Species-wide transposable element repertoires retrace the evolutionary history of the \textit{Saccharomyces cerevisiae} host

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Abstract

Transposable elements (TE) are an important source of genetic variation with a dynamic and content that greatly differ in a wide range of species. The origin of the intraspecific content variation is not always clear and little is known about the precise nature of it. Here, we surveyed the species-wide content of the Ty LTR-retrotransposons in a broad collection of 1,011 Saccharomyces cerevisiae natural isolates to understand what can stand behind the variation of the repertoire, i.e. the type and number of Ty elements. We have compiled an exhaustive catalog of all the TE sequence variants present in the S. cerevisiae species by identifying a large set of new sequence variants. The characterization of the TE content in each isolate clearly highlighted that each subpopulation exhibits a unique and specific repertoire, retracing the evolutionary history of the species. Most interestingly, we have shown that ancient interspecific hybridization events had a major impact in the birth of new sequence variants and therefore in the shaping of the TE repertoires. We also investigated the transpositional activity of these elements in a large set of natural isolates, and we found a broad variability related to the level of ploidy as well as the genetic background. Overall, our results pointed out that the evolution of the Ty content is deeply impacted by clade-specific events such as introgressions and therefore follows the population structure. In addition, our study lays the foundation for future investigations to better understand the transpositional regulation and more broadly the TE-host interactions.
Introduction

Transposable elements (TEs) are interspersed repetitive DNA sequences that are able to move from one genomic location to another via a process of intra-genomic propagation, called transposition. Several decades of studies, facilitated by whole genome sequence analyzes, have shown that the genome of almost all species is populated by TEs. However, TEs are incredibly diverse, both in terms of structure and mode of transposition, as illustrated by the TE classification (Wicker et al. 2007; Arkhipova 2017; Bourque et al. 2018; Kojima 2019). Transposable elements can be classified either into class I retrotransposons or class II DNA transposons, depending on their mechanism of transposition. In each class, TEs are grouped into orders, depending on the molecular mechanisms involved in transposition, and then divided into super-families, according to their structural characteristics. Finally, DNA sequence conservation and phylogenetic data defines myriads of TE families, subfamilies and sequence variants.

Even if TE elements are ubiquitous components, the content in terms of type, number and prevalence is very variable across the genomes. This variation was observed at different time scales, i.e. between species from the same phylum as well as between individuals from the same species (Huang et al. 2012; Thomas-Bulle et al. 2018; Fonseca et al. 2019; Gaiero et al. 2019; Ray et al. 2019). In the most recent studies, the comparison of TE contents has allowed a better understanding of the events that shaped populations, such as the domestication of rice (Carpentier et al. 2019), silk worm (Han et al. 2018), and sunflower (Mascagni et al. 2018). Biotic or abiotic stresses have also been shown to impact the TE content of plant and insect species (Quadrana et al. 2016; Stritt et al. 2018; Baduel et al. 2019; Lerat et al. 2019; Rogivue et al. 2019).

The *S. cerevisiae* species has a low TE diversity content with only a handful of families, called Ty1 to Ty5 (Lesage and Todeschini 2005). All Ty elements belong to the class I and more precisely to the order of LTR retrotransposons, divided into two super-families, namely copia (for the Ty1, Ty2, Ty4 and Ty5 elements) and gypsy (for the Ty3 element). The common feature of their coding region is the presence of two genes similar to the gag and pol genes of retroviruses. As the Ty elements were among the first LTR retrotransposons to be discovered, *Saccharomyces cerevisiae* emerged as a good model for TE biology (Cameron et al. 1979; Clark et al. 1988; Hansen et al. 1988). The Ty1 and Ty3 elements are the most characterized transposons, and have since been benchmarks for retroelement studies (Curcio et al. 2015; Sandmeyer et al. 2015). The in-depth knowledge of the Ty biology stands in contrast with the exploration of its species-wide diversity. This aspect remains overlooked although a large number of *S. cerevisiae* isolates have been sequenced in recent years (Strope et al. 2015; Gallone et al. 2016; Zhu et al. 2016; Peter et al. 2018). In the S288C reference genome, the Ty fraction is modest, around 3%, and in addition to the full-length elements, it includes a large fraction of solo-LTR resulting from the loss of the internal coding region by inter-LTR recombination. Ty elements have developed strong insertion preferences believed to generate neutral alleles (Lesage and Todeschini 2005; Bridier-Nahmias et al. 2015; Patterson et al. 2019). Previous studies have already shown the variability of the Ty contents, both
in terms of types and copy number (Carr et al. 2012; Bleykasten-Grosshans et al. 2013; Czaja et al. 2020). However, these studies focused on very limited sets of isolates, preventing a global view of the evolution of the TE repertoire.

The collection of 1,011 S. cerevisiae natural isolates that were recently sequenced is a valuable sample to study the TE content repertoires within the species (Peter et al. 2018). This population has a wide geographical distribution and their ecological origins are highly diverse. Its evolutionary history has delimited 26 different subpopulations as well as three groups of mosaic isolates characterized by admixture from different lineages. In addition, a few subpopulations are characterized by introgressed regions, which are the signatures of ancient hybridization events with the closest related species to S. cerevisiae, namely Saccharomyces paradoxus (Peter et al. 2018; D’Angiolo et al. 2020). These two species diverged from a common ancestor ~5 million years ago (Tirosh et al. 2009).

