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Author manuscript

Science. Author manuscript; available in PMC 2024 September 18.

Published in final edited form as:

Science. 2024 July 26; 385(6707): eadi3048. doi:10.1126/science.adi3048.

Selection for Robust Metabolism in Domesticated Yeasts is Driven by Adaptation to Hsp90 Stress

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Abstract

Protein folding both promotes and constrains adaptive evolution. We uncover this surprising duality in the role of the protein-folding chaperone Hsp90 in maintaining the integrity of yeast metabolism amid proteotoxic stressors within industrial domestication niches. Ethanol disrupts critical Hsp90-dependent metabolic pathways and exerts strong selective pressure for redundant duplications of key genes within these pathways yielding the classical genomic signatures of beer and bread domestication. This work demonstrates a mechanism of adaptive canalization in an ecology of major economic significance and highlights Hsp90-dependent variation as an important source of phantom heritability in complex traits.

Structured Summary:

INTRODUCTION: Living systems rely on buffering mechanisms to maintain their robustness to genetic and environmental perturbations, a phenomenon termed "canalization". One such mechanism, the protein-folding chaperone heat shock protein 90 (Hsp90), buffers perturbations

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Author contributions:

Conceptualization and Methodology: GIK; Software and resources: HA, DP, WS, NOL, BH; Formal analysis and investigation: DP, NC, HA, YS, BJ, GIK; Validation: NC, HA, DP, GIK; Writing: NC, GIK; Supervision: GIK; Funding acquisition: GIK.

Competing Interests:

The authors declare that they have no competing interests.

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on protein folding. Hsp90 stabilizes diverse proteins with immediate adaptive functions; mitigates the effects of deleterious mutations, permitting them to accumulate in a cryptic state within populations; and links the expression of such cryptic variation to proteotoxic environmental stressors that exceed the buffering capacity of Hsp90. Understanding how Hsp90 and other canalization mechanisms impact adaptive fitness and identifying the ecological stressors that compromise them will have important implications for human health, ecosystem sustainability, and biotechnology.

RATIONALE: The mechanisms driving canalization in nature are incompletely understood and their applications in medicine and industry underdeveloped. Budding yeast provides an ideal system to address these problems because its evolutionary success rests on well-defined metabolic traits that have been selected for their ability to endure various stresses in human-made environments. Because Hsp90 has multiple functions in yeast metabolism, this study aimed to uncover ecological stressors that compromise Hsp90 buffering and reveal cryptic variation. We employed subtoxic-level Hsp90 inhibition as a proxy for environmental stress and compared the robustness of 12 metabolic traits to Hsp90 inhibition across 711 domesticated and wild yeast strains. We delineated the mechanisms of robustness, identified environmental stressors that compromise them, and confronted predictions from five independent models of canalization with fitness data.

RESULTS: Hsp90 inhibition perturbed several metabolic traits in yeast. The sensitivity of these traits to Hsp90 inhibition varied substantially across closely related strains. Domesticated yeasts used in beer and bread production metabolized the most abundant sugars in wort and dough, maltose and maltotriose, more robustly under Hsp90 inhibition than did wild yeasts. Duplications in several metabolic genes were common in domesticated yeasts and reinforced the robustness of maltose and maltotriose metabolism to Hsp90 inhibition. Exposure to ethanol, a primary product of fermentation, compromises key Hsp90-dependent regulators of maltose and maltotriose metabolism and revealed cryptic genetic variation in these pathways. Gradual escalation of ethanol concentration in wort and liquid sourdough unleashed the cryptic fitness effects of redundant metabolic genes, leading to their rapid selection in these environments. Results from competition assays established the adaptive value of canalization in yeast domestication and refuted alternative byproduct and neutral canalization scenarios.

CONCLUSION: This work identifies a long-sought mechanism of environmental canalization driving the rapid adaptation of a eukaryote in an ecology of great economic importance. Our data collectively indicate that highly desirable budding yeast traits, maltose and maltotriose metabolism, underwent canalization against ethanol-induced Hsp90 stress during domestication and the industrialization of beer and bread making; two major mechanisms of robustness, gene redundancy and Hsp90 buffering, interact in promoting this canalization. Thus, this study highlights the evolutionary significance of Hsp90-contingent variation and clarifies how canalization allows species to quickly adapt to environmental changes. It also underscores the profound environmental sensitivity of protein folding by revealing Hsp90 as an ecologically relevant vulnerability to both exogenous and endogenous (metabolic) stressors. In establishing that Hsp90 is an important mechanism of canalization, this study should provoke further investigations into Hsp90-dependent variation in disease and biotechnology.

One-Sentence Summary:

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Metabolic robustness to Hsp90 stress evolves by gene copy number redundancy during yeast domestication.

Biological systems can tolerate many types of changes in their internal and external conditions, a widespread characteristic of life known as robustness. Classical experiments conducted by C.H. Waddington revealed that the robustness of developmental traits to environmental variation is in part genetically determined and can be subject to natural selection, a phenomenon he coined "canalization" (1, 2). Despite the long history of the concept, canalization remains a mechanistically obscure phenomenon with unexploited practical applications (3–7). Without deep knowledge of the canalization strategies that impact evolutionary adaptations and fitness, it will remain difficult to harness evolution for human benefit in medicine and industry alike.

Diverse unrelated genes, especially hubs in intracellular networks, can "buffer" genetic and environmental variation as well as stochastic fluctuations (biological noise), thereby contributing to canalization (4, 8–12). Buffer genes promote canalization by stabilizing the output of biological networks through gains in redundancy, compensation, regulatory feedback, and homeostatic control (2, 13–16). However, the fitness impact of such buffering mechanisms is debated (4, 6, 13, 15, 17–22). Buffering mechanisms can evolve because they render fitness-related traits robust to high mutation rates (13, 23–26). They can also evolve to maintain the integrity of fitness traits under environmental stress conditions (27), as well as non-adaptively, as pleiotropic byproducts of other adaptations (28) or as emergent properties of evolving gene networks (17, 29).

Perhaps the best studied genes underlying buffering mechanisms encode members of the heat shock protein 90 (Hsp90) family (30). Hsp90 is an ancient and highly conserved ATP-dependent protein-folding chaperone that evolved to safeguard protein homeostasis within the cell by assisting in the maturation and disposition of diverse protein substrates, termed "clients". Hsp90 is constitutively expressed in excess over the cell's needs for growth and survival (31, 32). This excess provides a protein-folding buffer that can enhance the phenotypic effects of some mutations while rendering others phenotypically cryptic (11, 33–46). However, the Hsp90 buffering capacity of the cell is finite and can be exceeded by proteotoxic challenges that lead to protein unfolding, titrating Hsp90 away from its normal clients and causing loss of their function (30). Thus, pathways linked through mutations to this finite buffering capacity of Hsp90 can be compromised by proteotoxic stressors in the cell's environment.

Despite the many cellular functions of Hsp90 (30), the significance of variation dependent on Hsp90 in the evolution of life on Earth is uncertain. Multiple lines of evidence suggest that Hsp90-dependent variation has adaptive value (37–39, 43, 44, 46–49). For example, Hsp90 can drive the evolution of drug resistance (39, 47, 49) and the accumulation of cryptic genetic variation in eye size that can become revealed and foster adaptive evolution in caves (46). However, Hsp90 and other buffering mechanisms have been traditionally studied in model traits in the laboratory or in silico (7, 13, 15, 19, 20, 22, 50, 51). As a result, the ecological mechanisms that reveal Hsp90-dependent variation in natural populations remain elusive.

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To tackle our limited understanding of Hsp90's role in adaptive evolution, we focused on yeast carbohydrate metabolism, a system that is regulated by Hsp90 (52–54) and has undergone rapid evolution in some cases driven by strong human selection (55). Our results demonstrate that ecologically important metabolic traits in domesticated yeasts have evolved robustness to stresses that exceed Hsp90's chaperone capacity. Specifically, gene duplications characteristic of beer and bread yeasts stabilize desirable metabolic traits against ethanol, a prevalent Hsp90 stressor. Thus, we propose that, during yeast domestication, metabolic traits relevant to beer and bread production have undergone genetic and environmental canalization through selection for robustness against Hsp90 stress.

Results:

Ecology shapes the robustness of metabolic traits to Hsp90 stress

To comprehensively survey natural variation in budding yeast metabolism, we examined 12 metabolic traits in *Saccharomyces sensu stricto* yeast laboratory strains from 73 distinct genetic backgrounds and 10 ecological niches (Fig. 1A, fig. S1, A to D, and table S1) (Materials and Methods). The efficiency of metabolic traits was significantly more variable among *S. cerevisiae* isolates than *S. paradoxus* isolates (F test, $P = 2.0585 \times 10^{-8}$; Fig. 1B). In fact, *S. cerevisiae* preferred maltose, the most abundant sugar in wort and dough, while *S. paradoxus* preferred palatinose, turanose, and methyl-α-D-glucopyranoside (methyl-αG). Hence, we identified a panel of rapidly evolving and ecologically relevant metabolic traits to probe Hsp90-dependent variation in yeast metabolism.

Next, we determined the growth effects of Hsp90 disruption on each of the above-mentioned strains/traits. Supplementation of each culture with the naturally occurring resorcinylic Hsp90 inhibitor radicicol induced low and moderate Hsp90 disruption at concentrations of 4 μM and 10 μM, respectively (44). At both concentrations, radicicol reduced the efficiency of diverse metabolic traits across diverse strains but had minimal impact on glucose, sucrose, and methyl-αG utilization overall (fig. S1, E to H). The observed differences under radicicol and control treatment conditions were independent of yeast ploidy, mating type, and the number of generations in continuous culture (fig. S1I).

Structurally diverse small-molecule Hsp90 inhibitors reproduced the effects of radicicol (fig. S2, A and B), validating the on-target effects of radicicol on metabolic traits. Moreover, expression of a hypomorphic Hsp82 mutant (A587T, or G313S, or T101I) as the sole source of Hsp90 (56) compromised palatinose metabolism in a laboratory strain (fig. S2C). In particular, the Hsp82-A587T mutant perturbed palatinose metabolism without impacting growth in glucose (fig. S2C). Conversely, yeasts overexpressing *HSC82*, a *HSP90* paralogue, metabolized palatinose even in the face of Hsp90 inhibition but showed slightly reduced growth in glucose (fig. S2C). Collectively, these results indicate that Hsp90 plays a broader role in yeast metabolism than was previously understood.

Next, we evaluated the sensitivity of metabolic traits to subtoxic impairment of Hsp90 function—called Hsp90 stress throughout. To facilitate comparisons between traits and strains, we calculated the robustness (Materials and Methods) of each metabolic trait to Hsp90 stress in every strain (Fig. 1C and fig. S2, D to H and data S1, and see supplementary

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materials (SM), text 1, for more details). The robustness of metabolic traits to Hsp90 stress varied dramatically between closely related strains (Fig. 1, C to E and fig. S2, I to K). Furthermore, maltose metabolism was significantly more robust to low- and moderate-level Hsp90 stress in domesticated than nondomesticated strains (Mann-Whitney test, RAD 4 μM: $P = 0.0096$, RAD 10 μM: $P = 0.0252$, n = 8 domesticated and n = 12 nondomesticated, maltose-utilizing strains only). In contrast, palatinose metabolism, a structurally similar trait not related to domestication was uniformly hypersensitive to Hsp90 stress across wild *S. paradoxus* strains. These findings suggest that the observed Hsp90-dependent metabolic variation in yeasts reflects ecological differences between domesticated and wild niches.

