Role of social wasps in *Saccharomyces cerevisiae* ecology and evolution

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S*accharomyces cerevisiae* is one of the most important model organisms and has been a valuable asset to human civilization. However, despite its extensive use in the last 9,000 y, the existence of a seasonal cycle outside human-made environments has not yet been described. We demonstrate the role of social wasps as vector and natural reservoir of *S. cerevisiae* during all seasons. We provide experimental evidence that queens of social wasps overwintering as adults (*Vespa crabro* and *Polistes* spp.) can harbor yeast cells from autumn to spring and transmit them to their progeny. This result is mirrored by field surveys of the genetic variability of natural strains of yeast. Microsatellites and sequences of a selected set of loci able to recapitulate the yeast strain's evolutionary history were used to compare 17 environmental wasp isolates with a collection of strains from grapes from the same region and more than 230 strains representing worldwide yeast variation. The wasp isolates fall into subclusters representing the overall ecological and industrial yeast diversity of their geographic origin. Our findings indicate that wasps are a key environmental niche for the evolution of natural *S. cerevisiae* populations, the dispersion of yeast cells in the environment, and the maintenance of their diversity. The close relatedness of several wasp isolates with grape and wine isolates reflects the crucial role of human activities on yeast population structure, through clonal expansion and selection of specific strains during the biotransformation of fermented foods, followed by dispersal mediated by insects and other animals.

evolutionary biology | genomics

The yeast *Saccharomyces cerevisiae* is one of the microorganisms most appreciated by humans because of its utility in the production of food and drink. The discovery of ancient *S. cerevisiae* DNA in Chinese pots (7,000–5,500 BC) (1) and in jars of the King Scorpion tomb in Abydos (3,150 B.C.) (2) have allowed us to date the first observed wine fermentations back to proto-historic periods. Although we have thorough knowledge regarding the genetic, molecular, and phenotypic traits arising from the wide use of *S. cerevisiae*, its origin and evolution are still debated. Some scientists hypothesize that this organism evolved in human-associated environments, where selective pressure (such as high ethanol concentrations in must fermentation) allowed yeast speciation (3). The recent finding of *S. cerevisiae* DNA in Miocene and Oligocene ambers indicates that the budding yeast existed long before human advent (4). The discussion on domestication then moved from the species level to the strain level, with genetic evidence suggesting that strains used for fermentation have been selected and domesticated from wild strains, and then dispersed (5). However, it is still unclear how *S. cerevisiae* cells spread among different environments. Polsinelli et al. reported that, before maturation, grapes are almost free of *S. cerevisiae* (~0.05%), whereas 25% of ripe damaged grapes harbor such cells (6, 7). Following this pioneering study, *S. cerevisiae* strains have been isolated from several natural sources (7–22). However, these reports are restricted to warm seasons, when ripe fruit is available, or to post harvest. Even if a natural origin of *S. cerevisiae* is no longer under debate, the crucial question regarding its ecological sanctuaries in the absence of sugar-rich fruits and far from protected human environments remains to be explained. Answering this question might furnish fundamental information regarding its evolution. It must be noted that *S. cerevisiae* is not airborne, but requires a vector to move (23). Several studies show a flow of *S. cerevisiae* cells among wineries and natural environments (24), probably favored by animal vectors (25). In a recent paper, Francesca et al. suggest the role of migratory birds as vectors of *S. cerevisiae* cells (26). Nevertheless, no indications of the periods of yeast isolation were given. Moreover, these authors showed that yeast cells persisted in the bird's gut for no longer than 12 h, indicating that birds cannot act as environmental reservoirs for this microorganism. It has been reported that yeasts are associated with insects (23, 27–29) during grape harvest season. Stevic et al. (30) showed that bees and wasps act as carriers of yeasts in autumn and that honey bee hives contain yeasts during the winter. Unfortunately, in these reports no details were provided about the presence of *S. cerevisiae* within the identified yeast species. Social wasps are very promising as potential yeast vectors. Their colony cycle initiates in spring when new nests are founded by females emerging in the previous autumn that overwintered after being fertilized. These foundresses feed the larvae with regurgitated food (trophallaxis), allowing a possible transgenerational passage of yeasts, which could be continued by food exchange between overlapping generations of workers and future foundresses as well. Moreover, the number of adult wasps in a given locality presents a significant peak in concomitance with grape maturation, and wasps are well known to feed on this fruit (*SI Appendix*, Fig. S1). In particular, *Vespa crabro* wasps, which are common in Mediterranean and Southern European countries, have a buccal apparatus that allows them to break hard substrates, such as the skin of pristine grapes. Social wasps comprise a very large niche including most of the environments where yeasts can be found. Adults feed mainly on sugar sources, need wood fibers to construct their nests,

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and often avail themselves of human structures to find suitable nesting sites. They feed their larvae with insect prey. By living and foraging in miscellaneous environments, they could gather most of the existing yeast diversity.

We have addressed two questions in this study: (*i*) Do social wasps represent an ecological niche where *S. cerevisiae* cells occur during a complete annual cycle through transgenerational transfer? (*ii*) Do social wasps host a specific yeast microbiota or do they host and move strains from different natural and human environments that they exploit? We characterized yeast strains isolated from the guts of social wasps collected during spring, summer, and autumn. Particular attention was given to *S. cerevisiae*. We also experimentally assessed the capacity of the wasps to harbor yeasts in their gut from summer to the end of winter and to inoculate larvae.