Here, we sought to survey the Ty content variation of S. cerevisiae at a species level. We first characterized all the Ty sequence variants present in the 1,011 S. cerevisiae genomes by identifying new and undescribed Ty sequence variants. We then explored the repertoire (i.e. the type and number of TEs) in each isolate and subpopulation. Our results clearly showed that the population structure could be defined by the variation of the Ty repertoires. In fact, each defined S. cerevisiae clade is characterized by its unique and specific Ty repertoire. In addition, our results highlight that introgression events had a significant impact on the appearance of new sequence variants and therefore on the variation of repertoires between different subpopulations. Finally, we also extended our study by a functional analysis of the permissive behavior with respect to the transpositional activity, which revealed a broad variability of it across genetic backgrounds.
Results

Species-wide overview of the full-length Ty sequence variants in the *S. cerevisiae* species

To explore the Ty diversity within *S. cerevisiae*, we have established an exhaustive catalog of the sequence variants of each Ty family (*Ty1* to *Ty5*) present in the 1,011 genomes with unprecedented high resolution. To this end, the first step was to determine a set of query sequences consisting of the internal gag-pol coding sequences to accurately capture full-length Ty diversity. We used one query for each of the five Ty families of the S288C reference genome (File S1) to perform a first BLASTn search (supplementary fig. S1 and see Materials and methods). The search was conducted on both *S. cerevisiae* and *S. paradoxus* genome assemblies to obtain the most representative set of query sequences and to further resolve potential inter-species Ty flows. We have sorted hundreds of sequences based on their similarity patterns (see Materials and methods) and obtained a final set of twelve Ty representative sequences distributed across the five families, allowing to cover *gag-pol* diversity across the whole *S. cerevisiae* species (File S2).

To detect already identified and even new chimeric TEs, we performed a competitive mapping of the sequencing reads of the 1,011 genomes on the twelve *gag-pol* representative sequences (supplementary fig. S1). This strategy allowed us to distinguish the different Ty sequence variants present in *S. cerevisiae*. We examined the coverage profiles along the 12 sequences for each isolate (supplementary fig. S1 and see Methods) and we defined a precise catalog of Ty sequence variants in *S. cerevisiae*. Out of the twelve queries, two of the *S. paradoxus* queries did not reveal significant coverage (*Ty3p* and *Ty56p*). Partial or complete coverage of the ten other queries resulted in the definition of a total of thirteen sequence variants (fig. 1A). Our results clearly illustrate a varying degree of diversification among the five Ty families of *S. cerevisiae* and most families do not have a large set of sequence variants. The *Ty2* and *Ty3* families are only represented by a single sequence variant (fig. 1A). The *Ty4* family consists of two sequence variants: one specific to *S. cerevisiae* (*Ty4c*) and another one coming from its sister species *S. paradoxus* (*Ty4p*). *Ty4p* is similar to *Tsu4* that likely came via horizontal transfer from *Saccharomyces uvarum* to *S. paradoxus* (Bergman 2018). The *Ty5* family is also composed of two sequence variants: a full-length element (*Ty5f*) is present in a limited number of isolates (n=78) while a majority of isolates carry a truncated version with a 1500 bp internal deletion (*Ty5Δ*).

By contrast, the *Ty1* family has a complex composition due to a high number of sequence variants (fig. 1A). Few of these sequence variants have already been described (Jordan and McDonald 1998; Kim et al. 1998; Bleykasten-Groshans et al. 2013; Czaja et al. 2020) and our results provide an exhaustive catalog as well as a detailed view of their mosaic structure. The variants can be organized in three different subfamilies according to the origin of the *gag* coding sequence. The divergence of the *gag* gene reaches 8 to 15% between subfamilies, with the first subfamily (*Ty1*) having a *gag* sequence...
specific to the *S. cerevisiae* species whereas the two other subfamilies (*Ty1* and *Ty101*) exhibit distinct *gag* sequences that are similar to *S. paradoxus* elements. In the first subfamily (*Ty1’*), there is only one sequence variant, which is composed of *gag* and *pol* sequences both specific to the *S. cerevisiae* species. By contrast, the variants present in the second subfamily (*Ty1*) exhibit a *gag* coding segment similar to *Ty1p* from the Eurasian *S. paradoxus* subpopulation, while its *pol* coding segment corresponds to a *S. cerevisiae* sequence (Czaja et al. 2020, fig. 1A and supplementary fig. S3B). The variants of this subfamily differ by the presence (*Ty1/2* and *Ty1c2*) or not (*Ty1c1*) of small *Ty2* segments (fig. 1A). Jordan and McDonald (1998) have already described the *Ty2 pol* segment present in the *Ty1/2* element, and here we have identified the *Ty1c2* *gag* version, characterized by the presence of a *Ty2* segment of 20 bp in the *gag* coding sequence (supplementary fig. S3A). The sequence divergence between *Ty1c1* and *Ty1c2* reaches 28% in this segment and results in important changes on five out of six consecutive amino-acids in the corresponding proteins (supplementary fig. S3C). In the S288C reference genome, 24 out of the annotated *Ty1* elements have the *Ty1c2* *gag* version while the remaining six elements have the *Ty1c1* *gag* version (supplementary fig. S2B). Finally, in the last and third subfamily (*Ty101*), we identified an additional interspecies transfer involving the American *S. paradoxus* lineage. This latest subfamily consists of Ty elements sharing an American *S. paradoxus* *gag* sequence similar to the *Ty101p* element (fig. 1A). The *Ty101c* and *Ty101m* variants differ from *Ty101p* in that they have a mosaic *pol* sequence involving *S. cerevisiae* *Ty1’ pol* segments of different lengths (fig. 1A and supplementary fig. S3B). The *Ty101m* variant is a newly described element. We extracted the complete sequence of this *Ty101m* elements present in three isolates for which de novo assemblies were generated using a long-read sequencing strategy (HE015, CBS7962 and RP11.4.11) (Istace et al. 2017) and we were therefore able to confirm the mosaic structure. These new sequence variants are inserted in solo-LTR rich regions commonly located near tRNA genes, indicating a comparable integration site preference than the other *Ty1* sequence variants.

