85% identity with an established Gt R3-111a-1 DUF3435 gene
785 (GenBank accession EJT80010.1). These two genes were then used for a local blastp v2.13.0
786 search against all nine *Gaeumannomyces* assemblies reported here, which identified 33 full
787 length hits (>95% identity) that were associated with insertions when visualised in Circos plots.
788 This manual approach was then compared to *Starship* element identification using starfish
789 v1.0 (Gluck-Thaler and Vogan 2023). One element identified by starfish was discounted as it
790 consisted solely of a single predicted captain gene with no cargo or flanking repeats. A gene
791 tree of all tyrosine recombinases predicted by starfish (including *Starship* captains), blastp-
792 identified DUF3435 homologues, and previously reported *Starship* captain genes (Gluck-
793 Thaler et al. 2022) was built using the same methods described above for phylogenetic
794 classification and the JTT+G4 amino acid substitution model, with the addition of alignment
795 trimming using trimAI v1.4.rev15 (Capella-Gutiérrez et al. 2009) with the -gappyout parameter.

796 Data visualisation was completed in R v4.3.1 (R Core Team 2022) using the packages ape
797 v5.7-1 (Paradis and Schliep 2019), aplot v0.2.2 (Yu et al. 2023), ComplexUpset v1.3.3
798 (Krassowski 2022), cowplot v1.1.1 (Wilke 2020), data.table v1.14.8 (Dowle and Srinivasan
799 2023), eulerr v7.0.0 (Larsson 2020), ggforce v0.4.1 (Pedersen 2021), ggh4x v0.2.6 (van den
800 Brand 2023), gggenomes v0.9.12.9000 (Hackl et al. 2023), ggmsa v1.6.0 (Zhou et al. 2022),
801 ggnewscale v0.4.9 (Campitelli 2020), ggplot2 v3.4.4 (Wickham 2016), ggplotify v0.1.2 (Yu
802 2021), ggpubr v0.6.0 (Kassambara 2020), ggrepel v0.9.3 (Slowikowski 2020), ggtree v3.9.1
803 (Yu et al. 2017), Gviz v1.44.2 (Hahne and Ivannik 2016), matrixStats v1.0.0 (Bengtsson
804 2021), multcompView v0.1-9 (Graves et al. 2019), patchwork v1.1.3 (Pederson 2022),
805 rtracklayer v1.60.1 (Lawrence et al. 2009), scales v1.2.1 (Wickham and Seidel 2020),
806 seqmagick v0.1.6 (Yu 2023), tidyverse v2.0.0 (Wickham et al. 2019). All analysis scripts are
807 available at <https://github.com/Rowena-h/GaeumannomycesGenomics>.

808 **DATA ACCESS**

809 WGS data and annotated genome assemblies are available on GenBank under the BioProject
810 accession PRJNA935249 (assemblies pending release). Additional data files are deposited in
811 Zenodo doi:10.5281/zenodo.10277622 (pending release). All bioinformatics scripts are
812 available at <https://github.com/Rowena-h/GaeumannomycesGenomics>.

813 **COMPETING INTEREST STATEMENT**

814 The authors declare no competing interests.

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840 **Author contributions**

841 RH, MM, NH, KH-K and JP-G conceived, managed and/or coordinated the work. NH, MM and
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843 of the research. GC, VEM, S-JO, JH, MG and MM collected the samples and/or isolated
844 strains. JP-G, JS and JH performed *Gt* inoculation experiments. MG and NI performed
845 molecular lab work. SJW performed genome assembly analyses. MOF performed genome
846 annotation analyses, supervised by D Swarbreck. RH performed functional annotation
847 analyses; designed and performed phylogenetic, comparative and statistical analyses; and
848 performed data visualisations. D Smith performed the exploratory *Starship* analyses. RH and

849 MM wrote the manuscript with contributions from MG, MOF, TC, D Smith, NI, NH, JP-G, and
850 KH-K. All authors read and approved the manuscript.

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1448

1449 **Figure 1** Intraspecific variation in *Gaeumannomyces tritici* (*Gt*) virulence assessed from
1450 inoculation of wheat plants. Representative photos of wheat roots (**a**) and above-ground
1451 features (**b**) following inoculation treatment. Inoculated strains from top left to bottom right:
1452 no *Gt* (control), *Gt*-8d, *Gt*-19d1, *Gt*-23d, *Gt*-4e and *Gt*-LH10. **c** Box and violin plots showing

1453 the impact of the five *Gt* strains sequenced in this study on above- and below-ground
1454 characteristics in winter wheat. Control, *Gt* type A and type B groups are indicated by
1455 different colours. Strains with a significant mean difference for the characteristic as
1456 calculated by either the Tukey HSD or Games-Howell test are shown by letters above the
1457 plots.