Results

Wasp Gut Yeast Flora Composition. Yeast strains were isolated from grapes and insects collected in several Italian locations. Adult *Vespa crabro* and *Polistes* spp. wasps and *Apis mellifera* were captured and dissected in spring, summer, and autumn. A *Polistes* spp. nest was collected to examine the larvae. Seventeen *S. cerevisiae* strains were isolated from wasp guts, constituting 4% of the yeast gut community of these insects. No *S. cerevisiae* strains were found in honey bee guts, confirming previous findings (31, 32). We therefore focused further analyses only on social wasp fungal microbiota. A total of 393 yeast strains (Dataset S1) were isolated from the guts of 61 wasps collected in Tuscany, Garda lake, and Elba island regions. *Candida* spp. strains were the most represented (150 isolates), 43% of which were determined as *Candida apicola* (64 isolates). *Pichia* spp. isolates comprised 32% (127 strains) of the total. The frequency of several species changed according to the season. Whereas the *C. apicola* isolates doubled after grape maturation (from 18 to 46 isolates), the number of *S. cerevisiae* strains isolated from wasp guts showed a minimal change (from 7 to 10 isolates) (Fig. 1*A*). On the contrary, the number of *Pichia* spp. isolates dropped by almost half at the time of harvest (from 92 to 35 isolates). The strongest connection between the occurrence of a yeast species and the time of insect collection was observed for *Saccharomycodes* spp. strains, which were isolated mainly when grapes were ripe. These findings indicate that, whereas the amount of *Pichia* spp., *Saccharomycodes* spp. and *C. apicola* isolates shows a seasonal trend, *S. cerevisiae* strains are almost constantly present in the insect guts. The comparison of the number of accumulated isolates per yeast species allows monitoring of the amount of organisms that could be exchanged between insects and other natural sources. Nevertheless, a high number of conspecific strains isolated in a specific period does not necessarily mean that all insects bear more cells of this yeast species in their guts in this period. For each analyzed wasp, the presence/absence of each yeast species (irrespectively of the number of isolates) and the collection period (before/after grape maturation) were scored and used as factorial variables. Correspondence analysis for yeast communities found in individual insect guts revealed three dimensions with eigenvalues > 1 (Fig. 1*B* and *SI Appendix*, Fig S3) (explained variance, 25.6%, 21.3% and 16.6%, respectively). The first axis was associated with the period of collection and confirmed the prevalence of *Saccharomycodes* spp. and *C. apicola* before grape maturation, and of *Pichia* spp. after harvest. *S. cerevisiae* showed a very low absolute value in the first axis, thus confirming the lack of association with season (Fig. 1*B*). *S. cerevisiae* cells are present in insect guts all year round, regardless of the possibility of finding yeast cells in the environment (i.e., on grapes).

Phylogenetic Relationships of *S. cerevisiae* Wasp Isolates. The relatedness between *S. cerevisiae* strains has thus far been assessed mainly by means of highly polymorphic microsatellite loci (33), sequencing of selected genes (5, 34), or whole genome sequencing (35). We used both variability of microsatellites and of selected allele sequences to assess the genetic structure of our *S. cerevisiae* population compared with a collection of conspecific isolates representing the entire known yeast genetic variability. The clustering analysis, based on the microsatellite sequences at 12 loci of 256 investigated *S. cerevisiae* strains (*SI Appendix*, Table S4) showed that the 17 wasp strains were spread among

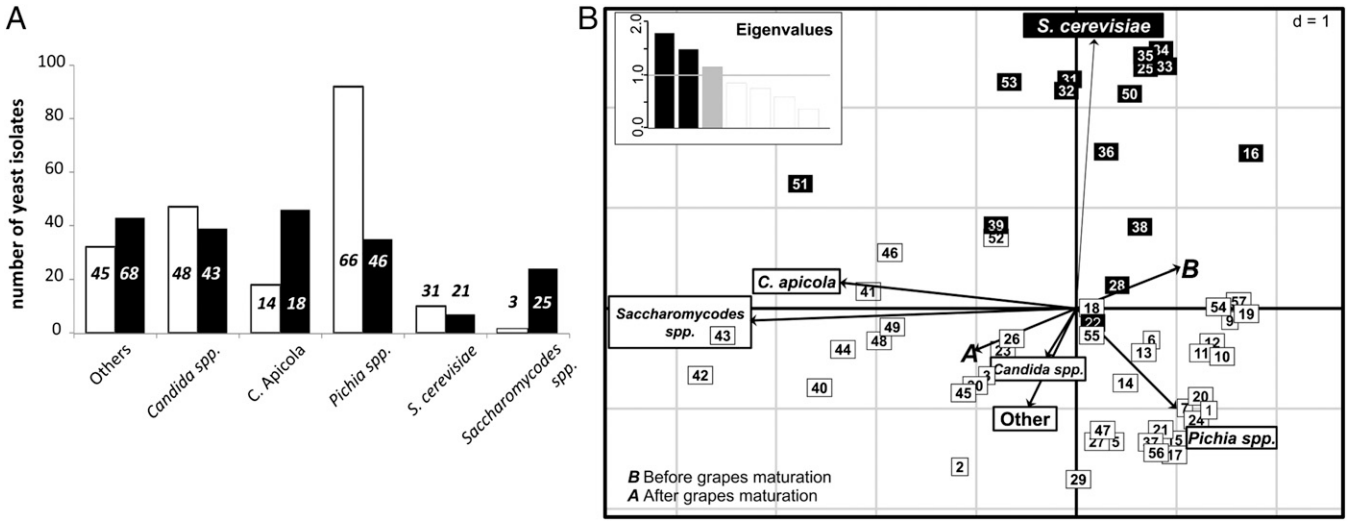

Fig. 1. Yeast flora in the collected wasps. (*A*) Distribution of yeast isolates ($n = 393$) from *Vespa crabro, Polistes spp,*. and *Apis mellifera* insects ($n = 61$); white bars indicate the number of isolates per species before grape maturation; black bars indicate the number of isolates per species after grape maturation; italic numbers: percentage of insects bearing at least one isolate per yeast species. (*B*) Duality diagram for the first two components obtained by correspondence analysis of seasonal profile of the yeast flora of *V. crabro* guts ($n = 57$). Cases for the analysis are the individual insects indicated by boxes numbered with their ID (Dataset S1). Variables are the period of collection (*A* and *B*) and the occurrence of the specific yeast species; rectangles bearing yeast species names represent the occurrence of yeast species. Black numbered boxes indicate insects bearing *S. cerevisiae* strains. The third axis having an eigenvalue > 1 is illustrated in *SI Appendix*, Fig. S2.