Metabolic robustness to Hsp90 stress is a phenotypic signature of beer and bread yeasts

To validate the association between yeast domestication and the robustness of maltose metabolism to Hsp90 stress, we used an independent cohort including 320 domesticated strains used in beer, wine, and bread making, and other fermentative processes, a reference of 178 wild strains derived from clinical settings, soil, and wasp gut, and 32 strains of unknown origin (n = 530; table S1). We generated 50,946 growth curves examining maltose, maltotriose, and palatinose metabolism comparing basal conditions with conditions of low- and moderate-level Hsp90 inhibition across the 530 strains (Fig. 2A). Overall, more than half of the examined strain/trait pairs were sensitive to Hsp90 stress (fig. S3, A to C, and data S2).

In agreement with the proposed adaptive roles of maltose and maltotriose utilization in yeast domestication (55, 57, 58), domesticated strains metabolized maltose and maltotriose more efficiently than did wild isolates (left panel; Fig. 2B). Furthermore, maltose metabolism was more robust to Hsp90 stress in domesticated than wild yeasts (right panels; Fig. 2B). Hierarchical clustering revealed a positive association between the robustness of maltose metabolism to Hsp90 stress and the basal efficiency of maltotriose metabolism (fig. S3, D and E), a phenotypic signature of beer yeast (55). Indeed, domesticated strains from niches rich in maltose and maltotriose (i.e., beer, bread, whiskey) metabolized both sugars with significantly higher basal efficiency and robustness to Hsp90 stress than domesticated strains from environments depleted of these sugars (i.e., wine, other domesticated; Fig. 2, A and C, and fig. S3, F and G). We also observed significant differences in the robustness of both traits to Hsp90 stress between ale and lager beer strains (fig. S3H). The effects of Hsp90 inhibition were reproducible at different maltose concentrations (fig. S3I), as well as in brewer's wort (fig. S3J). The basal efficiency of maltose metabolism explained less than 27% of the trait's robustness to Hsp90 stress (fig. S3K).

In contrast to our results with maltotriose, basal palatinose utilization efficiency was not associated with the robustness of maltose metabolism to Hsp90 stress (fig. S3E) and was highly sensitive to Hsp90 stress across diverse domesticated isolates (Fig. 2D and fig. S3F). We conclude that the evolved robustness or canalization of maltose and maltotriose—but not palatinose—metabolism against Hsp90 stress is a novel phenotypic signature of beer and bread yeast domestication.

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Mendelian inheritance of metabolic trait robustness to Hsp90 stress

Next, we asked whether the robustness of maltose metabolism to Hsp90 stress is genetically determined. To this end, we performed 11 crosses between a *S. cerevisiae* laboratory strain that does not metabolize maltose and diverse *S. cerevisiae* haploid strains that expressed the trait, in seven of which maltose metabolism was robust to Hsp90 stress. Inheritance of maltose metabolism followed a 2:2 Mendelian pattern in at least seven of the crosses (data S3). The parental pattern of robustness to Hsp90 stress was inherited in 125 of 168 (74.4%) dissected tetrads (fig. S4, A and B). These data suggest that a small number of genetic loci determines both the basal efficiency of maltose metabolism and the trait's robustness to Hsp90 stress. Furthermore, the robustness of maltose metabolism to Hsp90 stress was not associated with the robustness of other metabolic traits in strains that co-expressed two or more traits (fig. S4, C and D). These data suggest that robustness is not an indirect manifestation of differences among strains in the cell permeability of Hsp90 inhibitors or in Hsp90 buffering capacity. We conclude that the robustness of each metabolic trait to Hsp90 stress is fundamentally embedded within the genetic architecture of the trait.

Overlap between genomic signatures of yeast domestication and metabolic robustness to Hsp90 stress

To uncover the genetic architecture of metabolic robustness to Hsp90 stress, we performed whole-genome sequencing of 63 natural, maltose-utilizing yeast isolates representing the variance in trait robustness to Hsp90 stress within domesticated and wild groups (fig. S5A). We observed substantial copy number variation (CNV) at subtelomeric regions of the genome across the sequenced strains (fig. S5B). Next, we divided these strains into two groups based on the robustness of their maltose metabolism to moderate-level Hsp90 inhibition (robust vs. sensitive). Comparing CNV between the two groups revealed associations between metabolic trait robustness and amplifications of genes involved in oligosaccharide catabolism (fig. S5C and table S5). These genes (*MAL* genes) reside within unlinked subtelomeric loci called *MAL* clusters (*MAL1, MAL2, MAL3, MAL4, MAL6*) (59) and encode permeases and hydrolases, which are critical for the utilization of diverse sugars (60, 61). Specifically, amplifications of *MAL31* permease and *MAL32* and *IMA1* hydrolase genes previously linked to yeast domestication (62–65) were significantly associated with increased robustness of maltose metabolism to Hsp90 stress (Fig. 3A, fig. S5D, and tables S6 and S7). An independent CNV algorithm reproduced these results (fig. S5E). Hence, the genomic signatures of yeast domestication and robustness of maltose metabolism to Hsp90 stress overlap.

A closer investigation of CNVs between linked genes revealed important deviations from the established signatures of domestication. *MAL* genes with no known role in maltose or maltotriose metabolism, such as *YPR196W* and *IMA1-4* (60), have been reported to be amplified across diverse domesticated yeasts (62, 65). By performing correlation analyses among genes that are amplified in the group of domesticated yeasts we sequenced, we determined that the observed discrepancies resulted from hitchhiking, misalignment of sequencing reads between paralogues, and the absence of *MAL63* from the reference genome (fig. S5, F and G, and see supplementary materials (SM), text 2, for more details). Read consolidation adjustments confirmed that *MAL63*—but not *YPR196W* or *MAL33*—

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was significantly amplified in association with domestication (Fig. 3A and fig. S5H). These results are consistent with previous reports documenting the existence of multiple routes to yeast domestication (55, 57, 62, 63, 65–68), and implicate the amplification of *MAL31*-, *MAL32*-, and *MAL63*-contining *MAL* clusters (i.e., *MAL3* and *MAL6*).

Next, we evaluated the fraction of genetic variance in metabolic robustness to Hsp90 stress each amplified gene explains. The relative copy number of *MALx2* genes explained a striking 56% to 60% of the genetic variance in the robustness of maltose metabolism to Hsp90 stress (fig. S5I, tables S1 and S8, and data S2 and S4). We observed similar relationships for *MAL31* and *MAL63* (table S8). These observations were not artifacts of ploidy differences between domesticated and wild yeasts or bias towards domesticated strains, because they were reproduced when comparisons were limited to diploid strains or wild isolates from the gut of wasps (Fig. 3A, fig. S5, J and K, table S8). Moreover, these comparisons revealed a critical threshold in the number of *MAL* genes required for efficient maltose and maltotriose metabolism (fig. S5, L and M). There was no correlation between *MALx2* copy number and the basal efficiency of palatinose metabolism (fig. S5N). These findings provide refined genomic signatures of yeast domestication to help in establishing causal relationships between *MAL* gene duplications and canalization against Hsp90 stress.

Redundant *MAL* gene duplications canalize metabolic traits against Hsp90 stress

To investigate whether *MAL* gene duplications cause canalization, we tested the effect of *MAL* gene disruptions on the robustness of metabolic traits to Hsp90 stress in isogenic strains engineered to harbor different numbers of *MAL* gene copies. Because *MAL31* and *MAL32* genes are typically co-inherited, we disrupted both genes in one step and deleted the bidirectional promoter that drives their Mal63-dependent expression (fig. S6A). Mutation of a single copy of *MAL31* and *MAL32* in an industrial bread strain had no obvious effect on basal maltose utilization efficiency (left panel; Fig. 3B). Maltose metabolism was similarly robust to *MAL63* mutation across diverse industrial strains (left panels; Fig. 3C and fig. S6, B to D). We observed significant effects on basal maltose, maltotriose, and turanose metabolism only after deletion of two or three *MAL* genes (fig. S6, B and E), while diverse industrial strains tolerated up to three or four mutations with negligible effects on basal metabolic efficiency (Fig. 3, C and D, and fig. S6, C and D). We confirmed the gene disruptions in 36 strains derived from seven parental strains by whole genome sequencing and CNV analysis (Fig. 3E, fig. S6F, and data S5).

The striking robustness of these traits to mutation was unexpected given that *MAL* gene duplications are thought to support the basal efficiency of maltose and maltotriose metabolism (55, 61, 62, 69, 70). As further evidence contradicting this conventional view, maltose, maltotriose, and turanose metabolism were substantially more robust to mutations in domesticated yeasts than in wild isolates (two-sided unpaired t-test, $P = 1.60716 \times 10^{-5}$; Fig. 3F). These differences reflect the lower number of *MAL* genes in the genome of wild yeasts (data S4 and S5). Overall, these results demonstrate that ecologically relevant metabolic traits underwent genetic canalization during yeast domestication by virtue of acquiring redundant *MAL* gene duplications.

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Next, we investigated the role of these redundant *MAL* gene duplications in the robustness of metabolic traits to Hsp90 stress and the canalization of these traits in domesticated yeasts. Disruption of either *MAL31-MAL32* or *MAL63* profoundly reduced the robustness of maltose, maltotriose, and turanose metabolism to Hsp90 inhibition across diverse industrial strains (Fig. 3, B to D and fig. S6, B to E). Because sequential deletion of *MAL* genes progressively compromised the robustness of metabolic traits reproducibly across independent biological replicates (Fig. 3, B and C and fig. S6, B to E), the targeted genes must encode for functional proteins. *MAL* gene mutations did not affect the robustness to Hsp90 stress of an independent metabolic trait or its basal efficiency (fig. S6G). These findings demonstrate that the redundant *MAL* gene duplications that give rise to the genomic signatures of yeast domestication canalize maltose and maltotriose metabolism against Hsp90 stress. Because metabolic traits tend to be more robust to mutations than to Hsp90 stress (Fig. 3F), our data collectively suggest that the canalization of maltose and maltotriose metabolism evolved as an adaptation to Hsp90 stress within maltose-rich domestication niches—that is, environmental canalization.

Hsp90 stress from a proteotoxic metabolite de-canalizes metabolic traits

Having found evidence that yeast domestication entailed environmental canalization of ecological traits against Hsp90 stress, we next asked what environmental stressors found in industrial contexts were responsible for this stress. The yeasts used in traditional beer, bread, and whiskey making must endure diverse stresses associated with fermentation, including low pH, elevated temperature, osmotic pressure, salinity, and alcohols (71, 72). Of those stressors, ethanol exposure has been shown to compromise Hsp90 function and the folding of client proteins within yeast cells (73, 74), while elevated temperature can abrogate Hsp90-dependent resistance to anti-fungal agents (39). Therefore, we applied heat, ethanol, and other fermentation-associated stresses to test their ability to uncover Hsp90-dependent variation in metabolic traits.

Most of the examined stressors perturbed maltose metabolism in a strain-specific way but ethanol and heat stress produced effects most similar to those of Hsp90 inhibition (fig. S7A). Moreover, ethanol perturbed maltose, maltotriose, and palatinose metabolism at subtoxic concentrations well within the range at which it is found in most industrial fermentation niches (4% to 8%, v/v; Fig. 4A and fig. S7, A to C, and data S6). Therefore, we further investigated ethanol as an ecological driver of canalization.

In line with ethanol serving as an agent of selection in the environmental canalization of maltose metabolism, maltose and maltotriose metabolism were more robust to ethanol stress across domesticated yeasts from maltose-rich niches (beer, bread, and whiskey) than in other yeasts (Fig. 4B and fig. S7, D to F). Maltose and maltotriose metabolism were also more efficient in industrial strains under high Hsp90 stress elicited by the combined effect of ethanol exposure and moderate-level Hsp90 inhibition (Fig. 4A and fig. S7, G and H). In contrast, palatinose metabolism was hypersensitive to these exposures (Fig. 4B, and fig. S7, E, G and I).

