**Objectives:** We aimed to investigate the occurrence of loss of heterozygosity (LOH) in diploid fungal species and the subsequent impact on outbreak detections with whole genome sequencing (WGS).

**Methods:** *Candida parapsilosis*, *Candida tropicalis*, and *Wickerhamomyces anomalus* isolates from hospital outbreaks and consecutive *Kluyveromyces marxianus* isolates from a single patient were submitted to WGS single nucleotide polymorphism (SNP) analysis. SNP were inspected and assessed for LOH and the ensuing impact on clonal classification of isolates was determined.

**Results:** Initially, isolates of all species appeared to be unrelated due to large numbers of SNPs. Subsequently, SNP were visually inspected in regard to location and zygosity. For *C. tropicalis*, LOH was observed within sequential isolates of patients that resulted in the acquisition of up to 70 SNPs within two days. These LOH events were divided across multiple chromosomes. For *W. anomalus*, LOH tracts were larger than *C. tropicalis* and occasionally involved entire chromosomes, while other chromosomes were completely unaffected. When SNP analysis was conducted on individual chromosomes instead of the entire genome, clonal transmission was revealed, as on chromosomal levels isolates were nearly identical for some chromosomes, while other chromosomes displayed many SNPs due to LOH. Also for *C. parapsilosis*, SNPs between patients were frequently due to LOH events and for *K. marxianus*, LOH was found between isolates from the same patient.

**Conclusions:** Here we demonstrated that LOH often occurs in diploid fungi, which leads to a high number of SNPs accrued in a short period. Isolates with LOH that are involved in outbreaks appear unrelated with automated pipelines, highlighting the need to interpret WGS results with caution and the necessity to develop alternative approaches to detect LOH in outbreak settings.