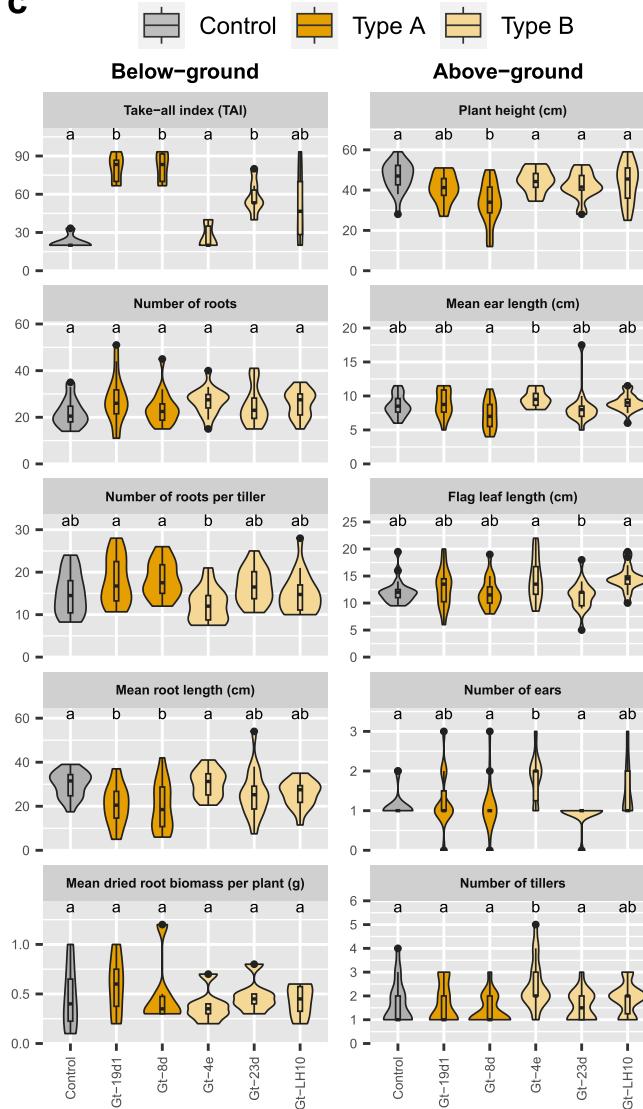
1458 **Figure 2** GENESPACE plot showing synteny across the nine *Gaeumannomyces* strains.
1459 A/B lineages are indicated for *G. tritici* strains. Fragments are labelled with numbers
1460 corresponding to pseudochromosomes, and an asterisk indicates that a fragment was
1461 inverted in the visualisation. Black bars on the ends of fragments indicate telomeres
1462 predicted using Tapestry.