different clusters. Ten wasp isolates were related to wine strains, three to a group of strains found in bread, one to a mixed group encompassing African beer, palm wine isolates and laboratory strains, and one to a group containing natural isolates found in African palm wines. Some wasp strains (F31x, Buc1, E32, and NPSM) sampled in different periods of the year contained almost identical genotypes, forming a subgroup within a larger cluster containing many wine and grape strains from Tuscany, a starter strain (Lallemand 6009) and three Italian clinical isolates (YJM975, YJM978, and YJM981). The Sgv114 wasp isolate clustered in a subgroup composed of almost only sympatric strains, two from wine (SG10 and SG60) and three from grapes (SGU165, SGU89, SGU25) (Fig. 2, IV) isolated from the same vineyard in the same year. As expected, several strains isolated from insects collected the same day in the same location show a strong genetic similarity (BIBVC4.3 and BIBVC5.3, collected near Florence, and YVC1E2, YVC2E6, and YVC4EST1, isolated from insects caught on Elba island) (Fig. 2, II and V). At the same time, strain BIBVC1.1, collected before grape maturation, and its sympatric BIBVC strains, show high genetic divergence, being the first strain strictly similar to the YVCE4 strain. BIBVC1.1 and YVCE4 strains were isolated from insects caught before grape maturation in different sites distant ~40 km from each other. Similarly, the BIBVC4.3 and BIBVC5.3 strains are highly similar to the YVPC7.6 strain, isolated from different insect species (*V. crabro* the first two, *P. dominula* the third) in locations almost 20 km apart.

The structure of a population composed of subgroups can be described by identifying the most probable ancestor for each group, namely an individual from which all of the organisms in the subgroup are directly descended. To support the global structure observed from microsatellite typing, we used a Bayesian algorithm to infer the most probable partition of the 256 strains into 13 groups or ancestral lineages (Fig. 2*B*). Wine strains were placed into five main groups. Tuscan grape strains were inferred to descend from the same ancestors as the Tuscan wine strains and from a fourth European ancestor, not shared with the Tuscan wine group (bright blue in Fig. 2*B*). This result indicates the presence of a specific yeast "microbiota" in geographically and climatically different regions having a millenary culture of production of fermented food and drink. Strains isolated from humans and other animals did not have a specific ancestor, but rather the vast majority of ancestors belonging to different groups. Wasp isolates were placed into four main groups, two of which were shared with European wine isolates, one an ancestor of bread isolates and the fourth typical of laboratory strains. Laboratory strains have a natural origin (i.e., rotten figs, soil) whose ancestor is also shared with US oak, palm wine, and clinical strains.

In addition to microsatellite analysis, we assessed strain relatedness by using three gene sequences recently shown to recapitulate the genetic relatedness and population structure that could be obtained by means of entire genome sequencing (34). The cluster obtained describes an overall scenario similar to that observed with microsatellite analysis (Fig. 3). Indeed, the Mantel test performed on the distance matrices obtained by microsatellites and gene sequence analyses showed a highly significant correlation ($P < 0.001$). Nevertheless, many strain pairs showing high distances in microsatellites have low dissimilarity in the three genes, thus resulting in a relatively low Pearson R (0.340) (*SI Appendix*, Fig. S5). This is expected in the case of mosaic genomes. When repeating the clustering using strains of *S. paradoxus* (the closest known relative of *S. cerevisiae*) as root (*SI*