Population-scale Ty content highlights prevalence of the *Ty2* family

A total of 48,765 full-length Ty elements were detected across the 1,011 genomes. The number of Ty elements per genome is highly variable with an average of about 24 Ty elements per isolate and ranging from none to 100 elements in the PW5 and SJ5L12 isolates, respectively (File S3). The Ty families were found to be very unequal in terms of the number of elements detected (fig. 1B). As previously shown in a small subset of strains (Kim et al. 1998; Gabriel et al. 2006; Carr et al. 2012; Bleykasten-Grosshans et al. 2013) *Ty1* and *Ty2* are the most represented families with a total of 17,127 (i.e. 35.1 %) and 24,517 elements (i.e. 50.3 %), respectively. By contrast, the *Ty3*, *Ty4* and *Ty5* families are in considerably lower abundance with approximately 2,000 to 2,500 elements in each family. The distribution among isolates indicates that *Ty3* and *Ty4* are absent in a large proportion of them (absent in 511 and 593 isolates, respectively) while *Ty5* is more widely distributed (absent in 209 isolates) (File S4). *Ty3*, *Ty4* and *Ty5*
solo-LTR were detected in almost all these isolates (not shown), suggesting that their transpositional activity did not balance Ty loss by inter-LTR recombination.

As mentioned previously, the Ty1 family is characterized by a large number of sequence variants and we observed a high variability regarding their distribution. Two sequence variants from distinct subfamilies are prevalent: the Ty1' and Ty1c2 elements. While the Ty1' variant, specific to S. cerevisiae, is present in a large number of isolates (n=887) with a low copy number, the Ty1c2 variant is the most abundant element but limited to certain isolates (n=459) (File S4). The other Ty1 sequence variants all show a restricted distribution in a very small subset of isolates, as well as a high degree of variability in terms of copy number. As an example, the Ty101c, Ty101p, Ty1c1, Ty1/2 and Ty101m elements are present in 3 to 82 isolates with an average number of one (Ty101c) to 20.4 (Ty1c1) elements per genome.

As already mentioned, the Ty2 element appears to be the predominant family in the S. cerevisiae species. Only 55 out of the 1,011 strains do not have full-length Ty2 elements, indicating its wide distribution throughout the species. In addition, this element is also prevalent in terms of copy number with 12.7 elements per haploid genome on average (File S4). It is also present in the most divergent Asian S. cerevisiae isolates and, taken together, it can be considered as the ubiquitous element of the species. Regarding the origin of Ty2, it was reported that a very similar sequence variant is present in some Saccharomyces mikatae strains but absent in the closely related S. paradoxus species. This observation raised the hypothesis of a horizontal transfer of Ty2 between S. cerevisiae and S. mikatae without addressing the direction of the transfer (Liti et al. 2005). Because Ty2 is widespread and has a homogeneous structure, Ty2 seems to be a recent TE element with a very active transposition in the S. cerevisiae species. The Ty2 insertion polymorphism studied in isolates of various origins provides additional support for this hypothesis (Bleykasten-Grosshans et al. 2013). Nevertheless, its prevalence as well as its presence in Asian isolates strongly suggest that the Ty2 family predates the diversification of the species (Bendixsen et al. 2021).

The Ty repertoires reveals the S. cerevisiae population structure

To have a global overview of the evolution of the Ty contents at a species-wide level, we sought to explore the conservation and variation of the Ty repertoires (i.e. the type and number of TEs) across the different subpopulations, which were defined based on the 1,011 sequenced genomes (Peter et al. 2018). By clustering the strains based on the genetic diversity, we found that most of the subpopulations are defined by a unique and specific Ty repertoire (fig. 2). The generated heatmap highlights a variation of the transposon content at two levels: (i) the presence/absence of given Ty sequence variants or elements as well as (ii) the variation in terms of copy number across the different subpopulations (fig. 2). In fact, isolates from the same subpopulation exhibit a very similar and specific repertoire, characterized by a
precise type and number of TEs. These clear Ty patterns could have only been highlighted via our previous and precise characterization of the Ty elements in the large sample.