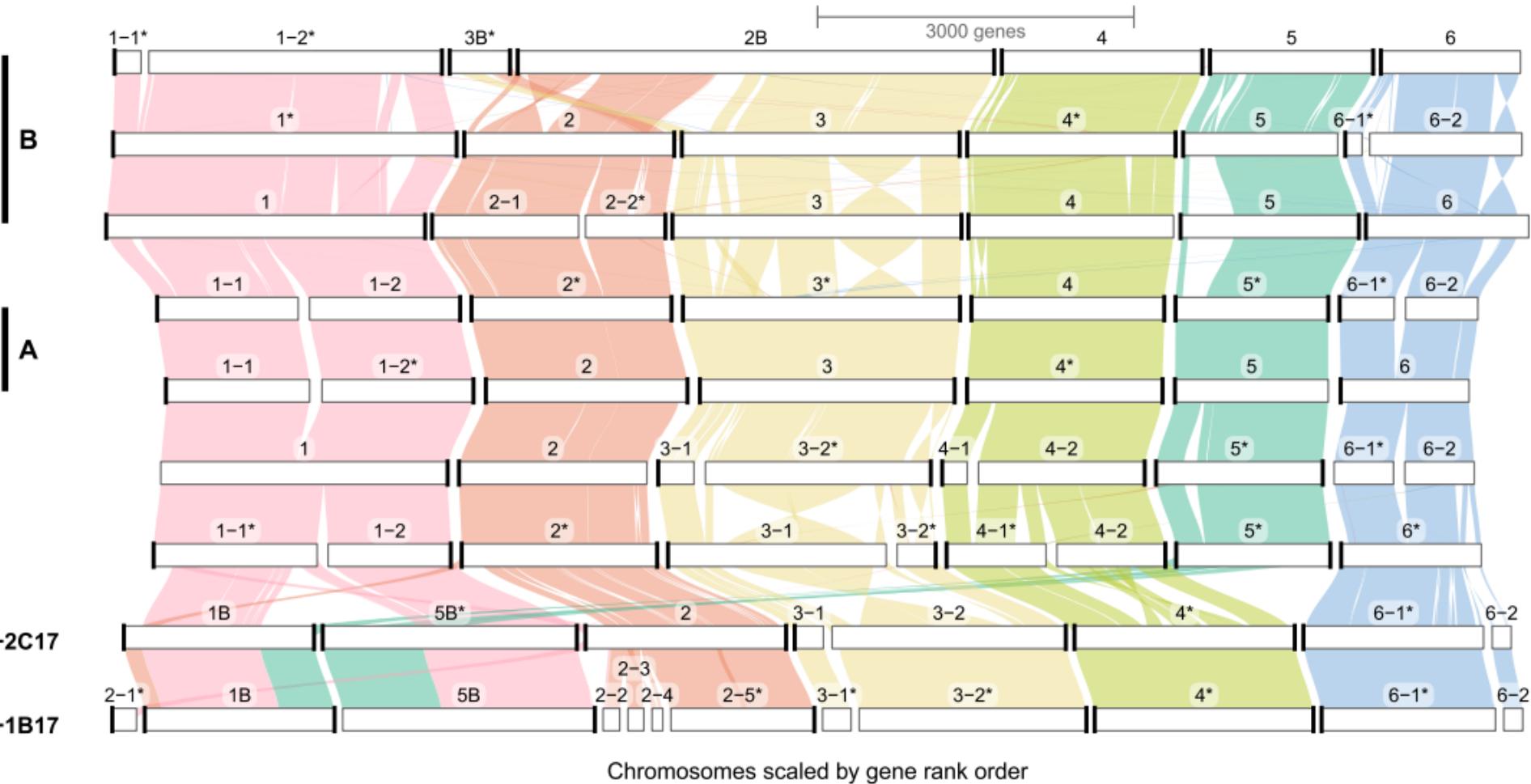
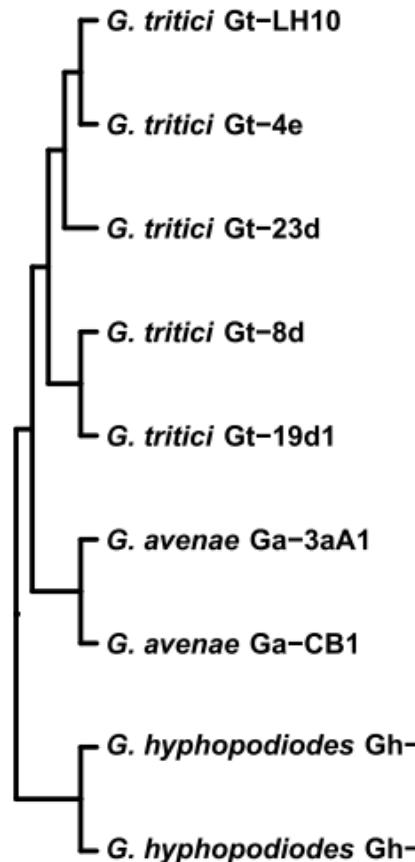
1463 **Figure 3** The relationship between candidate secreted effector proteins (CSEPs) and
1464 transposable elements (TEs) in *Gaeumannomyces*. **a** TE density (per 100,000 bp) and the
1465 location of CSEPs (black ticks) across fragments. Fragments are ordered syntenically
1466 according to GENESPACE (Fig. 2). **b** Scatterplot showing the relationship between CSEP
1467 density versus TE and gene density (per 100,000 bp) with local polynomial regression lines
1468 (ggplot2 function geom_smooth, method = “loess”). Significant correlation is indicated with
1469 Kendall’s tau (τ). **c** Box and violin plots showing the distance of genes to the closest TE, with
1470 CSEPs and other genes distinguished by colour. An asterisk indicates where a Wilcoxon
1471 rank sum test found the mean TE distance to be significantly different for CSEPs versus
1472 other genes. Strains with a significant mean difference in overall gene-TE distance as
1473 calculated by the Games-Howell test are shown by letters above the plots.