In agreement with a direct role for ethanol in the canalization, disruption of *MAL* genes hypersensitized both maltose and maltotriose metabolism to ethanol stress across diverse

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industrial strains (Fig. 4C and fig. S7, J and K). *MAL63* duplications—but not *MAL31-32* —also canalized maltotriose metabolism against moderate ethanol stress (fig. S7K). On the other hand, disruption of *MAL63* or *MAL31-32* did not affect the robustness of maltose or glucose metabolism to moderate-level salt, low pH, and sorbitol stresses (fig. S7J). These results demonstrate that *MAL* gene duplications canalize metabolic traits against not just any stress but specifically against Hsp90 stress and identify ethanol as a major ecological driver of canalization in yeast domestication.

In addition, moderate ethanol stress revealed cryptic Hsp90 dependencies in robust maltose metabolism across domesticated beer and bread yeasts (Fig. 4A, and fig. S7, G and H). Thus, ethanol broadly sensitized metabolic traits to both genetic and environmental variation —that is, it de-canalized them. These findings demonstrate that ethanol stress unmasks Hsp90-buffered genetic variation (CNVs) in metabolic traits; they further suggest that the Hsp90 dependencies of these traits are ancient and have endured domestication under escalating Hsp90 stress conditions.

Metabolic genes exert rheostatic control over the canalization of metabolism against Hsp90 stress

To assess ethanol's role in canalization more precisely, we evaluated the relationship between *MAL* gene copy number and metabolic robustness to ethanol stress. As expected, an increased copy number of relevant *MAL* genes was associated with maltose—but not palatinose—metabolism that was robust to low and moderate ethanol exposure (fig. S8, A and B). Notably, increasing the ethanol concentration raised the threshold number of *MAL* gene copies required for robust maltose and maltotriose metabolism (fig. S8C and table S9). This threshold was further raised at a higher temperature, while it was diminished at a lower temperature (fig. S8, C and D), suggesting interaction between ethanol and heat stress. In support of the idea that temperature influences ethanol's role in canalization, maltose and maltotriose metabolism were more robust to high-level Hsp90 stress in ale beer yeasts than in their cold-adapted lager beer relatives (fig. S3H and S8E and table S1). Further, ale beer yeasts generally harbored more *MAL* genes than lager beer yeasts, although these differences in gene copy numbers were not statistically significant (fig. S8F). Notably, *MALx2*, *MAL31*, and *MAL63* gene copy numbers explained the genetic variance in the robustness of maltose metabolism under 7% ethanol stress at 25 °C ($R^2 = 0.7186$, $F_{3,46} = 39.16$, $P = 1.01343 \times 10^{-12}$) much better than they did at lower or higher ethanol stress conditions at any temperature (table S10). Considering that ethanol stress is much more prevalent in domesticated niches than nondomesticated ones, these observations point to moderate ethanol stress as a major driver of canalization in domesticated yeasts.

We further found that the Hsp90-dependent transcription factor Mal63 is a key point of fragility of maltose and maltotriose metabolism against ethanol stress. Indeed, low ethanol exposure drove GFP-tagged Mal63 out of the nucleus and into the cytoplasm, ultimately leading to Mal63 depletion, similar to Hsp90 inhibition (Fig. 4D and fig. S8G). Furthermore, overexpression of Mal63 protein in a laboratory strain rheostatically canalized both maltose and maltotriose metabolism against Hsp90 inhibition and ethanol stress without affecting palatinose metabolism (fig. S8, H to J). The effects of Mal63

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overexpression on maltose metabolism were preserved even upon deletion of *MAL11*, which abolished maltotriose metabolism (fig. S8, I and J). On the other hand, overexpression of an unrelated Malx3 protein, Mal13, which is required for palatinose utilization, increased the efficiency of palatinose metabolism under Hsp90 stress without affecting maltose or maltotriose metabolism under basal or Hsp90 stress conditions (fig. S8H).

Taken together, these findings demonstrate that ethanol exposure compromises Hsp90 protein-folding chaperone capacity, thus uncovering cryptic Hsp90-buffered CNVs in metabolic genes. We conclude that yeast domestication entailed environmental canalization of metabolism against niche-related Hsp90 stress.

Redundancy in maltose metabolism genes supports fitness predominantly under Hsp90 stress

Our identification of ecological Hsp90 stressors that interact with redundant *MAL* gene duplications (namely, ethanol and heat) does not automatically suggest that the observed canalization of maltose metabolism is adaptive (fig. S9A). In fact, adaptive canalization is believed to be limited by alternative scenarios involving selection against biological noise or for metabolic flux (fig. S9B) (10, 11, 75), selection against lethals (fig. S9C) (4, 29, 76, 77), pleiotropic selection (by-product of other adaptations; fig. S9D) (13, 77–79), and neutral evolution (fig. SE) (16). These models make different predictions on the fitness effects of the canalizing *MAL* genes under basal and Hsp90 stress conditions.

To help distinguish which mechanism (or combination thereof) may have driven the canalization of maltose metabolism across industrial yeasts, we examined the effects of *MAL* gene duplications on the relative fitness of industrial yeast strains in maltose-rich media (Fig. 5, A to C and fig. S10, A to C). We observed a small selective advantage for a parental bread strain over the derivative strain with a single disrupted *MAL31-MAL32* copy (fig. S10D). This effect was markedly increased upon Hsp90 inhibition (fig. S10D), revealing that *MAL31-MAL32* duplications play a more important role in the robustness of maltose metabolism against Hsp90 stress than in the basal efficiency of the trait.

To support an adaptive role in canalization, *MAL* gene duplications must confer fitness effects even when they are underrepresented in the population, as may have been the case in early yeast domestication niches. To mimic the traditional practice of backslopping that resulted in the yeast strains used in modern beer and bread making (80–82), we passaged cultures through sequential bottlenecks in maltose-rich media (Fig. 5D). Again, Hsp90 inhibition drastically enhanced (by at least 20-fold) the relative fitness of *MAL31-MAL32* gene duplications (fig. S10, E and F). *MAL* mutations and Hsp90 inhibition had no fitness effect in glucose (fig. S10, E and F), suggesting negligible cost to maintaining redundant *MAL* genes under these conditions. Hence, *MAL31-MAL32* gene duplications confer frequency-independent fitness effects in maltose-rich media, and Hsp90 stress dramatically increases these fitness effects.

To simulate the genetic variation in early domestication yeast communities, we competed parental strains with single and double *MAL* gene-disrupted derivatives. At a higher cell density close to those in traditional fermentations (81, 82), *MAL* gene duplications

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conferred a strong selective advantage only under Hsp90 stress in both *mal31-32* and *mal63* mutant lineages (Fig. 5, E to G, and table S11), suggesting that selection is also independent of cell density. A single canalizing copy of *MAL31-MAL32* or *MAL63* conferred a fitness advantage of 20- to 30-fold in maltose under Hsp90 stress conditions (Fig. 5H and fig. S10J). Notably, the effect of disruption of two *MAL63* copies on relative fitness in maltose under Hsp90 stress was comparable to that of disruption of a single *MAL31-MAL32* copy. These results demonstrate that the canalization of maltose metabolism against Hsp90 stress by redundant *MAL* gene duplications provides a strong fitness benefit.

Ethanol exposure transforms the adaptive value of redundant metabolic gene duplications

In agreement with the adaptive environmental canalization model, ethanol exposure replicated the effects of Hsp90 inhibition in competition assays (Fig. 5, E to H, and fig. S10, G to J). In all experiments conducted in maltose-rich conditions, ethanol de-canalized maltose metabolism, thereby revealing the cryptic fitness effects of *MAL* gene duplications. Furthermore, this interaction between ethanol exposure and *MAL* gene copy number was also strong in wort and sourdough media, even at a much lower concentration of ethanol (5%, v/v; fig. S11, A to C). These data validate the role for ethanol as a major selection agent in the adaptive environmental canalization of maltose metabolism.

An adaptive environmental canalization model would predict that the de-canalizing stressor should influence the fitness effects of *MAL* gene duplications in a dose-dependent way (fig. S9A). To test this prediction in a context resembling the early domestication of beer yeasts, we competed an industrial beer yeast parent strain (WLP802; Fig. 5A) against isogenic *mal63* mutant derivatives in wort. Higher *MAL63* copy number conferred small fitness advantages under basal conditions (Fig. 5I and fig. S11D). As expected, gradual dose escalation of the Hsp90 inhibitor radicicol rapidly selected the high-*MAL*-gene-copy parent in wort (Fig. 5I and fig. S11E).

To simulate the gradual increase in ethanol content during the domestication of beer yeasts, we gradually increased the concentration of ethanol in wort over a period of 64 generations in continuous culture. Escalation of ethanol concentration drove rapid selection for canalizing *MAL63* duplications until the CNVs reached an equilibrium at 9% ethanol (Fig. 5I, fig. S11F, and table S12), suggesting diminishing returns at that point for higher *MAL* gene copy number. Strikingly, a small increase to 10% ethanol eliminated residual CNVs, rapidly fixing the amplified *MAL63* copy number. This rapid fixation was not the result of adaptation to chronic ethanol stress, because ethanol similarly induced expression of Hsp104, a heat shock marker, in both chronic and acute exposure conditions (fig. S11G). These data demonstrate that the adaptive value of *MAL* gene duplications resides in their ability to precisely canalize metabolism against niche-related Hsp90 stress, in agreement with the adaptive environmental canalization model (fig. S9A).

Refuting alternative scenarios to the adaptive environmental canalization model

Next, we examined whether alternative theories provide sufficient fitness effects to have driven canalization. *MAL* gene mutations impact relative fitness in maltose even under basal conditions; these effects are not minimal by most selective standards and could provide a

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selective force for indirect canalization (fig. S9B). However, the basal fitness effects of *MAL* gene duplications pale in comparison to the fitness advantage they confer under Hsp90 stress conditions (Fig. 5, F to I, fig. S10, E to J and S11, A to F, and table S11), arguing in favor of an adaptive environmental canalization scenario (fig. S9A). In fact, single disruption of *MAL63* had no effect on relative fitness of an industrial strain growing in maltose under basal conditions (basal: $f_{1xMAL63} = -0.4243\% \pm 0.7555\%$; fig. S10J) and the effect of two *MAL63* disruptions was small (basal: $f_{2xMAL63} = +2.525\% \pm 0.3202\%$; Fig. 5H). It would take more than 150 generations for two *MAL63* copies to reach 50% frequency in maltose under basal conditions, but merely 21 generations under Hsp90 stress (moderate Hsp90i: $f_{2xMAL63} = +22.2\% \pm 0.049\%$). These observations argue against a major role for selection against noise or on basal metabolic flux in the canalization of maltose metabolism (fig. S9B).

The rapid selection of *MAL* gene duplications under ethanol stress also does not provide enough time for spontaneous or ethanol-induced mutations (74) to drive canalization directly or indirectly through selection-against-lethals (fig. S9C and S11H, and see supplementary materials (SM), text 3, for more details). Additionally, the fitness effects of pleiotropic *MAL* gene duplications were greater on maltose metabolism than on pleiotropic traits, suggesting against a model of canalization by pleiotropy (fig. S9D, and see supplementary materials (SM), text 4, for more details). Finally, the strong selection of *MAL* gene duplications in domesticated yeasts refutes a neutral evolution model of canalization (fig. S9E).