Our analysis shows that some Ty sequence variants are only present in specific subpopulations, which is for example the case for the sequence variants of the Ty1 and Ty101 subfamilies (fig. 2). With the exception of the Ty1’ and Ty1c2 variants, the other Ty1 sequence variants are restricted to a few subpopulations and we will detail this aspect later. Another example corresponds to the Ty5f variant, which is specific to the subclade 4 of the European wine as well as to the African palm wine subpopulations. Beside the clade specific TE sequence variants, some subpopulations are also characterized by the absence of pervasive elements. As an example, the Ty2 element is absent in the French Guiana and African beer subpopulations. By contrast, the isolates from the sake clade as well as the M1 and M2 mosaic subpopulations show a very high number of Ty2 copies. We can also notice that the TE repertoires are characterized by specific patterns of different TE families. The most obvious example of pattern corresponds to the repertoire of the French Guiana isolates. Their Ty content is defined by the presence of Ty101p and Ty101m variants, whereas the other Ty1 variants and Ty families are completely absent. Overall, our observations emphasize the fact that the TE content is primarily shaped at the subpopulation level in S. cerevisiae.

Finally, the generated heatmap also highlights an interesting aspect of the evolution of the Ty1 family (fig. 2). As mentioned previously, Ty1’ is the S. cerevisiae specific and prevalent element of the Ty1 family. Ty1’ segments are almost ubiquitous across the 1,011 isolates as they are present in the mosaic Ty1 variants, such as the variants from the Ty1 and Ty101 subfamilies. Nevertheless, we observed an enrichment of the full-length Ty1’ variant in some specific subpopulations and more precisely in the Asian isolates (supplementary fig. S5). This is an interesting observation in respect to the single ‘out of China’ origin of the S. cerevisiae species (Peter et al. 2018). The geographic distribution as well as its omnipresence provide a strong support to the fact that Ty1’ is the ancestral representative element of the Ty1 family, as recently hypothesized (Czaja et al. 2020).

**Contrasting outcomes of independent interspecies hybridization events on the Ty contents**

Our population genomic study focusing on the 1,011 S. cerevisiae genomes highlighted the presence of introgressed ORFs (Open Reading Frames) coming from the closely related species, S. paradoxus (Peter et al. 2018). In fact, all studied isolates carry at least one introgressed ORF, with a mean of 32, indicating a ubiquitous gene flow between these yeast species. In this context, it was interesting to find a large number of TE sequence variants of the Ty1 family, being chimeric elements between S. cerevisiae and S. paradoxus. As previously described, the Ty1 diversity was shaped by introgression events with Eurasian and American S. paradoxus lineages, leading to the Ty1 and Ty101 subfamilies, respectively (fig. 2). These introgression events correspond to secondary contacts with S. paradoxus and occurred
after the out-of-China dispersal (Peter et al. 2018). They consequently had a major impact on the genome evolution in terms of gene content (introgressed ORFs) but also clearly shaped the Ty repertoire at the subpopulation level (fig. 2). Whereas the Ty1’ variant is probably the ancestral element of this subfamily, the Ty1 and Ty101 variants are the result of Ty flow between these sister species.

Regarding the introgression events involving the American S. paradoxus lineage, the Mexican agave and French Guiana subpopulations were found to have a large number of introgressed ORFs (n=207 and n=86 on average, respectively). These two subpopulations were impacted by this major event as they retained copies of the S. paradoxus Ty1 element (Ty101p) and contain mosaic elements (Ty101m) (fig. 3). Whereas the two subpopulations probably lost the Ty1’ element, only the Mexican agave subpopulation kept the Ty2 element, leading to a slightly different repertoire. Finally, we had the opportunity to determine the genomic location of the Ty101m elements in three isolates (HE015, CBS7962 and RP11.4.11) for which the genomes were completely assembled using Oxford Nanopore sequencing (Istace et al. 2017). By comparing the location of the introgressed genes and the TE, it was interesting to observe that there is no overlap between the Ty101m elements and this set of genes. This observation provides an evidence of the existence of active Ty101m elements.

The introgression events involving the Eurasian S. paradoxus lineage led to a contrasting evolution of the Ty repertoires. The Alpechin subpopulation, with the highest number of introgressed ORFs (n=287 on average), exhibit a Ty landscape with almost no S. cerevisiae / S. paradoxus mosaic elements (fig. 3). Only five out the 17 isolates carry some Ty1c2 elements. We then analyzed the Ty content of the hybrid ancestor of the Alpechin subpopulation, which was recently described (D’Angiolo et al. 2020). By using our set of Ty queries, we detected six Ty1p elements in this genome. This observation indicates that the S. paradoxus Ty1p is present in this ecological niche and was present in the founder population of the current S. cerevisiae Alpechin subpopulation. It is therefore clear that neither this original element nor a mosaic version of it did play a major role in shaping the Ty landscape. By contrast, the French dairy clade with a small of introgressed ORFs (n=48 on average) is characterized by the presence of a high number of Ty1c1 elements. This sequence variant is almost exclusive to this subpopulation and is only found in a few isolates, such as the African beer strains.