1474 **Figure 4** Summary of predicted gene content for the *Gaeumannomyces* strains reported in
1475 this study. **a** Number of total genes, candidate secreted effector proteins (CSEPs),
1476 carbohydrate-active enzymes (CAZymes) and biosynthetic gene clusters (BGCs) for each
1477 *Gaeumannomyces* strain. The A/B lineages are indicated for *Gaeumannomyces tritici* (*Gt*)
1478 strains. The dashed line in the phylogeny indicates bootstrap support <70 found within the *GtB*
1479 lineage (see Supplemental Fig. S13b for the full genome-scale *Gaeumannomyces* species

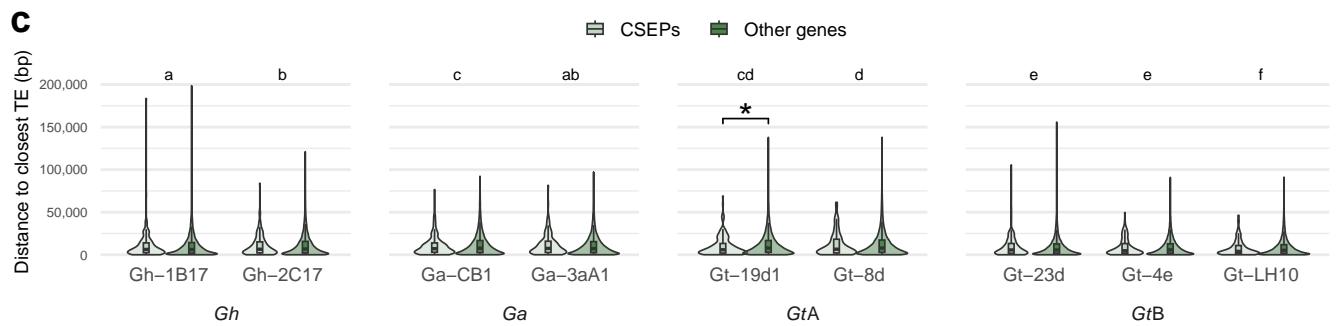
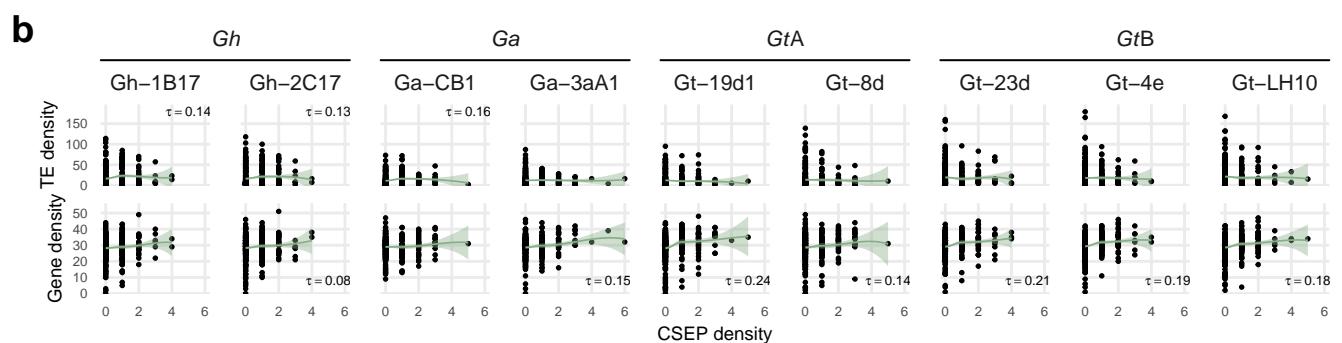
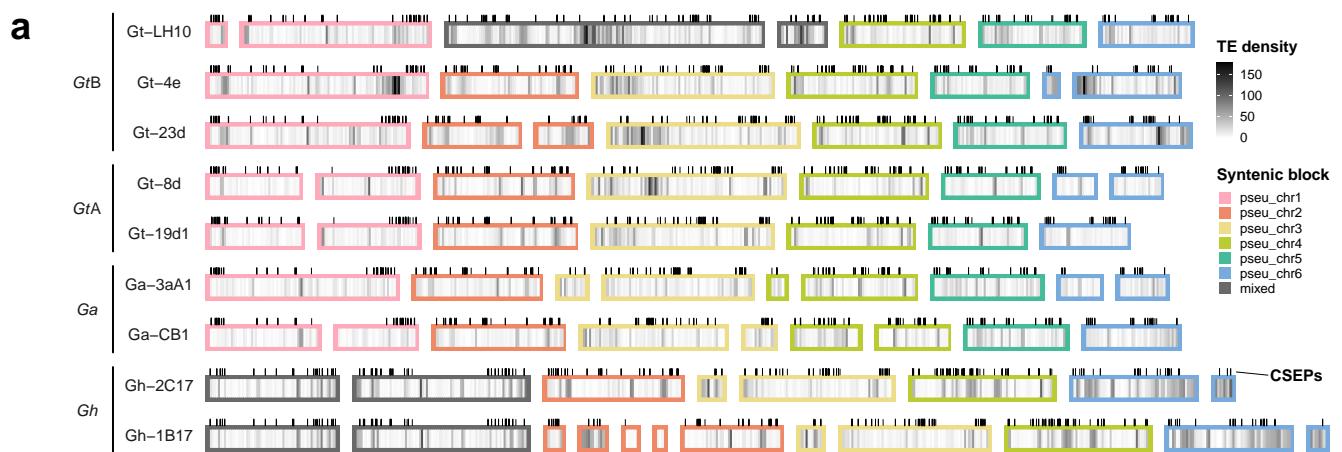
1480 tree). The *Gt* pangenome (within dashed box) is categorised as core (present in all strains),
1481 soft-core (present in all but one strain), accessory (present in at least two strains) and specific
1482 (present in one strain). The lefthand inset box shows the results of PERMANOVA statistical
1483 tests which calculate the descriptive power of relatedness (phylogeny) versus lifestyle
1484 categorisation (*Gt* and *G. avenae* as pathogenic in wheat, *G. hyphopodiooides* as non-
1485 pathogenic) on gene variance. Gene copy-number is shown on a scatterplot to the right, with
1486 points jittered vertically to improve visualisation. **b** Accumulation curves of pan and core genes
1487 for the *Gt* pangenome (Siozios 2021). **c** Euler diagram summarising whether high copy-
1488 number genes in each lineage are present but in low copy-number in *GtA*, or completely
1489 absent.