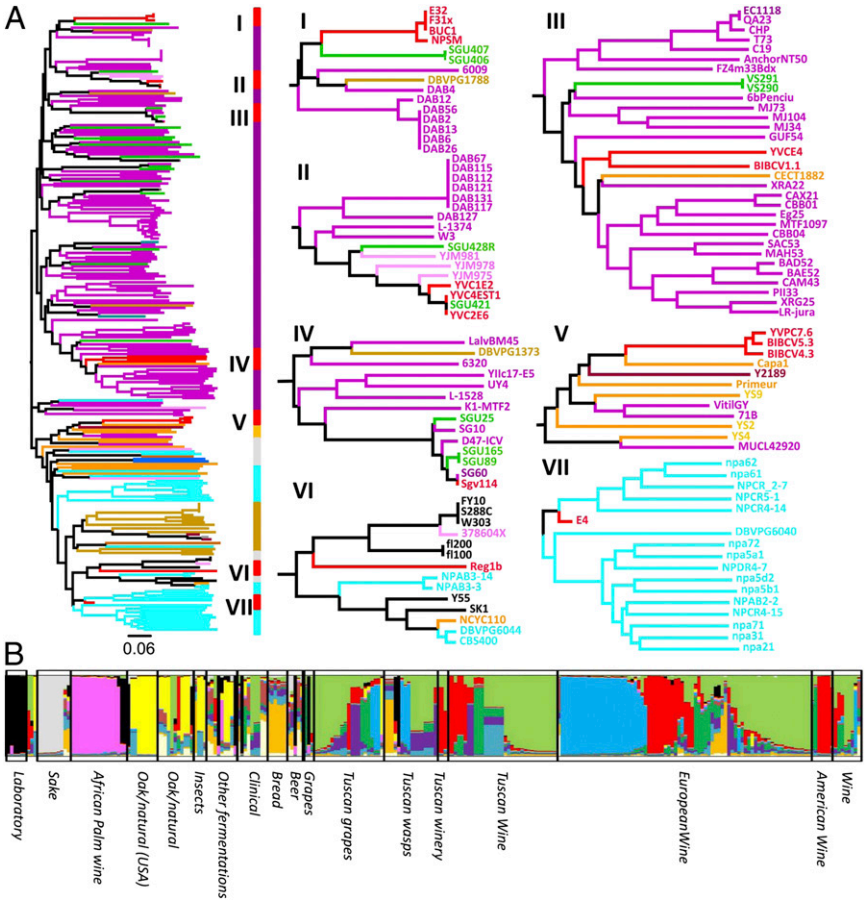

Fig. 2. Microsatellite analysis results. (*A*) Neighbor-joining tree showing the clustering of 17 *S. cerevisiae* wasp isolates among 256 yeast strains obtained from different sources. The tree was constructed from the Chord distance between strains based on the polymorphism at 12 loci and was rooted according to the midpoint method. Branches are colored according to the substrate from which strains have been isolated. Color code: red, insect isolates; purple, wine; green, grapes; orange, baked products and beer; pink, clinical; light blue, other fermentations; light brown, other natural sources. The position of wasp isolates within the tree is indicated by a red bar. I, II, III, IV, V, VI, and VII: detail of the subclusters encompassing wasp isolates. (*B*) Ancestry of the 256 *S. cerevisiae* strains analyzed by microsatellite analysis. The figure shows the proportion of each strain's ancestry in each cluster. This set of strains as a whole was inferred to fall into 13 clusters. Vertical lines are partitioned into 13 colored components (each representing one of the most probable inferred ancestors, or K clusters), which represent the individual's estimated membership coefficients in the K clusters. Ancestry was inferred by Instruct analysis (53) and drawn with Distruct (54).

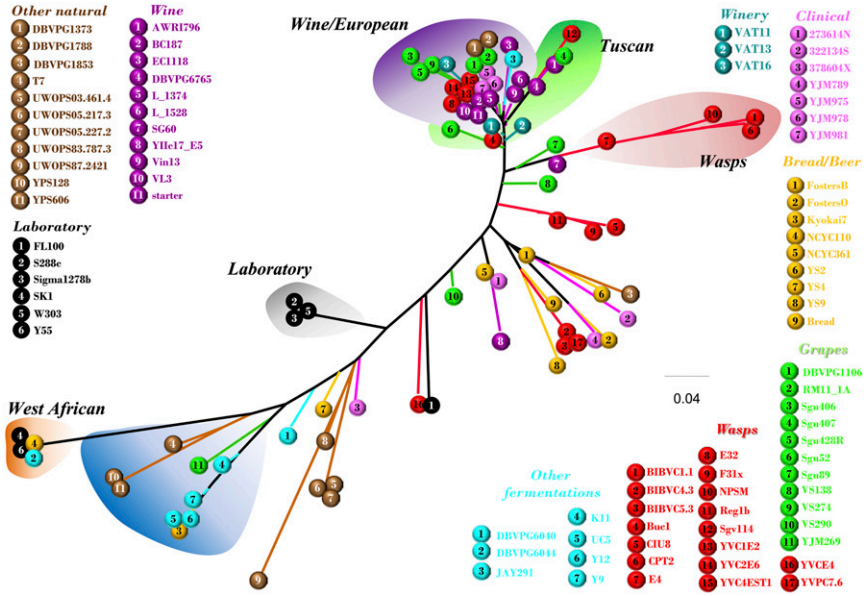

Fig. 3. Yeast strain cluster based on the SNPs differences of the genome-mimicking genes. Neighbor-joining tree based on SNP differences of the *EXO5*, *IRC8*, and *URN1* sequences of yeast strains. The strain membership of a specific cluster was assessed by inferring their most probable ancestor with the Bayesian algorithm implemented in Structure (48).

Appendix, Fig. S6), we were able to confirm two main clusters, one composed of yeast isolated by sampling of wine and other wild European sources, and a second cluster encompassing sake strains and isolates from non-European regions. The majority of the *S. cerevisiae* strains isolated from insect guts belong to the "core" of the first cluster, mainly composed of strains isolated from European grapes and wines (Fig. 3), in agreement with the result from microsatellite analysis. Three wasp strains, BIBVC4.3, BIBVC5.3, and YVPC7.6, cluster with the clinical isolates 322134S and YJM789, and strains isolated from fermentations of different types of cereals. The "starter" strain, used for wine fermentation, and the strains isolated from vats in the same winery, VAT11, VAT13, and VAT16, were genetically distant from the BIBVC wasp strains isolated from the nearby vineyards, indicating the role of wasps in disseminating an endemic Tuscan yeast microbiome. It appears that wasps have the potential to maintain and promote yeast biodiversity also by means of disseminating interspecific hybridization, as suggested by the clustering of the CPT2 strain, a wasp-derived *S. cerevisiae–S. paradoxus* hybrid, very near to the root of the tree (*SI Appendix*, Fig S6). The finding that CPT2 is a *S. cerevisiae–S. paradoxus* hybrid with a mosaic genome is supported by its *MET2* gene sequence, indicating two genomes, by the failure of the microsatellite analysis based on the 12 *S. cerevisiae*–specific loci, in agreement with previous observations (36), and by the lack of two of three genome-mimicking genes that are *S. cerevisiae* specific (37).