Altogether, these results highlight the clade-specific and differential impacts of the introgression events on the Ty repertoire. They also reveal the origin of the broad diversity of Ty1 variants. With the exception of the Ty1c2 variant, the other sequence variants are mostly private or specific to few subpopulations. This high diversity of Ty1 variants stands in contrast with the low number of sequence variants present in the other subfamilies.

Variable transpositional activity across genetic backgrounds
Finally, we explored the correlation between the transpositional activity and the Ty contents for both the haploid and the natural isolates. Our results show an absence of correlation between the Ty activity...
and the number of copies of the main families or subfamilies (Ty1, Ty1' and Ty2) in both sets of strains (supplementary fig. S8). This observation is somewhat at odds with previous studies showing that the Ty1 activity is decreased when additional copies of the Ty1 element are present in the genome, a mechanism termed Copy Number Control (CNC) (Saha et al. 2015; Tucker et al. 2015; Garfinkel et al. 2016; Błaszczyk et al. 2017; Czaja et al. 2020). If this mechanism would have been a predominant mechanism acting on pervasive transposition at a population-scale, we would expect to observe an anti-correlation between the Ty content and activity for such a large sample. CNC has been studied in a very limited number of isolates and this mechanism might be genetic background specific. In addition, we cannot exclude that CNC may be a preponderant response of the host during a burst of transposition but that additional regulation mechanisms might act in isolates already carrying Ty1 elements as well as various compositions in Ty1 sequence variants.

Discussion

To investigate the composition of the repetitive genomic fraction represented by the full-length Ty retrotransposons, we developed a strategy to dissect this aspect across the whole genome sequences of 1,011 S. cerevisiae isolates. We first determined the catalog of Ty sequence variants present in this species with an unprecedented level of precision. Among the five Ty families (Ty1 to Ty 5), we have shown that the Ty1 family is the more diversified one as three subfamilies were identified, each including one to four sequence variants. Our analyses therefore broaden our knowledge of the diversity of Ty1 sequences, which will constitute a useful reference in the in-depth understanding of the control of TE transposition, a subject of great importance in the understanding of the TE-host relationship. Additionally, the combination of these potential sequence variant effects with the yet to be identified background host effects could explain why we found such a wide range of transposition activity across the large sample of isolates we tested.

Our results highlighted that the Ty landscape retraces the population structure with each subpopulation having a unique and specific TE repertoire. The distribution pattern of the Ty families and subfamilies in the entire species makes it possible to distinguish between the widespread TEs which originate from the ancestral Ty repertoire of S. cerevisiae and the TEs which appeared more recently during the diversification of the species. Whereas the Ty1' subfamily most likely arise from the ancestral Ty1 representative, the Ty1 and Ty1O1 subfamilies are the results of subpopulation events, namely hybridizations with distinct lineages of the S. paradoxus species.

The diversification of the panoply of Ty elements in S. cerevisiae comes from the generation of mosaic elements that combine segments originating from several species-specific TEs. The process that can lead to such mosaic elements is known as inter-element recombination and it was already proposed to explain the existence of the Ty1/2 hybrids (Jordan and McDonald 1998). While ectopic homologous
recombination between Ty copies can occur (Roeder and Fink 1980; Rachidi et al. 1999; Hou et al. 2014), these mosaic elements can also be the result of the reverse-transcriptase jumping between distinct RNA templates that are encapsidated in the same virus-like particle, like it was described for recombinant retroviruses (Hu and Temin 1990; Negroni et al. 1995; Anderson et al. 1998). The latest mechanism was already shown to be highly efficient in the generation of Ty1 sequence diversity in the S288C reference strain (Bleykasten-Grosshans et al. 2011).

In fact, Ty1 is the only family presenting mosaic S. cerevisiae / S. paradoxus variants. By contrast, the most preponderant element in S. cerevisiae, Ty2, is not present in S. paradoxus and therefore does not present such a mode of diversification. The apparent homogeneity of the Ty2 structure across the population calls for additional analyzes to assess the origin and age of this element. It would eventually address the already long-term issue of the direction of the transfer between S. cerevisiae and S. mikatae (Liti et al. 2005; Carr et al. 2012). To perform analyses that discriminate between vertical and horizontal transposon transfer like the VHICA method for example (Wallau et al. 2016), additional data on the Ty2 sequences of S. mikatae would be necessary.