Taken together, our findings argue against various indirect and neutral models to explain the environmental canalization of industrial maltose metabolism against Hsp90 stress. Instead, they provide strong support for a model of adaptive environmental (and congruent genetic) canalization in which niche-related proteotoxic stress likely exerted by ethanol drove the profound selection of canalizing *MAL* gene duplications during yeast domestication (Fig. 6).

Discussion:

Budding yeast is intertwined with human history, but the mechanisms underlying the species profound economic importance remain incompletely understood (57, 58, 83). Over the last few centuries, *S. cerevisiae* underwent strong selection for the ability to ferment sugars into alcohol. Alcoholic fermentation of maltose and maltotriose relies on well-understood and genetically similar metabolic pathways critical for producing diverse foods and beverages, and these pathways have markedly evolved during yeast domestication (58, 83). However, the adaptive value of these evolved pathways has eluded experimental demonstration (84, 85). Now, we show that redundant gene duplications in the genomic signatures of yeast domestication confer fitness advantages to industrial yeasts because they canalize desirable metabolic traits against compromise of Hsp90’s chaperone function by specific proteotoxic stressors that are prevalent in industrial niches.

Ethanol exposure, at industrially relevant concentrations—but not other fermentation-associated stressors—disrupts key Hsp90-dependent regulators of metabolism and exerts strong selective pressure for gene duplications characteristic of domesticated beer and bread yeasts. Results from competition assays demonstrate the adaptive value of environmental

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canalization under ethanol escalation and refute alternative indirect and neutral evolution hypotheses.

Identification of maltose utilization in industrial yeasts as a bona fide example of adaptive canalization helps answer long-standing questions that have confronted the study of canalization. First, it resolves the controversy around the role of redundancy as a mechanism of canalization in nature (4, 7, 8, 19, 29, 50). We show that redundant *MAL* genes encoding for the Hsp90-dependent transcription factor Mal63 (52, 53) and its targets, are causally responsible for a striking 60% or more of the genetic variance in the robustness of maltose metabolism to Hsp90 stress. Hence, maltose metabolism serves as an ecologically relevant paradigm for canalization by redundancy.

Second, this work uncovers interactions between redundancy (gene duplications) and distributed robustness (nonredundant regulatory genes) in canalization. Removing layers of redundancy from canalized metabolic traits in industrial yeasts exposes a cryptic layer of distributed robustness provided by Hsp90's buffering capacity that canalizes these traits against ethanol stress. These findings reconcile two schools of thought on canalization (4, 8, 29) by showing that seemingly disparate mechanisms of robustness are in epistasis: *MAL* gene duplications mask the effects of Hsp90 stress on metabolic traits. This observation also adds structural variations to the spectrum of genetic alterations Hsp90 can buffer.

Importantly, we find that different robustness mechanisms differ in their adaptive value. Redundant *MAL* genes adaptively canalize industrial traits against niche-related Hsp90 stress. On the other hand, Hsp90 buffering appears to be an intrinsic feature of yeast metabolism. Considering the abundance of cryptic Hsp90-dependent variation in metabolic traits, Hsp90 may have facilitated the adaptive evolution of metabolic pathways in yeast (see supplementary materials (SM), text 5, for more details). In contrast, Hsp90 has also constrained the evolution of metabolic pathways in maltose-rich industrial niches, as evidenced by the strong genomic signatures of yeast domestication, because most *MAL* gene configurations that support efficient metabolic traits under basal conditions do not support these same traits under conditions of niche-related Hsp90 stress. Hence, Hsp90 can either accelerate or limit adaptive evolution, depending on the ecological setting and evolutionary time scales at play.

Another important contribution of this study is its identification of ecological sources of de-canalization by Hsp90 stress. We show that de-canalization can arise from mutations as well as from extrinsic and intrinsic environmental Hsp90 stressors. Ethanol is the end product of fermentation and a protein denaturant known to compromise protein folding and Hsp90 function (73, 74) and to interfere with beer wort fermentation processes within yeast cells (86). Ethanol exposure, at subtoxic concentrations, compromises the maturation of Mal63 and reveals, in a dose-dependent fashion, preexisting Hsp90-buffered genetic variation enabling the canalization of industrial traits in beer and bread yeasts. Added support for this model comes from the substantial amounts of ethanol produced during dough leavening and the interchangeable use of the same yeast strains for traditional bread and beer making (87, 88). Nonetheless, ethanol is not the only ecological Hsp90 stressor in this context; heat is also important (86). The weaker canalization of maltotriose metabolism

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against Hsp90 stress in lager beer yeasts, as compared to ale and bread yeasts, likely reflects the reduced demand on Hsp90 chaperone capacity at the lower temperatures used for lager brewing.

These observations uncouple canalization from general stress tolerance mechanisms and reveal that the robustness of ecological traits in beer and bread yeasts is precisely fine-tuned to compensate for the varying levels of niche-related Hsp90 stress in different industrial fermentation contexts. Indeed, we show strong dose-dependent threshold relationships between ethanol and *MAL* gene copy number. Identifying ecologically relevant Hsp90 stressors and showing how they interact leads us to conclude that Hsp90 stress is a major force of selection shaping the eco-evolutionary dynamics of canalization in nature and demonstrates that context matters in evolution, in contrast with the predominant probabilistic-statistical view.

These conclusions have important practical implications. First, the genetic and phenotypic biomarkers of canalization we identified could be useful in strain selection and trait optimization for industrial purposes. Additionally, the principles of robustness we uncovered could aid in the optimization of synthetic traits involving heterologous Hsp90 client proteins (89, 90). Although caution is warranted when drawing comparisons between budding yeasts and humans, this work provides a de-canalization model to explain inter-individual differences in susceptibility to ethanol-related diseases (91, 92) and the selection of amplifications of Hsp90-dependent oncogenes in tumors (47, 93, 94). Clearly, our understanding of the role of canalization in evolutionary processes and human disease is in its infancy (20, 95–97), but our findings highlight the untapped potential of Hsp90-buffered variation as an important source of phantom heritability in ecologically relevant traits.

Materials and methods summary:

To find natural Hsp90-dependent variation across diverse laboratory and natural yeasts (table S1), we used a high-throughput growth assay to estimate the basal efficiency and robustness of 12 distinct metabolic traits to Hsp90 inhibition. Metabolic trait, here, refers to the ability to utilize a specific sugar/carbon source, as represented by growth in culture. To allow quantitative comparisons of growth between different strains and conditions, we generated growth curves in high throughput and extracted growth parameters for each strain/condition. As a proxy for relative growth, we used the area under the curve (AUC) to capture the cumulative effect of all other growth parameters. To represent the relative efficiency of each metabolic trait in each strain and conditions, we normalized the measurements to those for glucose. Biological robustness is a measure of the sensitivity of a trait to a specific genetic or environmental perturbation. To account for the nonspecific growth effects of Hsp90 disruption and other perturbations, we calculated the robustness of each metabolic trait to each stressor as the logarithm of the ratio of metabolic efficiency—that is, the ability to utilize a given sugar as represented by growth relative to glucose control—under stress (e.g., Hsp90 inhibitor), over metabolic efficiency under basal conditions. Robustness was calculated only for expressed (>0.25 efficiency) strain/trait pairs to allow for statistical comparisons between domesticated and wild strain groups to assess the evolution of robustness—that is, canalization.

To find associations between the robustness of metabolic traits and ecological niche, we compared the robustness of two domestication-associated metabolic traits (maltose and maltotriose metabolism) and one non-domestication control trait (palatinose metabolism) across diverse yeast strains in two independent cohorts.

To estimate natural variation in gene copy number, we performed whole-genome sequencing and coverage analysis. This method includes an additional clustering step to consolidate highly similar genes into consensus gene groups and used custom reference sequences for the *MAL63* gene derived from an industrial strain. To benchmark our CNV analysis, we used an independent CNV algorithm, established robustness to broad parameter changes, and validated our CNV estimates in natural strains harboring three to five copies of each *MAL* gene and their derivatives we generated carrying a precise number of engineered *MAL* gene deletions.

Perturbations to growth do not invariably impact the organism's overall fitness. To determine the fitness effects of genetic and environmental perturbations and their interactions, we used a cell competition assay. We competed *MAL* gene deleted derivatives of industrial strains to their parental strains, which were underrepresented in the original population, in wort and synthetic sourdough and exposed these cultures to a ethanol escalation regime simulating ethanol accumulation within early domestication and industrial niches. We validated fitness estimates derived from flow cytometry with colony counting on plates and contrasted our observations with predictions from 5 independent canalization models.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

This work is dedicated to Susan Lindquist who inspired its pursuit. We are grateful to L. K. Clayton, L. Whitesell, and A. L. Ninetto (MD Anderson Research Medical Library) for editing the manuscript, and D. F. Jarosz, S. Arur, C. McLellan, R. Behringer, P. McCrea, H. Lodish, and R.-L. Yan for critical comments on earlier versions of the manuscript. We thank C. Klingemann and D. Russey at Saint Arnold Brewing Company for providing guidance on industrial fermentation conditions, N. Azubuine, S. Amarell, W. Gomarga, N. Kota, I. Qin, S. Chittoor, and S. Ding for technical assistance, P. Thiru, G. Bell, B. Gracia, X.-H. Zhang, S. Bujnowski for bioinformatics support, T. C. Pham for assistance with confocal microscopy, A. Paulucci-Holthauzen, core-director for BSRB Microcopy Laboratory, Department of Genetics, for guidance on microscopy acquisition and image processing, D. Landgraf, and J. Bonner for providing pDML215 and pDML209 plasmids, J. Schacherer for protocols on yeast genome purification and scripts for tetrad analysis, and D. Thompson, G. Fink, and D. Cavalieri for providing yeast strains.

Funding:

Research reported in this publication was supported by the National Cancer Institute of the National Institutes of Health under Award Number K22 CA222938 (GIK). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. GIK is a CPRIT Scholar in Cancer Research. This work was supported by the Cancer Prevention Institute of Texas grant RR180005 (GIK), and UTHealth start-up funds (BH). We acknowledge support from the Cytometry and Cell Sorting Core at Baylor College of Medicine with funding from the CPRIT Core Facility Support Award (CPRIT-RP180672), the NIH (CA125123 and RR024574) and the assistance of Joel M. Sederstrom.

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Data and materials availability:

All unique/stable reagents generated in this study, including the antibody against yeast Hsp90 (generated by Susan Lindquist) are available from the Lead Contact with a completed Materials Transfer Agreement. Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact Georgios Karras (gkarras@mdanderson.org). All original code is available and will be distributed upon request. The whole genome sequence data has been deposited at NCBI under the Bioproject ID (PRJNA997361). The BiosampleID numbers are listed in table S4. All other data are available in the Supplementary files data S1 to S6 and code on Zenodo (98).