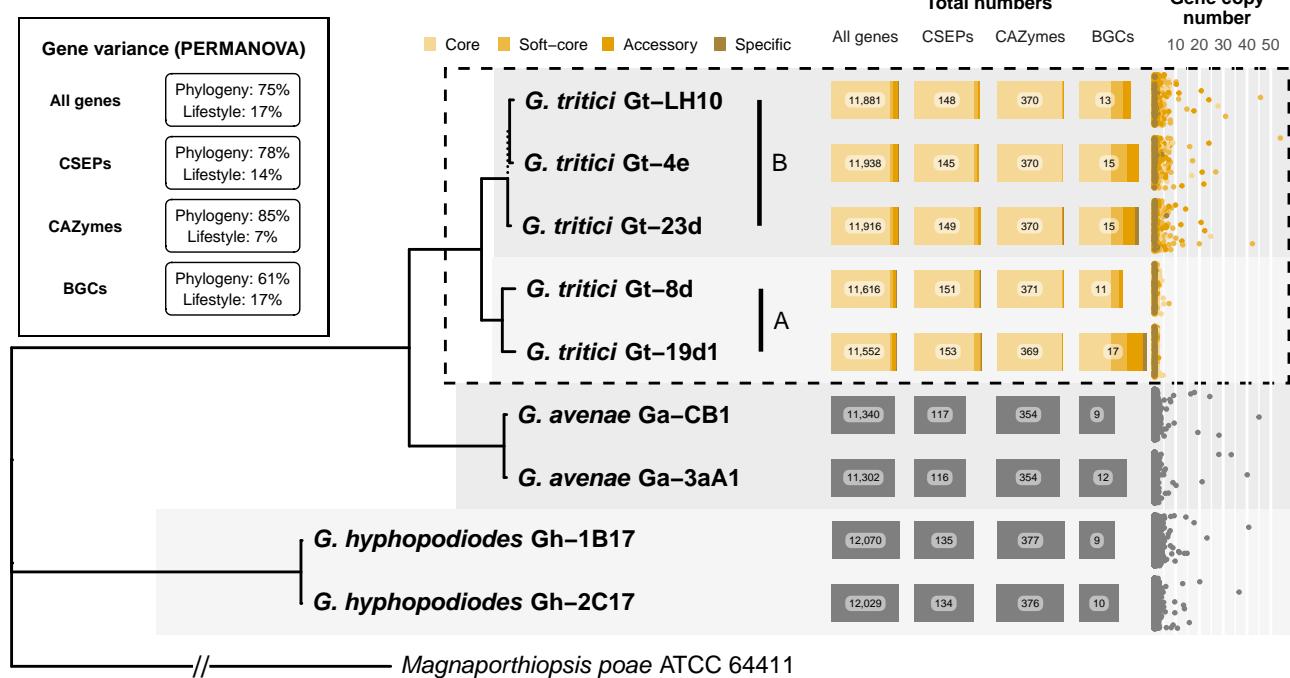
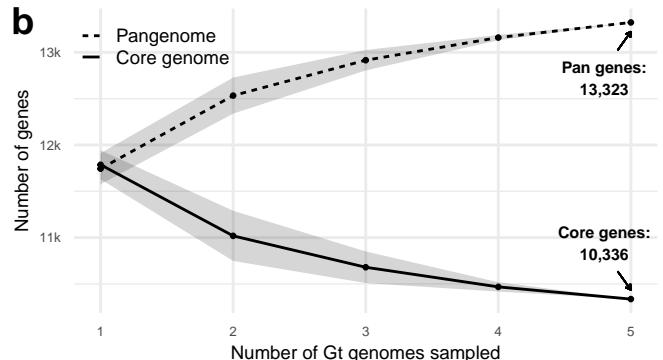
1490 **Figure 5** *Gaeumannomyces* genomes contain *Starship* giant transposable elements. **a** Gene
1491 tree of *Starship* 'captain' genes, including captains and other tyrosine recombinases identified
1492 from our assemblies via starfish, captain homologues identified via blastp, and previously
1493 published captain genes. **b** A summary of the *Starship* elements identified by starfish with the
1494 composite RIP index (CRI) shown above each element. The yellow highlight distinguishes a
1495 nested element. cap=captain gene, DR=direct repeat, RIP=repeat-induced point mutation,
1496 TE=transposable element gene, TIR=terminal inverted repeat, tyr=tyrosine recombinase
1497 gene.