Overall, it is of particular interest to see the rise of new Ty repertoires generated by the successful interspecific hybridization of distant genomes and associated Ty elements. By contrast to the case of hybrid dysgenesis syndrome in some Drosophila species (Mérel et al. 2020), it is not possible to predict if these TEs will lead or not to a genomic shock (McClintock 1984) and detrimental TE proliferation that can ultimately result in reproductive isolation. Furthermore, there is growing evidence that hybridization events and their associated TE contents is not obligatory subjected to massive TE deregulation. Such outcomes were described in yeast species (Hénault et al. 2020; Smukowski Heil et al. 2021), as well as in other groups including plants (Kawakami et al. 2011; Heyduk et al. 2021) and insects (Coyne 1989; Vela et al. 2014). Our results show that these hybridization events can lead to different and unpredictable evolutionary trajectories as well as Ty repertoires. Interestingly, hybridization events can lead to new TE sequence variants and this reveals some possible long-term impact of natural hybridization on the TE landscape. By analogy with the transgressive phenotypic traits that arise through hybridization (Gabaldón 2020a; Gabaldón 2020b), these new TE repertoires can therefore being shaped during the so-called reticulated evolution. Exploration of species that result from hybridization events, exhibit admixed populations or contain recursive hybrids (Pryszcz et al. 2015) will therefore be insightful to dissect the formation, the evolution and the impact of these newly emerging TE repertoires.
Materials and methods

Yeast isolates

The 1,011 isolates investigated for Ty content were described in (Peter et al. 2018) and are provided in File S3. We used the raw Illumina reads generated in this project to perform our analyses. Reads are 102 bp long and the coverage information is in File S3. The strains and isolates used for the transposition assays are listed in File S5.

Set-up of the gag-pol query collection to detect full-length Ty elements

For a single representative of each of the Ty1 to Ty4 families from the S. cerevisiae S288C reference genome, we used the internal gag-pol segment (without the 5’ and 3’ LTR segments), as query sequence (File S1). For the Ty5 family, as the single Ty5 copy in S288C carries a 1500 bp long internal deletion, we used the gag-pol segment sequence of the full-length Ty5 from the YJM178 strain (Strope et al. 2015). In order to take into account of the maximum of variants already present in the sequenced and assembled S. cerevisiae and S. paradoxus isolates, BLASTn searches were conducted on the NCBI non-redundant nucleotide database, using the blastn option with default parameters. Target sequences with complete coverage along each query were sampled and aligned (Clustal Omega, https://www.ebi.ac.uk/), exact duplicates were excluded. Among sequences sharing more than 95 % identity, a single representative was retained. Additional manual inspections of the alignments were performed, leaving a final set of twelve sequences that we considered representative of the Ty diversity in both S. cerevisiae and S. paradoxus species (File S2). This set of gag-pol sequences was used as reference to detect potentially full-length Ty elements in the 1,011 genomes.

Detection of sequence reads mapping on Ty segments

Read mapping was performed with bwa (Li and Durbin 2009) against a reference consisting in the above described set of twelve gag-pol segment sequences. Samtools mpileup (Li et al. 2009) was used to generate pileup files that were processed to estimate the read depth along the reference sequences (File S2) using 10 bp non-overlapping windows.

Assignment to Ty families or Ty sequence variants and estimation of the corresponding copy number

Coverage profiles were obtained by plotting the reads depth along the corresponding gag-pol Ty query (examples in supplementary fig. S2). The coverage plots were manually inspected to precisely define
the structure of the mosaic Ty sequence variants (Figure 1 and supplementary Fig. S3). This allows us to (i) delimit the appropriate segments for accurately assigning the detected elements to a Ty category (family, subfamily or sequence variant), and (ii) set up the most appropriate computing scheme to assess its copy number. The corresponding Ty copy number per haploid-equivalent genome was estimated by determining the ratio between the average read depth over the defined region and the total depth of the nuclear genome (File S3). Determination of the number of copies per haploid-equivalent genome allows further comparisons between isolates of varying ploidy levels (File S3). Finally, the total number of elements by type and isolate was obtained by multiplying the number of copies by the ploidy of the isolates.

A summary of the defined regions used to assess the copy number is provided hereafter.

- **Ty1’**: region from 100 to 1,000 of the Ty1’ query, that corresponds to the gag segment specific to this subfamily;

- **Ty1c2**: region from 100 to 1,000 of the Ty1c2 query, that corresponds to the gag segment specific to this subfamily;

- **Ty2**: region from 100 to 4,500 of the Ty2 query;

- **Ty3c/p and Ty4c/p**: the whole respective query;

- **Ty5f**: region from 1,300-3,400 of the Ty5 query. Ty5Δ was obtained by the difference of coverage between the 3,450-4,800 and the 1,300-3,400 segments on the Ty5 query.

The Ty1/2 elements deserved specific attention. Indeed, they were characterized by an excess of coverage at the 4,900-5,100 region of the Ty2 query, balanced by a drop in the coverage of the 4,900-5,300 region of the Ty1c2 query (supplementary fig. S2 A, strain S288C). When Ty1/2 is the sole representative of the Ty1 subfamily (i.e. the coverage on the Ty1c2 4,900-5,300 segment resulted in a value less than 0.5), we inferred the value computed on the 100-1,100 segment of the Ty1c2 query as the actual Ty1/2 copy number. When Ty1c2 (and/or Ty1c1) and Ty1/2 are altogether present, the Ty1/2 copy number was assessed by the difference of coverage between the Ty2 4,900-5,100 and 100-4,500 segments. The corresponding value was then subtracted to the value computed on the 100-1,100 segment of the Ty1c2 query, in order to adjust the Ty1c2 copy number.