References and Notes:

1. Waddington CH, Canalization of development and the inheritance of acquired characters. Nature 150, 563 – 565 (1942).
2. Scharloo W, Canalization: Genetic and developmental aspects. Annual Review of Ecology and Systematics 22, 65 – 93 (1991).
3. Gibson G, Wagner G, Canalization in evolutionary genetics: a stabilizing theory? Bioessays 22, 372–380 (2000). [PubMed: 10723034]
4. de Visser JA et al. , Perspective: Evolution and detection of genetic robustness. Evolution 57, 1959–1972 (2003). [PubMed: 14575319]
5. Stelling J, Sauer U, Szallasi Z, Doyle FJ 3rd, Doyle J, Robustness of cellular functions. Cell 118, 675–685 (2004). [PubMed: 15369668]
6. Flatt T, The evolutionary genetics of canalization. Q Rev Biol 80, 287–316 (2005). [PubMed: 16250465]
7. Hallgrimsson B. et al. , The developmental-genetics of canalization. Semin Cell Dev Biol 88, 67–79 (2019). [PubMed: 29782925]
8. Wagner A, Distributed robustness versus redundancy as causes of mutational robustness. Bioessays 27, 176–188 (2005). [PubMed: 15666345]
9. Richardson JB, Uppendahl LD, Traficante MK, Levy SF, Siegal ML, Histone variant HTZ1 shows extensive epistasis with, but does not increase robustness to, new mutations. PLoS Genet 9, e1003733 (2013). [PubMed: 23990806]
10. Burga A, Casanueva MO, Lehner B, Predicting mutation outcome from early stochastic variation in genetic interaction partners. Nature 480, 250–253 (2011). [PubMed: 22158248]
11. Casanueva MO, Burga A, Lehner B, Fitness trade-offs and environmentally induced mutation buffering in isogenic C. elegans. Science 335, 82–85 (2012). [PubMed: 22174126]
12. Levy SF, Siegal ML, Network hubs buffer environmental variation in Saccharomyces cerevisiae. PLoS Biol 6, e264 (2008). [PubMed: 18986213]
13. Wagner GP, Booth G, Bagheri-Chaichian H, A Population Genetic Theory of Canalization. Evolution 51, 329–347 (1997). [PubMed: 28565347]
14. Kitano H, Biological robustness. Nat Rev Genet 5, 826–837 (2004). [PubMed: 15520792]
15. Felix MA, Barkoulas M, Pervasive robustness in biological systems. Nat Rev Genet 16, 483–496 (2015). [PubMed: 26184598]
16. Payne JL, Wagner A, The causes of evolvability and their evolution. Nat Rev Genet 20, 24–38 (2019). [PubMed: 30385867]
17. Bergman A, Siegal ML, Evolutionary capacitance as a general feature of complex gene networks. Nature 424, 549–552 (2003). [PubMed: 12891357]
18. Wagner GP, Zhang J, The pleiotropic structure of the genotype-phenotype map: the evolvability of complex organisms. Nat Rev Genet 12, 204–213 (2011). [PubMed: 21331091]

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19. Keane OM, Toft C, Carretero-Paulet L, Jones GW, Fares MA, Preservation of genetic and regulatory robustness in ancient gene duplicates of Saccharomyces cerevisiae. Genome Res 24, 1830–1841 (2014). [PubMed: 25149527]
20. Crespi E et al. , Resolving the Rules of Robustness and Resilience in Biology Across Scales. Integr Comp Biol 61, 2163–2179 (2022). [PubMed: 34427654]
21. Harrison R, Papp B, Pal C, Oliver SG, Delneri D, Plasticity of genetic interactions in metabolic networks of yeast. Proc Natl Acad Sci U S A 104, 2307–2312 (2007). [PubMed: 17284612]
22. Naseeb S, Ames RM, Delneri D, Lovell SC, Rapid functional and evolutionary changes follow gene duplication in yeast. Proc Biol Sci 284, (2017).
23. Goldhill D, Lee A, Williams ES, Turner PE, Evolvability and robustness in populations of RNA virus Phi6. Front Microbiol 5, 35 (2014). [PubMed: 24550904]
24. McBride RC, Ogbunugafor CB, Turner PE, Robustness promotes evolvability of thermotolerance in an RNA virus. BMC Evol Biol 8, 231 (2008). [PubMed: 18694497]
25. Montville R, Froissart R, Remold SK, Tenaillon O, Turner PE, Evolution of mutational robustness in an RNA virus. PLoS Biol 3, e381 (2005). [PubMed: 16248678]
26. Sanjuan R, Cuevas JM, Furio V, Holmes EC, Moya A, Selection for robustness in mutagenized RNA viruses. PLoS Genet 3, e93 (2007). [PubMed: 17571922]
27. Ancel LW, Fontana W, Plasticity, evolvability, and modularity in RNA. J Exp Zool 288, 242–283 (2000). [PubMed: 11069142]
28. Lehner B, Crombie C, Tischler J, Fortunato A, Fraser AG, Systematic mapping of genetic interactions in Caenorhabditis elegans identifies common modifiers of diverse signaling pathways. Nature Genetics 38, 896–903 (2006). [PubMed: 16845399]
29. Siegal ML, Bergman A, Waddington's canalization revisited: developmental stability and evolution. Proc Natl Acad Sci U S A 99, 10528–10532 (2002). [PubMed: 12082173]
30. Zabinsky RA, Mason GA, Queitsch C, Jarosz DF, It's not magic - Hsp90 and its effects on genetic and epigenetic variation. Semin Cell Dev Biol 88, 21–35 (2019). [PubMed: 29807130]
31. Bhattacharya K. et al. , Translational reprogramming in response to accumulating stressors ensures critical threshold levels of Hsp90 for mammalian life. Nat Commun 13, 6271 (2022). [PubMed: 36270993]
32. Borkovich KA, Farrelly FW, Finkelstein DB, Taulien J, Lindquist S, hsp82 is an essential protein that is required in higher concentrations for growth of cells at higher temperatures. Mol Cell Biol 9, 3919–3930 (1989). [PubMed: 2674684]
33. Kadibalban AS, Bogumil D, Landan G, Dagan T, DnaK-Dependent Accelerated Evolutionary Rate in Prokaryotes. Genome Biol Evol 8, 1590–1599 (2016). [PubMed: 27189986]
34. Bogumil D, Dagan T, Chaperonin-dependent accelerated substitution rates in prokaryotes. Genome Biol Evol 2, 602–608 (2010). [PubMed: 20660111]
35. Aguilar-Rodriguez J. et al. , The Molecular Chaperone DnaK Is a Source of Mutational Robustness. Genome Biol Evol 8, 2979–2991 (2016). [PubMed: 27497316]
36. Maisnier-Patin S. et al. , Genomic buffering mitigates the effects of deleterious mutations in bacteria. Nat Genet 37, 1376–1379 (2005). [PubMed: 16273106]
37. Queitsch C, Sangster TA, Lindquist S, Hsp90 as a capacitor of phenotypic variation. Nature 417, 618–624 (2002). [PubMed: 12050657]
38. Rutherford SL, Lindquist S, Hsp90 as a capacitor for morphological evolution. Nature 396, 336–342 (1998). [PubMed: 9845070]
39. Cowen LE, Lindquist S, Hsp90 potentiates the rapid evolution of new traits: drug resistance in diverse fungi. Science 309, 2185–2189 (2005). [PubMed: 16195452]
40. Iyengar BR, Wagner A, GroEL/S Overexpression Helps to Purge Deleterious Mutations and Reduce Genetic Diversity during Adaptive Protein Evolution. Mol Biol Evol 39, (2022).
41. Iyengar BR, Wagner A, Bacterial Hsp90 predominantly buffers but does not potentiate the phenotypic effects of deleterious mutations during fluorescent protein evolution. Genetics 222, (2022).
42. Fares MA, Ruiz-Gonzalez MX, Moya A, Elena SF, Barrio E, Endosymbiotic bacteria: groEL buffers against deleterious mutations. Nature 417, 398 (2002). [PubMed: 12024205]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

43. Karras etal GI., HSP90 Shapes the Consequences of Human Genetic Variation. Cell 168, 856–866 e812 (2017). [PubMed: 28215707]
44. Jarosz DF, Lindquist S, Hsp90 and environmental stress transform the adaptive value of natural genetic variation. Science 330, 1820–1824 (2010). [PubMed: 21205668]
45. Yeyati PL, Bancewicz RM, Maule J, van Heyningen V, Hsp90 selectively modulates phenotype in vertebrate development. PLoS Genet 3, e43 (2007). [PubMed: 17397257]
46. Rohner N. et al. , Cryptic variation in morphological evolution: HSP90 as a capacitor for loss of eyes in cavefish. Science 342, 1372–1375 (2013). [PubMed: 24337296]
47. Whitesell L, Lindquist SL, HSP90 and the chaperoning of cancer. Nat Rev Cancer 5, 761–772 (2005). [PubMed: 16175177]
48. Sangster TA et al. , HSP90-buffered genetic variation is common in Arabidopsis thaliana. Proc Natl Acad Sci U S A 105, 2969–2974 (2008). [PubMed: 18287064]
49. Whitesell L. et al. , HSP90 empowers evolution of resistance to hormonal therapy in human breast cancer models. Proc Natl Acad Sci U S A 111, 18297–18302 (2014). [PubMed: 25489079]
50. Wilkins AS, Canalization: a molecular genetic perspective. Bioessays 19, 257–262 (1997). [PubMed: 9080776]
51. Xu Y, Lindquist S, Heat-shock protein hsp90 governs the activity of pp60v-src kinase. Proc Natl Acad Sci U S A 90, 7074–7078 (1993). [PubMed: 7688470]
52. Bali M, Zhang B, Morano KA, Michels CA, The Hsp90 molecular chaperone complex regulates maltose induction and stability of the Saccharomyces MAL gene transcription activator Mal63p. J Biol Chem 278, 47441–47448 (2003). [PubMed: 14500708]
53. Ran F, Bali M, Michels CA, Hsp90/Hsp70 chaperone machine regulation of the Saccharomyces MAL-activator as determined in vivo using noninducible and constitutive mutant alleles. Genetics 179, 331–343 (2008). [PubMed: 18458105]
54. Gopinath RK, Leu JY, Hsp90 Maintains Proteostasis of the Galactose Utilization Pathway To Prevent Cell Lethality. Mol Cell Biol 36, 1412–1424 (2016). [PubMed: 26951197]
55. Gallone B et al. , Domestication and Divergence of Saccharomyces cerevisiae Beer Yeasts. Cell 166, 1397–1410 e1316 (2016). [PubMed: 27610566]
56. Nathan DF, Lindquist S, Mutational analysis of Hsp90 function: interactions with a steroid receptor and a protein kinase. Mol Cell Biol 15, 3917–3925 (1995). [PubMed: 7791797]
57. Bai FY, Han DY, Duan SF, Wang QM, The Ecology and Evolution of the Baker's Yeast Saccharomyces cerevisiae. Genes (Basel) 13, (2022). [PubMed: 36672755]
58. Steensels J, Gallone B, Voordeckers K, Verstrepen KJ, Domestication of Industrial Microbes. Curr Biol 29, R381–R393 (2019). [PubMed: 31112692]
59. Charron MJ, Read E, Haut SR, Michels CA, Molecular evolution of the telomere-associated MAL loci of Saccharomyces. Genetics 122, 307–316 (1989). [PubMed: 2548922]
60. Brown CA, Murray AW, Verstrepen KJ, Rapid expansion and functional divergence of subtelomeric gene families in yeasts. Curr Biol 20, 895–903 (2010). [PubMed: 20471265]
61. Duval EH, Alves SL Jr., Dunn B, Sherlock G, Stambuk BU, Microarray karyotyping of maltose-fermenting Saccharomyces yeasts with differing maltotriose utilization profiles reveals copy number variation in genes involved in maltose and maltotriose utilization. J Appl Microbiol 109, 248–259 (2010). [PubMed: 20070441]
62. Bigey F et al. , Evidence for Two Main Domestication Trajectories in Saccharomyces cerevisiae Linked to Distinct Bread-Making Processes. Curr Biol 31, 722–732 e725 (2021). [PubMed: 33301710]
63. Goncalves M et al. , Distinct Domestication Trajectories in Top-Fermenting Beer Yeasts and Wine Yeasts. Curr Biol 26, 2750–2761 (2016). [PubMed: 27720622]
64. Steenwyk J, Rokas A, Extensive Copy Number Variation in Fermentation-Related Genes Among Saccharomyces cerevisiae Wine Strains. G3 (Bethesda) 7, 1475–1485 (2017). [PubMed: 28292787]
65. Duan SF et al. , The origin and adaptive evolution of domesticated populations of yeast from Far East Asia. Nat Commun 9, 2690 (2018). [PubMed: 30002370]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