a**b****c**



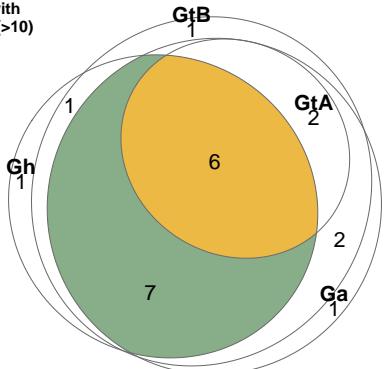
Chromosomes scaled by gene rank order



a**b****c**

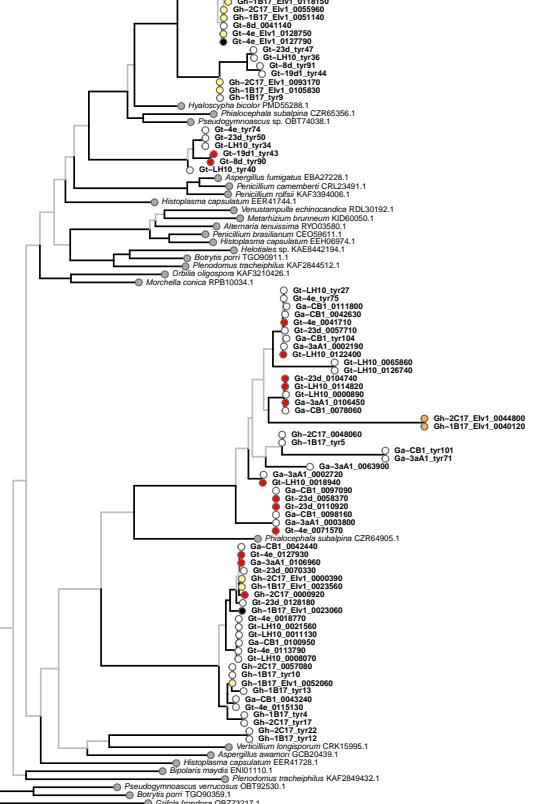
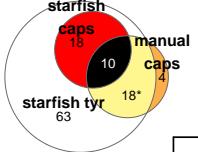
Distribution of genes with copy-number outliers (>10)

- Absent in GtA (green)
- Present in GtA (orange)



a

- published cap
- cap
- starfish cap
- manual cap
- starfish tyr, manual cap
- starfish tyr

**b**