Among the Ty1 family, the following sequence variants were carefully considered:

- **Ty1c1** differs from Ty1c2 by a very short segment in the gag region. Ty1c1 was revealed by an excess of coverage of this short segment on the Ty1p query visualized during manual inspection of the coverage profiles (supplementary fig. S2 A, strain S288C). We could only assess its copy number when Ty1c1 is the only representative of the Ty1 subfamily within a strain. Its copy number was inferred
from the coverage of the *gag* segment (100-1,100 segment) of the *Ty1c2* query. Otherwise, they are in a minority and were identified by default as *Ty1c2* elements;

- *Ty101m* variant was revealed by balanced coverage and absence of coverage of adjacent segments of the *Ty101c, Ty101p* and *Ty1* reference sequences (supplementary fig. S2 A, strain HE015). Its copy number was computed from the coverage along the *Ty101c* 500-1,000 segment;

- *Ty101c* and *Ty101p* variants were detected during manual inspection of the coverage plots and their copy number were computed from the coverage along the 500-1,000 segment of the corresponding query.

**LTR detection**

We aimed to detect the presence/absence of the Ty LTR, in order to identify isolates where solo-LTRs are the sole representatives of a given a Ty family. We performed the read mapping against a set of queries including only LTR segments: three LTR sequences that are representative of the very similar *Ty1* and *Ty2* LTRs and a single LTR sequence for each of the *Ty3* to *Ty5* families (File S7).

**Yeast methods**

Yeast cells were grown on YPD (yeast extract 1%, peptone 2%, dextrose 2 %) at 30°C. When required, the temperature was adjusted to 34°C or to 22°C which are respectively restrictive or permissive to *Ty1* transposition (Paquin and Williamson 1986; Lawler et al. 2002). Geneticin (Euromedex) to a final concentration of 200 µg/ml was added to obtain the selective YPD-G418 media. The solid selective media YPD-hygro was supplemented with hygromycin (Euromedex) to a final concentration of 200 µg/ml and with 2% agar (Bacto agar Difco). Composition of the nitrogen-depleted media was as follows: 0.67% Yeast Nitrogen Base without amino acids and ammonium sulfate (MP Biomedicals), 2% D-glucose, 0.05 mM ammonium sulfate. Yeast strains were transformed by the *Ty1hygro-AI* plasmid with the EZ-Yeast Transformation Kit (MP Biomedicals).

**Ty1hygro-AI** plasmid construction and validation

The pCeTyX *Ty1hygro-AI* carrying plasmid was constructed by assembling four overlapping DNA fragments thanks to the Gibson Assembly Cloning Kit (NEB). The 5,472 bp plasmid backbone was obtained after restriction of p41Neo 1-F GW (https://www.addgene.org/58545/) by *XhoI* and *XbaI*. We designed and purchased the *hygro-AI* cassette at Genescript and further amplified it by PCR using Iproof high fidelity DNA polymerase (BioRad). The 5'-LTR-*gagpol* and the 3'-LTR segments were obtained by PCR amplification from the pOY1 template (Lee et al. 1998). The sequence of the final construction
was verified by Sanger sequencing (Eurofins). Details on pCeTyX cloning and primer sequences are available in File S6.

We first validated the new Ty1hygro-AI reporter by testing its activity in different conditions in the S288C genetic background. The results obtained with the Ty1hygro-AI reporter recapitulates the available results of Ty1his3-AI reporters, as illustrated in (supplementary fig. S6A). Transposition was observed to be higher in the haploid state (FY5) compared to the diploid state (FY3) (Morillon et al. 2000), optimally at 22°C. Further the transposition activity was undetectable at 34°C (Lawler et al. 2002). We also have recorded the transpositional activity of the Ty1hygro-AI reporter in the CLQCA_20-259 background (File S5), where no copia elements were detected. Importantly, this shows that the Ty1hygro-AI reporter is autonomous.

**Ty1hygro-AI mobility assay**

To reliably assess the Ty1hygro-AI transpositional activity in a large number of strains, we designed the following protocol. This protocol was carried out on three independent cultures for each of the tested strains. Cells of each of the cultures were transformed with pCeTyX, and bulk G418 resistant transformants were selected in 1.8 ml YPD-G418, at 34°C (restrictive temperature for Ty1 transposition) during 48 h. Subsequently, 10 µl of culture were transferred to 150 µl fresh YPD-G418 and grown to saturation for 36 h at 34°C. To allow for Ty1 transposition, 100 µl of the saturated culture were inoculated in 1.8 ml YPD and incubated at the permissive temperature of 22°C for 24h. A 50 µl aliquot of the induced cultures was saved for further cell counting. Cell counting was performed by counting of the events with FCS ≥ 1.10^4 and SSC ≥ 5.10^5 by flow cytometry (BD-Accuri-C6). Variable fractions ranging from 0.1 to 95 % of the cells were harvested, plated on solid YPD-hygro, and incubated for 48 to 72 h at 34°C. Hygromycin-resistant colonies were counted. The transposition activity was represented as the ratio of the hygromycin-resistant colonies to the total cells. Depending on the efficiency of the transformation step, this protocol ensures that at least three independent transformants were assessed for each of the strains. In parallel, to control for the absence of preexisting transposition events that might have occurred prior to the induction step at 22°C, 20 µl of the aforementioned saturated cultures at 34°C were deposited on solid YPD-hygro and incubated during 48 h at 34°C; if hygromycin-resistant colonies grew on this control, the corresponding assay was discarded.