66. Michels CA, Needleman RB, The dispersed, repeated family of MAL loci in Saccharomyces spp. J Bacteriol 157, 949–952 (1984). [PubMed: 6321448]
67. Dunn B, Richter C, Kvitek DJ, Pugh T, Sherlock G, Analysis of the Saccharomyces cerevisiae pan-genome reveals a pool of copy number variants distributed in diverse yeast strains from differing industrial environments. Genome Res 22, 908–924 (2012). [PubMed: 22369888]
68. Day RE, Higgins VJ, Rogers PJ, Dawes IW, Characterization of the putative maltose transporters encoded by YDL247w and YJR160c. Yeast 19, 1015–1027 (2002). [PubMed: 12210897]
69. Voordeckers K. et al. , Reconstruction of ancestral metabolic enzymes reveals molecular mechanisms underlying evolutionary innovation through gene duplication. PLoS Biol 10, e1001446 (2012). [PubMed: 23239941]
70. De Chiara M et al. , Domestication reprogrammed the budding yeast life cycle. Nat Ecol Evol 6, 448–460 (2022). [PubMed: 35210580]
71. Gibson BR, Lawrence SJ, Leclaire JP, Powell CD, Smart KA, Yeast responses to stresses associated with industrial brewery handling. FEMS Microbiol Rev 31, 535–569 (2007). [PubMed: 17645521]
72. Attfield PV, Stress tolerance: the key to effective strains of industrial baker's yeast. Nat Biotechnol 15, 1351–1357 (1997). [PubMed: 9415886]
73. Alford BD, Brandman O, Quantification of Hsp90 availability reveals differential coupling to the heat shock response. J Cell Biol 217, 3809–3816 (2018). [PubMed: 30131327]
74. Voordeckers K et al. , Ethanol exposure increases mutation rate through error-prone polymerases. Nat Commun 11, 3664 (2020). [PubMed: 32694532]
75. Gavrilets S, Hastings A, A Quantitative-Genetic Model for Selection on Developmental Noise. Evolution 48, 1478–1486 (1994). [PubMed: 28568416]
76. Stearns SC, Progress on canalization. Proc Natl Acad Sci U S A 99, 10229–10230 (2002). [PubMed: 12149521]
77. Nowak MA, Boerlijst MC, Cooke J, Smith JM, Evolution of genetic redundancy. Nature 388, 167–171 (1997). [PubMed: 9217155]
78. Milloz J, Duveau F, Nuez I, Felix MA, Intraspecific evolution of the intercellular signaling network underlying a robust developmental system. Genes Dev 22, 3064–3075 (2008). [PubMed: 18981482]
79. Bauer CR, Li S, Siegal ML, Essential gene disruptions reveal complex relationships between phenotypic robustness, pleiotropy, and fitness. Mol Syst Biol 11, 773 (2015). [PubMed: 25609648]
80. Thesseling FA, Bircham PW, Mertens S, Voordeckers K, Verstrepen KJ, A Hands-On Guide to Brewing and Analyzing Beer in the Laboratory. Curr Protoc Microbiol 54, e91 (2019). [PubMed: 31518063]
81. Whittington HD, Dagher SF, Bruno-Bárcena JM, Production and Conservation of Starter Cultures: From "Backslopping" to Controlled Fermentations. (Springer, 2019).
82. De Vuyst L, Comasio A, Kerrebroeck SV, Sourdough production: fermentation strategies, microbial ecology, and use of non-flour ingredients. Crit Rev Food Sci Nutr, 1–33 (2021).
83. Lahue C, Madden AA, Dunn RR, Smukowski Heil C, History and Domestication of Saccharomyces cerevisiae in Bread Baking. Front Genet 11, 584718 (2020). [PubMed: 33262788]
84. Steenwyk JL, Rokas A, Copy Number Variation in Fungi and Its Implications for Wine Yeast Genetic Diversity and Adaptation. Front Microbiol 9, 288 (2018). [PubMed: 29520259]
85. Goddard MR, Greig D, Saccharomyces cerevisiae: a nomadic yeast with no niche? FEMS Yeast Res 15, (2015).
86. Zheng X, D'Amore T, Russell I, Stewart GG, Factors Influencing Maltotriose Utilization During Brewery Wort Fermentations. Journal of the American Society of Brewing Chemists 52, 41–47 (1994).
87. Jayaram VB et al. , Ethanol at levels produced by Saccharomyces cerevisiae during wheat dough fermentation has a strong impact on dough properties. J Agric Food Chem 62, 9326–9335 (2014). [PubMed: 25174613]
88. Patel GB, Ingledew WM, Trends in wort carbohydrate utilization. Appl Microbiol 26, 349–353 (1973). [PubMed: 16349967]

89. Denby CM et al. , Industrial brewing yeast engineered for the production of primary flavor determinants in hopped beer. Nat Commun 9, 965 (2018). [PubMed: 29559655]
90. Ignea C et al. , Improving yeast strains using recyclable integration cassettes, for the production of plant terpenoids. Microb Cell Fact 10, 4 (2011). [PubMed: 21276210]
91. Rehm J et al. , The relationship between different dimensions of alcohol use and the burden of disease-an update. Addiction 112, 968–1001 (2017). [PubMed: 28220587]
92. Sokol RJ, Delaney-Black V, Nordstrom B, Fetal alcohol spectrum disorder. JAMA 290, 2996–2999 (2003). [PubMed: 14665662]
93. Condelli V et al. , HSP90 Molecular Chaperones, Metabolic Rewiring, and Epigenetics: Impact on Tumor Progression and Perspective for Anticancer Therapy. Cells 8, (2019). [PubMed: 31861404]
94. Lauer S, Gresham D, An evolving view of copy number variants. Curr Genet 65, 1287–1295 (2019). [PubMed: 31076843]
95. Fitzgerald DM, Hastings PJ, Rosenberg SM, Stress-Induced Mutagenesis: Implications in Cancer and Drug Resistance. Annu Rev Cancer Biol 1, 119–140 (2017). [PubMed: 29399660]
96. Gibson G, Lacek KA, Canalization and Robustness in Human Genetics and Disease. Annu Rev Genet 54, 189–211 (2020). [PubMed: 32867542]
97. Koonin EV, Wolf YI, Is evolution Darwinian or/and Lamarckian? Biol Direct 4, 42 (2009). [PubMed: 19906303]
98. Shropshire WC, Lermi NO, Hanson B, Karras GI (2024; 10.5281/zenodo.10871210).

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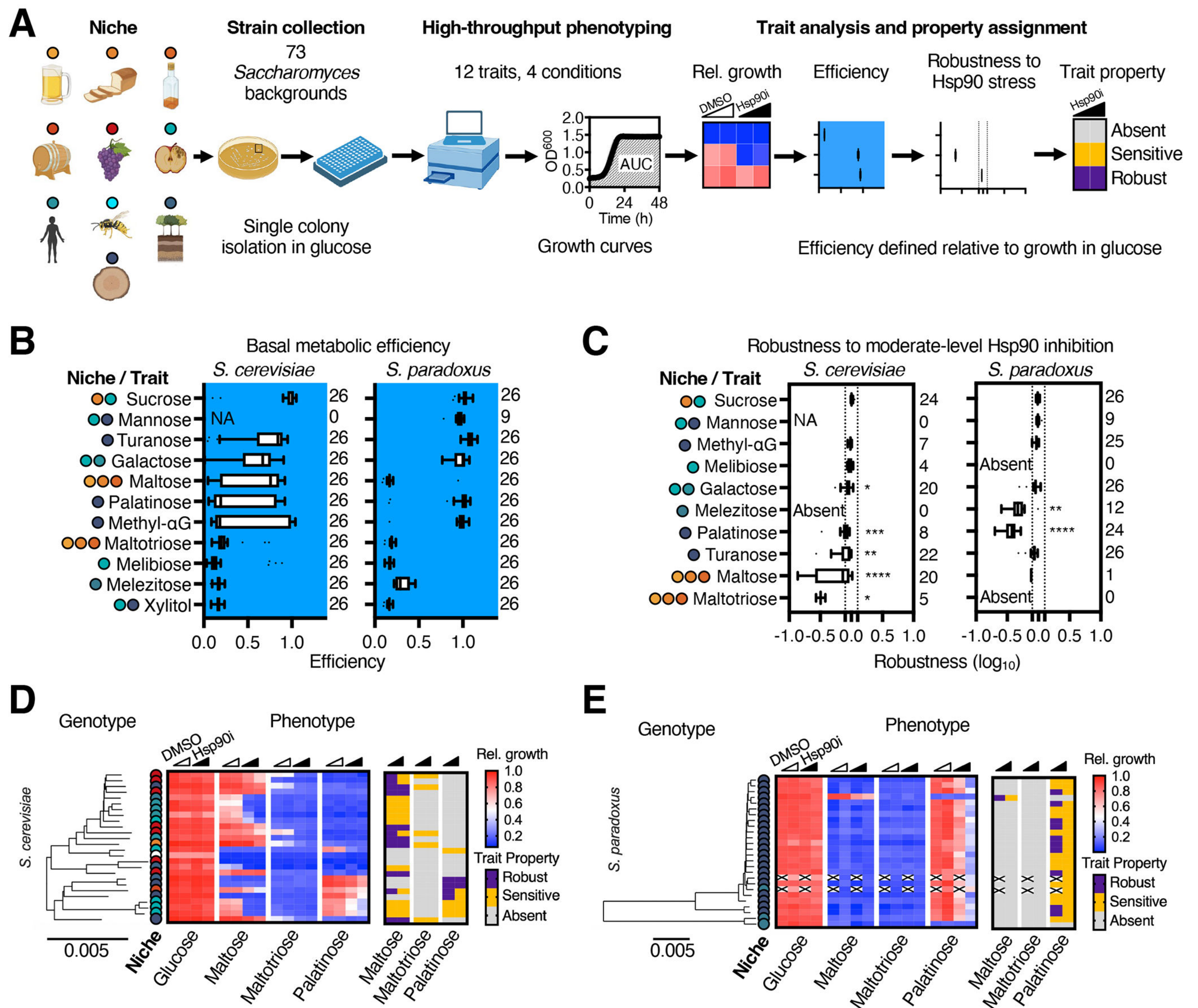

Fig. 1. Ecology shapes the robustness of metabolic traits to Hsp90 stress.