Altogether the performed genetic analyses show that the diversity of wasp isolates reflects the geographical variability shown by *S. cerevisiae* strains isolated from grapes, wines, bread and other environmental sources in the Mediterranean region to which we had access. Such a pattern could be the result of a process of clonal selection resulting from selective expansion of fitter strains emerging during the biotransformation of fermented foods, to be then moved by insects to occupy new environmental niches.

Experiments on Overwintering and Colony-Founding Wasps. To assess whether social wasps can harbor yeast cells during the whole wintering period and pass them to their offspring the next spring, we performed a controlled experiment, using *Polistes* wasps as a model. Preoverwintering females had been collected, fed 10^8 cells of labeled *S. cerevisiae* strain (BY4742-GFP/*FOX3*; *Methods* and Fig. 4*B*) and then allowed to hibernate in glass cages. After 3 mo, some wasps were dissected and the remaining were allowed to found new nests in the cages. We found that five of six of the wasps dissected just after the end of hibernation still bore yeast cells in their guts (11.8 colonies obtained ± 6.4 SD, $n = 5$ positive insects) (Fig. 4*A*). We also found BY4742-GFP/*FOX3* cells (3.5 colonies obtained ± 2.5 SD, $n = 16$ positive insects) in the larvae of the newly founded nests and in the workers emerging both in the nest and in sterile conditions.

Wasps feed their larvae through regurgitation of the content of a small part of their digestive tract, the crop. Thus, the observation that several larvae and newborn workers bear a mean of one-third of the number of BY4742-GFP/*FOX3* cells found in the digestive tracts of overwintering wasps provides strong evidence that naturally yeast-inoculated wasps can transfer the microorganism to their progenies. Overall, these findings confirm that yeast cells can be passed from one individual wasp to another in anticipation of the season in which they can then be transferred to fruit (Fig. 4*A*).

Discussion

It is well known that fermentation occurs in ripe grapes, even without artificial *S. cerevisiae* inoculation (natural fermentation). However, as pristine fruits do not harbor *S. cerevisiae* cells, it was not known how yeast cells are preserved during the winter or in the absence of fermentable sources in natural environments and then reach the ripe fruit in the following summer and autumn. The role of animals as vectors for *S. cerevisiae* has been frequently advocated because *S. cerevisiae* cells have been isolated from birds and insects (26, 30). However, their persistence in bird cloacae has been shown to be very short (26). Insects are also limited by their relatively brief adult lifespan (usually less than 1 y). Social wasps, on the other hand, represent a different scenario, because of their adult overwintering habitus and trophic contact among generations. Some species (*Saccharomycodes* spp. and *C. apicola*) have been isolated mainly from wasp guts after grape maturation, suggesting that the presence of these yeasts is an ephemeral condition linked to their explosion during the favorable season. *Saccharomycodes* spp. is strictly associated with fermentation (38, 39) rather, here it has been isolated in a wild environment. The frequency of *S. cerevisiae* in wasp guts is instead constant in different seasons suggesting an intimate and continuing relationship between these organisms. The continuous presence of *S. cerevisiae* in wasp guts is not sufficient evidence for assuming that the yeast can overwinter in these insects

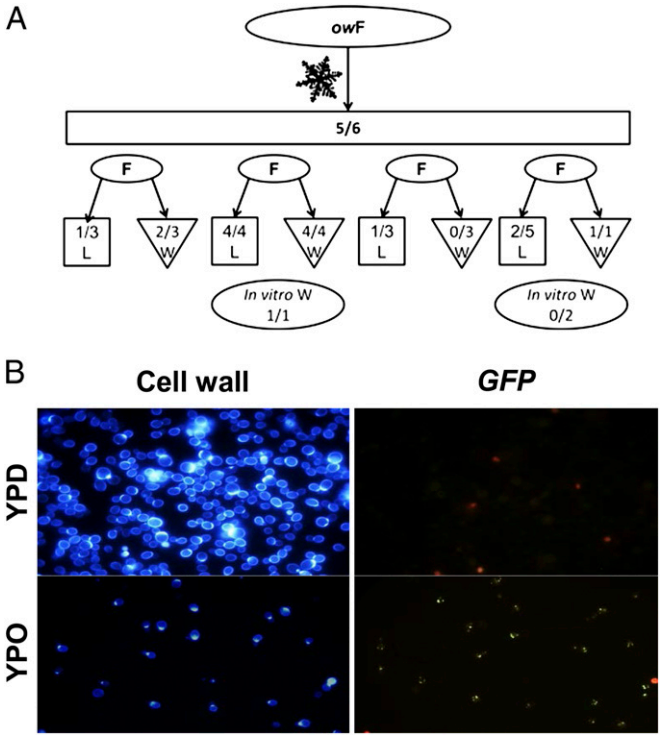

Fig. 4. *Polistes* spp. insects fed with *S. cerevisiae* cells can maintain the yeast in their gut during the winter and pass them to their progeny after nest foundation in the spring. (*A*) Five out of six overwintering wasps fed with the labeled yeast strain BY4742-GFP/*FOX3* preserved the yeast throughout the winter. Four of four foundresses allowed to form a colony were able to spread yeast cells to their offspring both at the larval stage and after their emergence. F = foundress; in vitro W = worker emerged in vitro; L = larva; *ow*F = overwintering foundress; W = worker, (*B*) Visualization of BY4742-GFP/*FOX3* cells in several conditions (original magnification, 100×), yeast cell wall was visualized with Calcofluor white (blue). Green fluorescence is only produced by the tagged strain when grown in YPO medium (Yeast Peptone 0.2% Oleate medium). Red auto-fluorescence is due to intracellular NADH accumulation in necrotic cells (55).

because wasps can harbor ingested yeast cells for a short time, as it occurs for birds, and continuously renew their microflora by trophic events. However, we experimentally demonstrated that hibernating female foundresses can harbor yeast cells from autumn to spring and then pass them to the next generation in a theoretically unending transmission phenomenon. The role of wasps in maintaining yeast cells during the winter and disseminating them before, during and after the grape harvest, fills the gap left by previous findings indicating a yeast flow between the winery and the vineyard (24, 40–42) but which failed to explain the annual persistence of yeast strains in the soil or in grapes (43).