Some genetic backgrounds display very low ratio of hygromycin-resistant colonies in the standard assay conditions. In order to rule out issues with the assay, two additional controls were performed. In the first control, we subjected the corresponding strains to conditions of nitrogen starvation during the step of Ty1 induction. Nitrogen starvation is known to stimulate the Ty1 transposition (supplementary fig. S6A and Morillon et al. 2000) and all the tested strains show substantial increases of the ratio in hygromycin-resistant colonies in this condition (examples in supplementary fig. S6B). This indicates
that the Tyhygro-AI reporter is actually functional even for the strains that display low transpositional activity very close to the detection threshold in the usual assay conditions. In the second control, we checked if pCeTyX may be lost during the step of Ty1 induction. This control was performed in several strains with variable transpositional activity. The results indicate that at least 40% of the cells retained pCeTyX after 24 h of growth at 22°C in non-selective media. The tested strains display variable rates of pCeTyX plasmid loss, however the strains with the lowest activity have the highest rates of plasmid retention and vice-versa (supplementary fig. S6C).

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Figure legends

**Figure 1.** Mosaic structure and species wide prevalence of the Ty sequence variants in *S. cerevisiae.*

(A) The Ty sequence variants are listed according to their classification in families and subfamilies. The color or shaded color of the *gag* and *pol* coding regions (boxes) and LTRs (triangles) reflect segment identity or high similarity. Dotted lines underline the Ty segments highly similar to *S. paradoxus* Ty elements. The divergence values of *gag* and *pol* sequences correspond to the nucleotide sequence divergence between the *gag* and *pol* sequences of the Ty1', Ty1p and Ty101p elements. The star highlights the newly described Ty101m variant. The Ty elements are not represented to scale. (B) The size of the bars represents the total number of elements per Ty family (transparent color) and per Ty sequence variant (solid color) detected among the 1,011 genomes. The exact number of copies is indicated for each Ty family (colored numbers) and for each sequence variant, if different from the family copy number (black numbers).

**Figure 2.** Correspondence between the Ty contents and the isolate phylogenetic relationships. The main heatmap represents the haploid-equivalent copy number of the Ty sequence variants for each of the 1,011 studied isolates, by using white (no Ty detected), light blue (low Ty copy number < 3) and shades of orange color. The related clades are represented (left panel) by alternating of blue and gray colors. Subpopulations are labeled darker and black is associated to strains with no clade. The yellow bars mark the isolates of Asian geographic origin. The violet shaded bars indicate the content in *S. paradoxus* ORFs ranging from 33 (light violet) to 319 (dark violet).

**Figure 3.** Outcomes of ancestral hybridization in terms of Ty1 and introgressed ORF contents in four subpopulations. In the bar-plot, the upper bars represent the mean number of the Ty1 variants. The lower bars represent the number of introgressed *S. paradoxus* ORFs. The number of isolates in the subpopulations is indicated in brackets.

**Figure 4.** Variation of the transpositional activity of a set of natural isolates and haploid strains. For each strain, the value of the transpositional activity is the mean of three independent replicates of the ratio between the hygromycin-resistant colonies and the total cells (see Materials and Methods). Panel (A) compares the boxplot representations of the distributions for the two sets of strains. Panel (B) shows the distribution of the values (central panel) and the corresponding probability density functions.

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Fig 1. Structure and species wide prevalence of the Ty families, subfamilies and sequence variants in S. cerevisiae.

Family        Subfamily   Sequence  Variant
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Ty1'           Ty1'        GAG       POL
Ty1p           Ty1p       GAG       POL
Ty1c1          Ty1c1      GAG       POL
Ty1c2          Ty1c2      GAG       POL
Ty1             Ty1        GAG       POL
Ty101p         Ty101p     GAG       POL
Ty101c          Ty101c    GAG       POL
Ty101m          Ty101m   GAG       POL

Ty2             Ty2        GAG       POL
Ty3             Ty3        GAG       POL
Ty4             Ty4        GAG       POL
Ty4c           Ty4c        GAG       POL
Ty4p           Ty4p        GAG       POL
Ty5             Ty5        GAG       POL
Ty5'           Ty5'        GAG       POL

% divergence: gag / pol

Subfamily  
---        
Ty1p Eurasian 14 / 11.5
Ty101p American 15 / 12
Ty101 8 / 6

Fig 2. Correspondence between the Ty contents and the isolate phylogenetic relationships.
Fig 3: Outcomes of ancestral hybridization in terms of Ty1 and introgressed ORF contents in four subpopulations.

Fig 4. Comparison of the species wide variation in the Ty1hygro-AI transpositional activity between the sets of natural isolates and haploid strains.

A.

B.