(**A**) Schematic of the approach used to survey natural Hsp90-dependent variation in yeast metabolism. Strains originating from 10 distinct ecological niches were evaluated for 12 ecologically relevant metabolic traits. Metabolic traits were assessed in high throughput by generating growth curves in media containing the indicated sugar as the sole source of carbon, under low (4 μM) and moderate radicicol concentrations (10 μM) and vehicle control (low and moderate DMSO, basal) conditions. Growth parameters from each growth curve were used to estimate relative growth (heatmap; red: maximum, 1.0; blue: minimum, 0.0), efficiency (box plots with blue background), robustness (box plots with white background), and trait property (orange: sensitive, –0.1; purple: robust, > –0.1; grey: trait absent; white with black cross: not determined) for each trait/strain. (**B** and **C**) Left: The niches where each sugar is found are indicated alongside the sugars. Right: Box plots of the (B) basal efficiency and (C) robustness to radicicol by trait and yeasts species. Differences in

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variance are indicated as ns (not significant), $P > 0.05$; *P 0.05; **P 0.01; ***P 0.001; ****P 0.0001; Kruskal-Wallis test with Dunn multiple comparisons test. (**D** and **E**) Left: Dendrograms of yeast genomes. Middle: Yest origins. Right: Heat maps of relative growth and estimates of robustness to radicicol are shown for maltose, maltotriose, and palatinose metabolism across (D) 26 *S. cerevisiae* and (E) 26 *S. paradoxus MATα* isolates. Filled triangles indicate samples exposed to increasing concentrations of radicicol; empty triangles indicate treatment with vehicle (DMSO). Values are averages of 3 independent experiments. Left: Niches: orange: bread; yellow: beer; red: whiskey; purple: wine; deep purple: other fermentation-associated; lime: bark; green: clinical; light blue: wasp gut; blue: soil; pink: fruit; grey: unknown. Depictions of yeast cells and niches were created with BioRender (https://biorender.com/).

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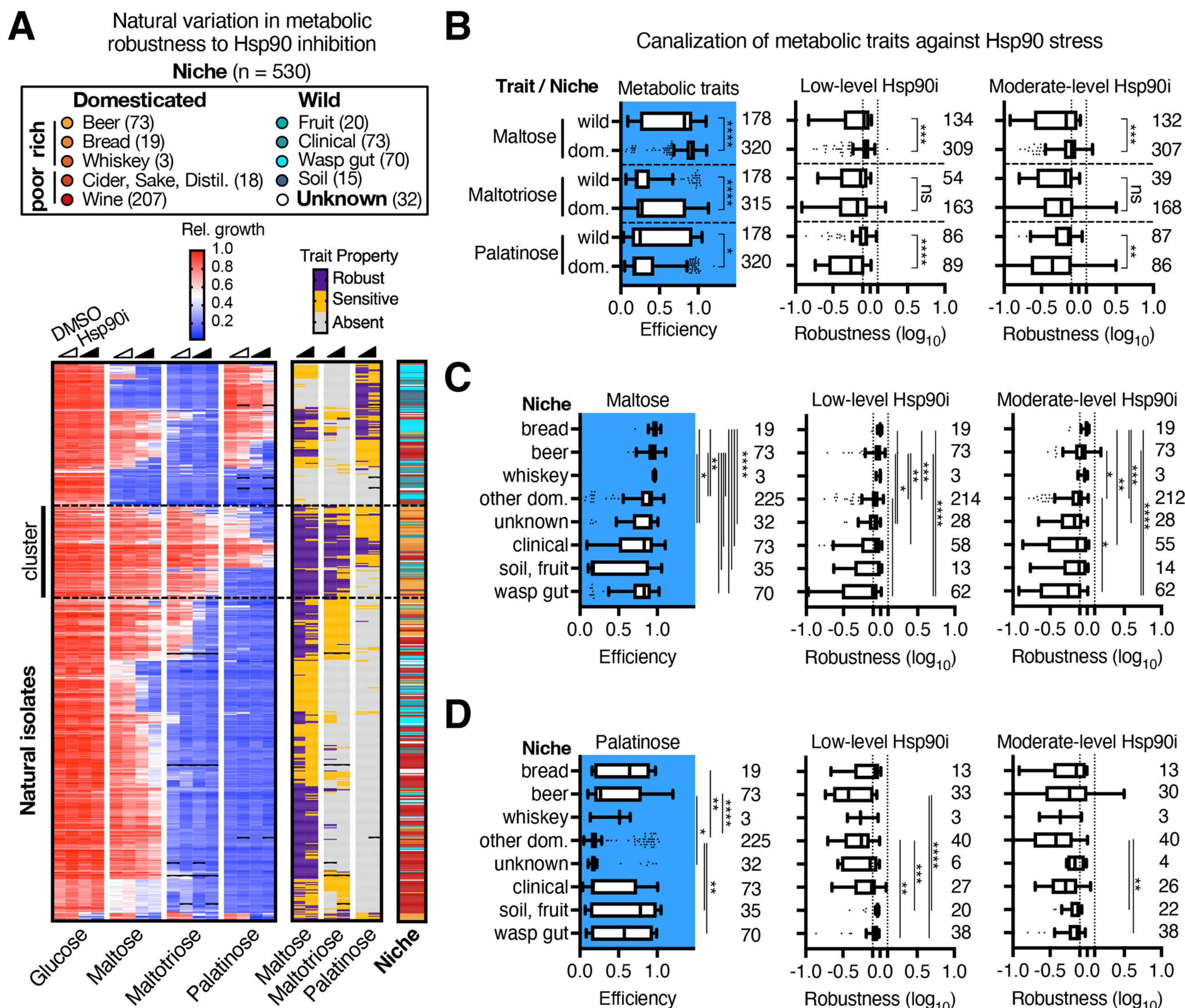

Fig. 2. Selection for trait robustness to Hsp90 stress during yeast domestication.

(**A**) Heat map of relative growth and robustness estimates for the indicated metabolic traits in 530 natural *Saccharomyces* isolates, under basal conditions (empty triangles) and under low (4 μM) and moderate (10 μM) concentrations of radicicol (Hsp90i, filled triangles) and DMSO (control). Strain origin (niche) is indicated on the top and the right. (**B** to **D**) Box plots of the efficiency (left) of maltose, maltotriose, and palatinose metabolism and the robustness of these metabolic traits to low-level (middle) and moderate-level (right) Hsp90 inhibition. (C and D) Efficiency and robustness data by strain origin (niche), comparing domesticated (wine, beer, bread, distillation, other) and wild (clinical, soil, fruit, wasp gut) strains. Maltose-rich ("rich": bread, beer, whiskey) and maltose-poor niches ("poor": wine, other fermentation-associated) are indicated. Values are averages of 3 independent experiments. Whiskers based on Tukey method. Differences between distributions are

indicated as ns, $P > 0.05$; *P 0.05; **P 0.01; ***P 0.001; ****P 0.0001; Kruskal-Wallis test with Dunn multiple comparisons test.

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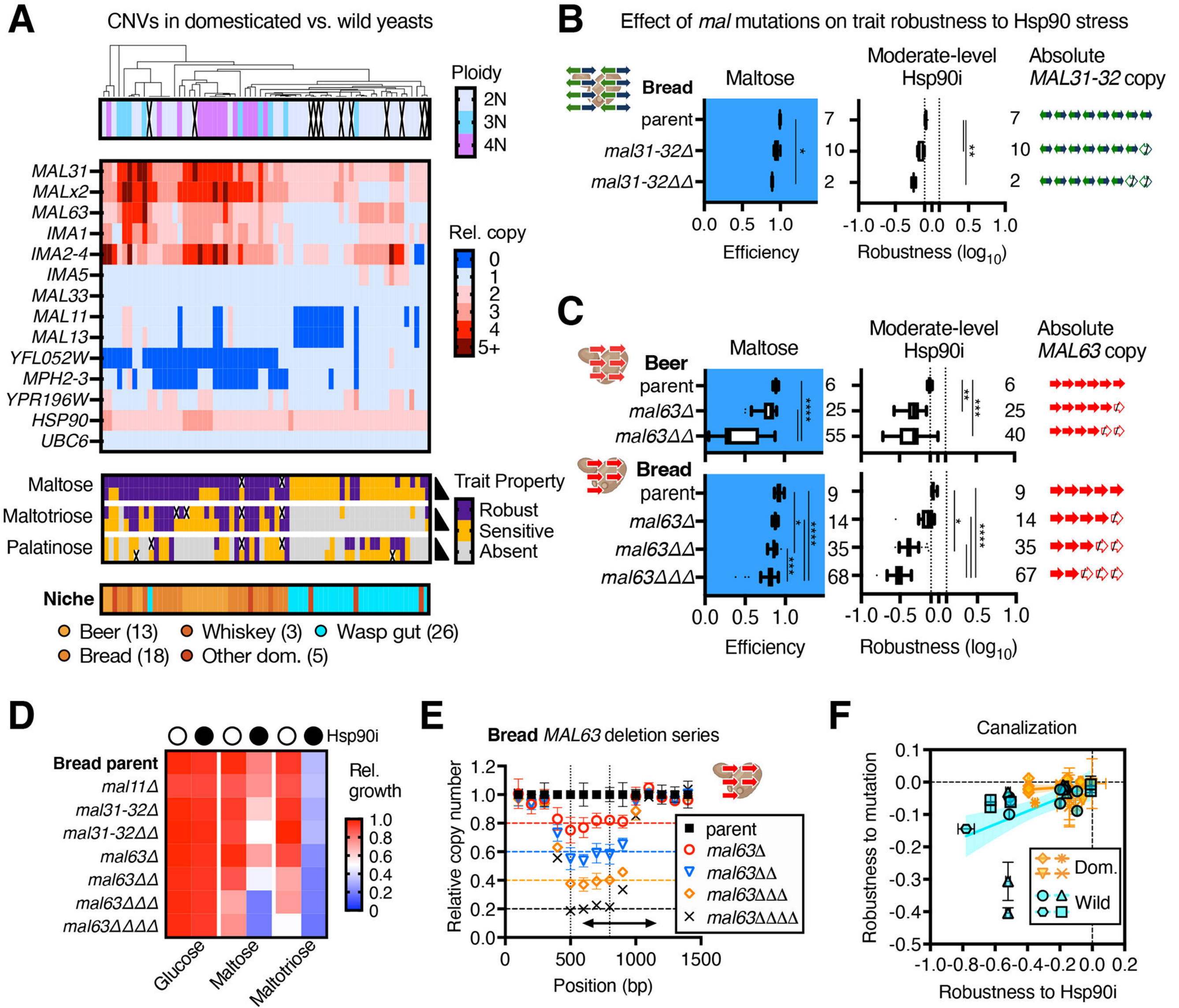

Fig. 3. Copy-number signatures of beer and bread domestication canalize metabolism against Hsp90 stress.

(**A**) Heat map showing (top to bottom): ploidy, CNVs of the indicated genes, robustness of metabolic traits to low- (4 μM) and moderate-level (10 μM) Hsp90i with radicicol, and niches for 63 natural yeast strains sequenced at 242× median coverage (min: 133.2×, max: 386.7×). (**B** and **C**) Genotype schematics where arrows indicate the absolute copy number of the indicated *MAL* genes in the parental strain (green: *MAL31;* blue: *MAL32;* red: *MAL63*). Box plots of basal efficiency and robustness of maltose metabolism to Hsp90 stress (radicicol, 10 μM) in lineages derived by disruption of (B) *MAL31-MAL32* copies, and (C) *MAL63* copies in industrial strains NCYC 1529 (bread) and WLP802 (Czech Budejovice lager beer). The number of biological replicates is shown next to each plot. (**D**) Heat map of relative growth estimates for the indicated traits in *MAL* gene-disrupted NCYC 1529 derivatives, evaluated under basal and radicicol treatment (10 μM) conditions. (**E**)

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CNV analysis of the *MAL63* gene in NCYC 1529 (contains five *MAL63* copies) and *mal63* derivatives. Relative coverage is shown at each non-overlapping 100-bp window spanning the length of the open reading frame (ORF) for 2 to 5 replicate strains per genotype. (**F**) Correlation between robustness of maltose, maltotriose, and turanose metabolism to Hsp90 stress (x-axis) and the robustness of these traits to mutations (single copy deletion of *MAL31* or *MAL63;* y-axis) across 4 domesticated (NCYC 1529, WLP802, WLD whiskey, Rum Turbo) and 4 wild yeast backgrounds (wasp gut: 89, 93, 436, 449) indicated by different symbol/shapes. Simple linear regression, Dom.: $y = 0.0303 \times x - 0.0107$, Wild: $y = 0.2031 \times x - 0.0085$ (difference between slopes: $F_{1,97} = 5.209$, two-sided $P = 0.0247$). Averages and standard deviations were derived from 3 independent replicate experiments. Differences between distributions are indicated as ns, $P > 0.05$; $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$; $****P \leq 0.0001$; Kruskal-Wallis test with Dunn multiple comparisons test (Fig. 3, B and C) and ANCOVA (Fig. 3F).