The use of two different markers, microsatellites and genome-mimicking genes, permits estimation of the genetic evolution of yeast strains at different levels. Both microsatellite and sequence analyses revealed that *S. cerevisiae* did not evolve specific strains associated with animals. Conversely, the genome complexity borne by yeast wasp isolates revealed ancestors common to wine, grapes, bread, and oak yeast isolates. This suggests that yeasts are not subject to strong constraints when in association with animals and that there exists a continuous exchange of cells from animals to different sources and vice versa. The great genetic distance observed between some wasp strains and those isolated from grapes from the same area and a nearby winery strengthens the hypothesis about a multidirectional flow of *S. cerevisiae* occurring not only between wineries and vineyards, but among different sources as well.

Our findings provide a unique illustration of the entire natural cycle of *S. cerevisiae* in at least one ecological environment the gut of social wasps—that, in association with a series of other human and wild environments, significantly contributes to complete the niche, population structure, and diversity of yeast. Wasps can maintain a potentially unending transmission of yeast strains through favorable and unfavorable seasons and also function as vectors to suitable targets (ripe fruits) in suitable seasons (the end of summer). We do not claim that the social wasp gut is the only niche where *S. cerevisiae* is able to survive throughout the year, but we propose that hibernating social wasps have a preferential role in disseminating yeasts compared with other insects.

Our results also reveal that yeast strains in wasps, grapes, and fermentation from the same vineyard, even in different months and years, are more similar than strains deriving from other environmental and geographical locations. In this perspective, wasps could play a role both in maintaining ecological diversity and in conserving the yeast populations evolved in human "ersatz" environments established throughout the centuries by means of vine culture and wine production. The conservation of such diversity may have potential industrial importance in preserving the quality of typical fermented products. This suggests that any environmental change affecting insect biodiversity may create a substantial risk of reducing yeast biodiversity and consequently have an impact on the quality of fermented products.

Methods

Insect Collection and Dissection, and Yeast Isolation and Identification. Adult wasps and bees were dissected in sterile Petri dishes using sterile clamps under a stereomicroscope. Intestines were extracted and their content suspended in sterile water. The obtained solution was plated on YPD supplemented with penicillin and streptomycin (44). The identification of the isolated yeast strains was carried out by sequencing the ribosomal intergenic region as described by Sebastiani et al. (45). Associations between the presence of different yeast species and the collection period in each examined insect gut have been assessed by correspondence analysis by using the *ade4* R package (46).

Sequencing DNA of *S. cerevisiae* Strains. Three genes, *URN1*, *EXO5* and *IRC8*, able to recapitulate the entire genome (34), have been sequenced. Primers used for both amplifications and sequencing are listed in *SI Appendix*, Table S3. Sequences (analyzed samples are listed in *SI Appendix*, Table S2) were compared with *S. cerevisiae* pre-edited sequences downloaded from the Sanger institute (35) and from the SGD (47) websites. Phylogenetic analysis and tree drawing were carried out as described by Ramazzotti et al. (34). Populations were inferred using *Structure* (48) and dapc (differential analysis of principal components in *SI Appendix*. The results of 10 independent *Structure* chains were combined with CLUMPP (49). These sequence data have been deposited in the GenBank database under accession nos. JQ946429–JQ946518.

Microsatellite Characterization. A set of 256 *Saccharomyces cerevisiae* strains, listed in *SI Appendix*, Table S4, was characterized for allelic variation at 12 microsatellites (50). The chord distance Dc matrix (51) was calculated. The tree was obtained from the distance matrices with Neighbor of the Phylip 3.67 package, and drawn using MEGA5.05 (52). The tree was rooted by the midpoint method. To assess the assignment of each insect yeast strain to a specific origin, Instruct (53) was used to evaluate the number of populations that can be observed in this set of strains. The results of 10 independent chains were combined with CLUMPP (49), and the consensus file was illustrated with Distruct (54).

Overwintering Wasps. In November, at the beginning of the hibernation, 20 *Polistes* spp. wasps were fed 10^8 cells of BY4742-GFP/*FOX3* (Mat α *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*). *S. cerevisiae* isolates were identified by sequencing the ribosomal intergenic region as described by Sebastiani et al. (45). *S. cerevisiae* isolates were then grown in rich medium supplemented with 0.2% oleate. BY4742-*GFP/FOX3* isolates were identified with fluorescence microscopy able to express the GFP-labeled Fox3 protein. *FOX3* gene expression is positively regulated by the presence of oleate as a carbon source. The strain used herein is able to express a Fox3p labeled with green at the

peroxisomal level, as shown in Fig. 4*B*. Fed wasps were allowed to hibernate. At the end of the hibernation, some wasps were dissected and the contents of their guts were treated as previously described.

Colony-Founding Wasps. Four colonies of *Polistes dominula* were collected at the pre-emerging phase before worker emergence. At that stage, colonies were composed of the adult foundresses and by larvae and pupae of their first daughters. We fed each foundress 10^8 cells of the BY4742-GFP/*FOX3* strain. During the following 10 d, a number of workers emerged having then adult–adult trophic interactions. *Some Polistes* pupae were removed from nests before their emergence. Such pupae were allowed to emerge in clean tubes to avoid any adult–adult contact. Larvae, worker wasps emerging in the colony, and worker wasps emerging without contact with the adults were dissected, and the contents of their guts were treated as previously described.

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1. McGovern PE, et al. (2004) Fermented beverages of pre- and proto-historic China. *Proc Natl Acad Sci USA* 101:17593–17598.
2. Cavalieri D, McGovern PE, Hartl DL, Mortimer R, Polsinelli M (2003) Evidence for S. cerevisiae fermentation in ancient wine. *J Mol Evol* 57(Suppl 1):S226–S232.
3. Teresa Fernández-Espinar M, Barrio E, Querol A (2003) Analysis of the genetic variability in the species of the Saccharomyces sensu stricto complex. *Yeast* 20:1213–1226.
4. Veiga-Crespo P, Poza M, Prieto-Alcedo M, Villa TG (2004) Ancient genes of Saccharomyces cerevisiae. *Microbiology* 150:2221–2227.
5. Fay JC, Benavides JA (2005) Evidence for domesticated and wild populations of Saccharomyces cerevisiae. *PLoS Genet* 1:66–71.
6. Mortimer RK, Romano P, Suzzi G, Polsinelli M (1994) Genome renewal: A new phenomenon revealed from a genetic study of 43 strains of Saccharomyces cerevisiae derived from natural fermentation of grape musts. *Yeast* 10:1543–1552.
7. Polsinelli M, Romano P, Suzzi G, Mortimer R (1996) Multiple strains of Saccharomyces cerevisiae on a single grape vine. *Lett Appl Microbiol* 23:110–114.
8. Murphy HA, Kuehne HA, Francis CA, Sniegowski PD (2006) Mate choice assays and mating propensity differences in natural yeast populations. *Biol Lett* 2:553–556.
9. Owuama CI, Saunders JR (1990) Physiological variants of Saccharomyces cerevisiae and Kloeckera apiculata from palm wine and cashew juice. *J Appl Bacteriol* 68: 491–494.
10. Garza S, Teixidó JA, Sanchis V, Viñas I, Condón S (1994) Heat resistance of Saccharomyces cerevisiae strains isolated from spoiled peach puree. *Int J Food Microbiol* 23: 209–213.
11. Las Heras-Vazquez FJ, Mingorance-Cazorla L, Clemente-Jimenez JM, Rodriguez-Vico F (2003) Identification of yeast species from orange fruit and juice by RFLP and sequence analysis of the 5.8S rRNA gene and the two internal transcribed spacers. *FEMS Yeast Res* 3:3–9.
12. Sossou SK, Ameyapoh Y, Karou SD, de Souza C (2009) Study of pineapple peelings processing into vinegar by biotechnology. *Pak J Biol Sci* 12:859–865.
13. Duarte FL, Pais C, Spencer-Martins I, Leão C (1999) Distinctive electrophoretic isoenzyme profiles in Saccharomyces sensu stricto. *Int J Syst Bacteriol* 49:1907–1913.
14. Nyanga LK, et al. (2007) Yeasts and lactic acid bacteria microbiota from masau (Ziziphus mauritiana) fruits and their fermented fruit pulp in Zimbabwe. *Int J Food Microbiol* 120:159–166.
15. Henselová M, Hudecová D (2001) Differences in the microflora of scarified and unscarified seeds of Karwinskia humboldtiana (Rhamnaceae). *Folia Microbiol (Praha)* 46: 543–548.
16. Ivannikova IuV, Naumova ES, Martynenko NN, Naumov GI (2007) Characterization of the genome of Saccharomyces yeasts from red berry wines. *Mikrobiologiia* 76: 225–235.
17. Morrissey WF, Davenport B, Querol A, Dobson AD (2004) The role of indigenous yeasts in traditional Irish cider fermentations. *J Appl Microbiol* 97:647–655.
18. Naumov GI, Naumova ES, Sniegowski PD (1998) Saccharomyces paradoxus and Saccharomyces cerevisiae are associated with exudates of North American oaks. *Can J Microbiol* 44:1045–1050.
19. da Silva GA (1996) The occurrence of killer, sensitive, and neutral yeasts in Brazilian Riesling Italico grape must and the effect of neutral strains on killing behaviour. *Appl Microbiol Biotechnol* 46:112–121.
20. De La Torre MJ, Millan MC, Perez-Juan P, Morales J, Ortega JM (1999) Indigenous yeasts associated with two Vitis vinifera grape varieties cultured in southern Spain. *Microbios* 100:27–40.
21. Masneuf I, Hansen J, Groth C, Piskur J, Dubourdieu D (1998) New hybrids between Saccharomyces sensu stricto yeast species found among wine and cider production strains. *Appl Environ Microbiol* 64:3887–3892.
22. Nurgel C, Erten H, Canbaş A, Cabaroğlu T, Selli S (2002) Influence of Saccharomyces cerevisiae strains on fermentation and flavor compounds of white wines made from cv. Emir grown in Central Anatolia, Turkey. *J Ind Microbiol Biotechnol* 29:28–33.
23. Mortimer R, Polsinelli M (1999) On the origins of wine yeast. *Res Microbiol* 150: 199–204.
24. Schuller D, Casal M (2007) The genetic structure of fermentative vineyard-associated Saccharomyces cerevisiae populations revealed by microsatellite analysis. *Antonie van Leeuwenhoek* 91:137–150.
25. Goddard MR, Anfang N, Tang R, Gardner RC, Jun C (2010) A distinct population of Saccharomyces cerevisiae in New Zealand: Evidence for local dispersal by insects and human-aided global dispersal in oak barrels. *Environ Microbiol* 12:63–73.
26. Francesca N, Canale DE, Settanni L, Moschetti G (2012) Dissemination of wine-related yeasts by migratory birds. *Envir Microbiol Rep* 4:105–112.
27. El-Tabey Award Shihata AM, Mrak EM (1952) Intestinal yeast floras of successive population of Drosophila. *Evolution* 6:325–332.
28. Mortimer RK (2000) Evolution and variation of the yeast (Saccharomyces) genome. *Genome Res* 10:403–409.
29. Phaff H (2000) *The Life of Yeasts: Their Nature, Activity, Ecology, and Relation to Mankind* (Harvard Univ Press, Cambridge).
30. Stevic S (1962) The significance of bees (Apis sp.) and wasps (Vespa sp.) as carriers of yeast for the microflora of grapes and the quality of wine. *Arkhiv Poljjoprivredna Nauke* 50:80–92.
31. Basukriadi A, Sjamsuridzal W, Putra BB (2010) Molecular identification and diversity of yeasts associated with Apis cerana foraging on flowers of Jatropha integerrima. *Microbiol Indones* 4:44–48.
32. Gilliam M, Wickerham LJ, Morton HL, Martin RD (1974) Yeasts isolated from honey bees, Apis mellifera, fed 2,4-D and antibiotics. *J Invertebr Pathol* 24:349–356.
33. Legras JL, Ruh O, Merdinoglu D, Karst F (2005) Selection of hypervariable microsatellite loci for the characterization of Saccharomyces cerevisiae strains. *Int J Food Microbiol* 102:73–83.
34. Ramazzotti M, Berná L, Stefanini I, Cavalieri D (2012) A computational pipeline to discover highly phylogenetically informative genes in sequenced genomes: Application to Saccharomyces cerevisiae natural strains. *Nucleic Acids Res* 40:3834–3848.
35. Liti G, et al. (2009) Population genomics of domestic and wild yeasts. *Nature* 458: 337–341.
36. Erny C, et al. (2012) Ecological success of a group of Saccharomyces cerevisiae/Saccharomyces kudriavzevii hybrids in the northern european wine-making environment. *Appl Environ Microbiol* 78:3256–3265.
37. Sipiczki M (2008) Interspecies hybridization and recombination in Saccharomyces wine yeasts. *FEMS Yeast Res* 8:996–1007.
38. Daniel HM, et al. (2009) Yeast diversity of Ghanaian cocoa bean heap fermentations. *FEMS Yeast Res* 9:774–783.
39. Combina M, et al. (2005) Yeasts associated to Malbec grape berries from Mendoza, Argentina. *J Appl Microbiol* 98:1055–1061.
40. Stevic S (1962) The significance of bees (Apis sp.) and wasps (Vespa sp.) as carriers of yeast for the microflora of grapes and the quality of wine. *Arkhiv. Poljjoprivredne Nauke* 50:80–92.
41. Schuller D, et al. (2007) Genetic characterization of commercial Saccharomyces cerevisiae isolates recovered from vineyard environments. *Yeast* 24:625–636.
42. Valero E, Schuller D, Cambon B, Casal M, Dequin S (2005) Dissemination and survival of commercial wine yeast in the vineyard: A large-scale, three-years study. *FEMS Yeast Res* 5:959–969.
43. Cordero-Bueso G, Arroyo T, Serrano A, Valero E (2011) Remanence and survival of commercial yeast in different ecological niches of the vineyard. *FEMS Microbiol Ecol* 77:429–437.
44. Rose MD, Winston F, Hieter P (1990) *Methods in Yeast Genetics* (Cold Spring Harbor, New York).
45. Sebastiani F, Barberio C, Casalone E, Cavalieri D, Polsinelli M (2002) Crosses between Saccharomyces cerevisiae and Saccharomyces bayanus generate fertile hybrids. *Res Microbiol* 153:53–58.
46. Dray S, Dufour AB (2007) The ade4 package: Implementing the duality diagram for ecologists. *J Stat Softw* 22:1–20.
47. Cherry JM, et al. (2012) Saccharomyces Genome Database: The genomics resource of budding yeast. *Nucleic Acids Rest*; 40(Database issue):D700–D705.
48. Pritchard JK, Stephens M, Rosenberg NA, Donnelly P (2000) Association mapping in structured populations. *Am J Hum Genet* 67:170–181.
49. Jakobsson M, Rosenberg NA (2007) CLUMPP: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 23:1801–1806.
50. Legras JL, Merdinoglu D, Cornuet JM, Karst F (2007) Bread, beer and wine: Saccharomyces cerevisiae diversity reflects human history. *Mol Ecol* 16:2091–2102.
51. Cavalli-Sforza LL, Edwards AW (1967) Phylogenetic analysis. Models and estimation procedures. *Am J Hum Genet* 19:233–257.
52. Tamura K, et al. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28:2731–2739.
53. Gao H, Williamson S, Bustamante CD (2007) A Markov chain Monte Carlo approach for joint inference of population structure and inbreeding rates from multilocus genotype data. *Genetics* 176:1635–1651.
54. Rosenberg NA (2004) Distruct: A program for the graphical display of population structure. *Mol Ecol Notes* 4:137–138.
55. Liang J, et al. (2007) Study the oxidative injury of yeast cells by NADH autofluorescence. *Spectrochim Acta A Mol Biomol Spectrosc* 67:355–359.