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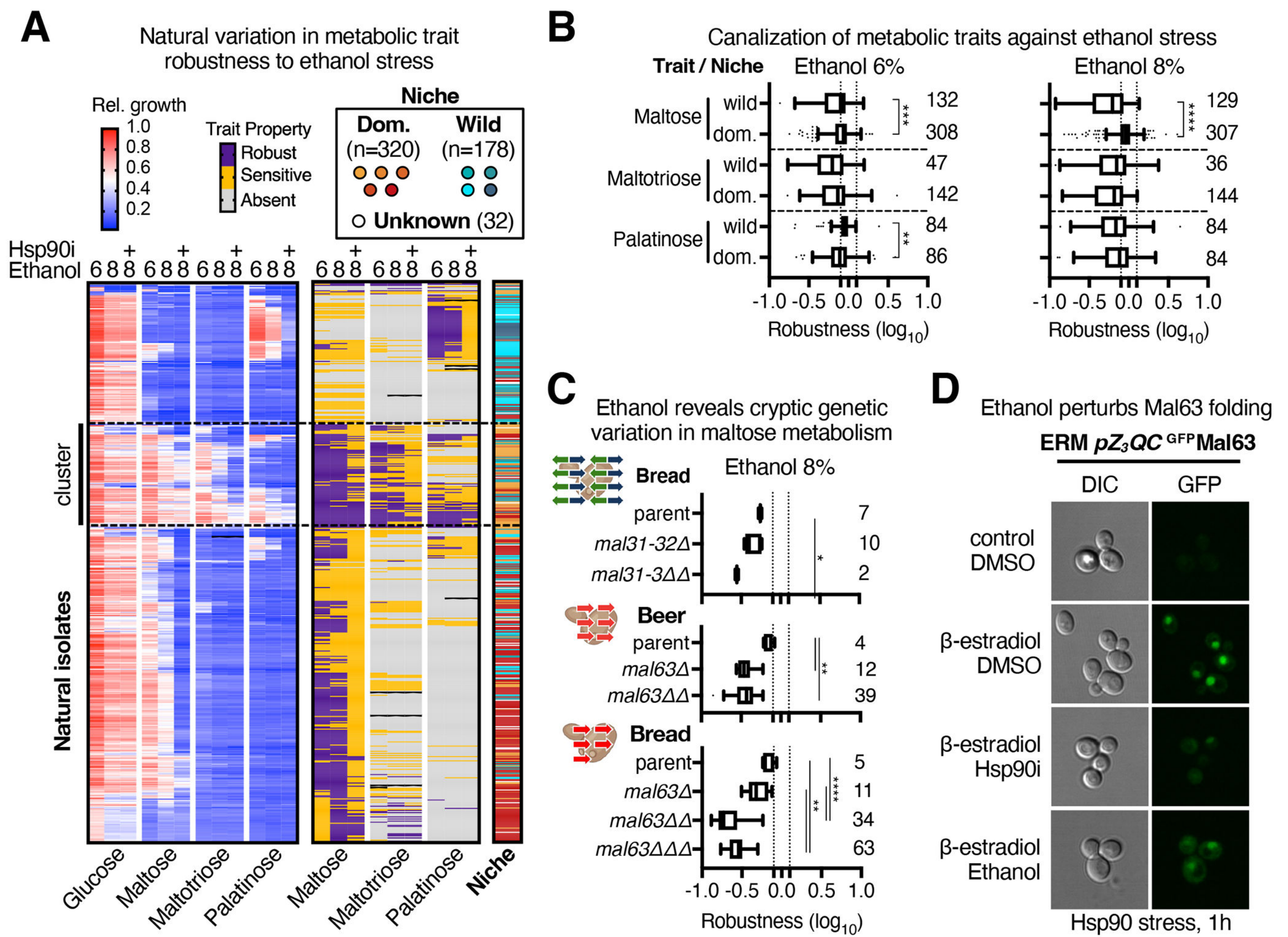

Fig. 4. Ethanol exposure de-canalizes metabolic traits by eliciting Hsp90 stress.

(**A**) Heat map of relative growth and trait robustness estimates for 530 natural *Saccharomyces* isolates evaluated under different levels of ethanol stress. Left to right: Ethanol 6% (v/v), ethanol 8% with DMSO, and ethanol 8% with radicicol (10 μM). (**B**) Box plots of maltose metabolism robustness to ethanol stress (6%, v/v) by strain origin. (**C**) Genotype schematic and box plots of maltose metabolism robustness against ethanol stress (8%, v/v) across *mal31-32* and *mal63* mutants of industrial strains NCYC 1529 (bread) and WLP802 (Czech Budejovice lager beer). (**E**) Confocal microscopy of BY4741 cells expressing GFP-Mal63 under the control of a β-estradiol–inducible promoter and growing in synthetic drop out media containing maltose (SM-URA) and β-estradiol (100 nM) under basal and Hsp90 stress conditions (1 hour exposure to radicicol 10 μM, vs. ethanol 6%, v/v). Averages and standard deviations were derived from 3 independent experiments. Differences between distributions are indicated as ns, $P > 0.05$; *P 0.05; **P 0.01; ***P 0.001; ****P 0.0001; Kruskal-Wallis test with Dunn multiple comparisons test.

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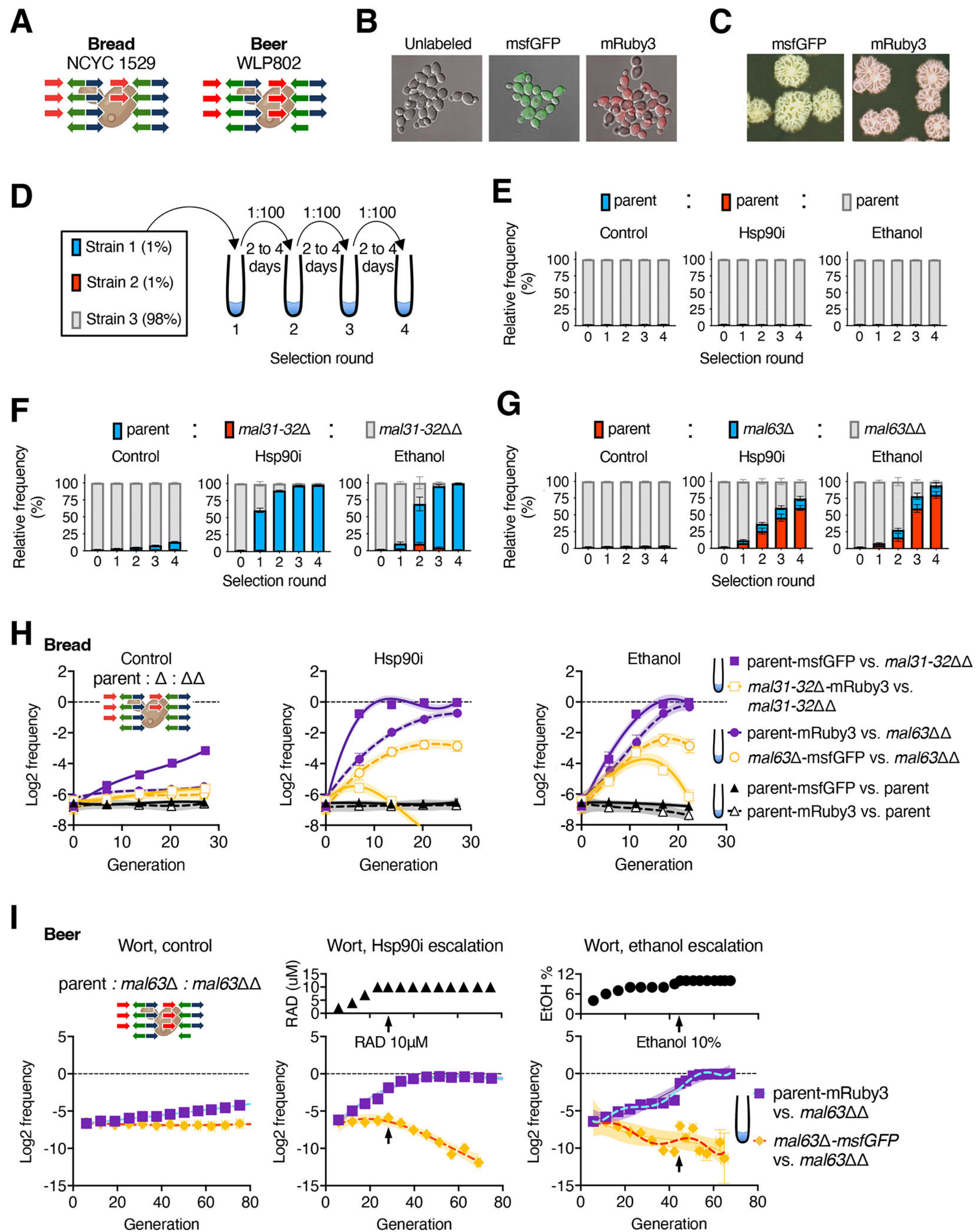

Fig. 5. Ethanol selects for redundant gene duplications conducive to adaptive canalization of metabolism against Hsp90 stress.

(**A**) Schematic of genotype of parental NCYC 1529 bread strain and parental WLP802 beer strain. (**B**) Confocal microscopy of microcolonies of NCYC 1529 derivatives stably expressing different fluorescent proteins under the control of a strong constitutive (GPD) promoter. Strains were grown for 3 days in SM complete media. (**C**) Image of fluorescent NCYC 1529 colonies under visible light after growth on YPD agar plates for 9 days at room temperature. (**D**) Schematic of serial passaging in competition assays involving isogenic

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strains. Dilutions and timing of each selection round are shown. (**E** to **G**) Flow cytometry analysis of 1:1:98 mixed populations passaging through sequential broad bottlenecks (1:100) and allowed to expand in YPM under basal conditions, moderate-level Hsp90 inhibition (radicicol, 10 μM), or ethanol stress (8%, v/v), using the following strain combinations: (E) parental strains competing against each other, (F) single (mRuby3) and double *mal31-32* disrupted lineages competing against the parent (msfGFP), and (G) single (msfGFP) and double *mal63* disrupted lineages competing against the parent (mRuby3). (**H** and **I**) Log2 ratios of genotypic frequencies plotted over the number of generations under basal conditions (left panels) relative to the frequency of unlabeled double disrupted derivatives of (H) NCYC 1529 (*mal31-32*, *mal63*) (raw data: Fig. 5, E to G; regression analyses: table S11), and (I) WLP802 beer strain, competing in wort against labeled parent derivatives in 1:1:98 ratio (raw data: fig. S11, D to F; regression analyses: table S12). Strains are in competition in the same culture are indicated with a test tube symbol. Differences between slopes and distributions are indicated as ns, $P > 0.05$; *P 0.05; **P 0.01; ***P 0.001; ****P 0.0001; Kruskal-Wallis test with Dunn multiple comparisons test.

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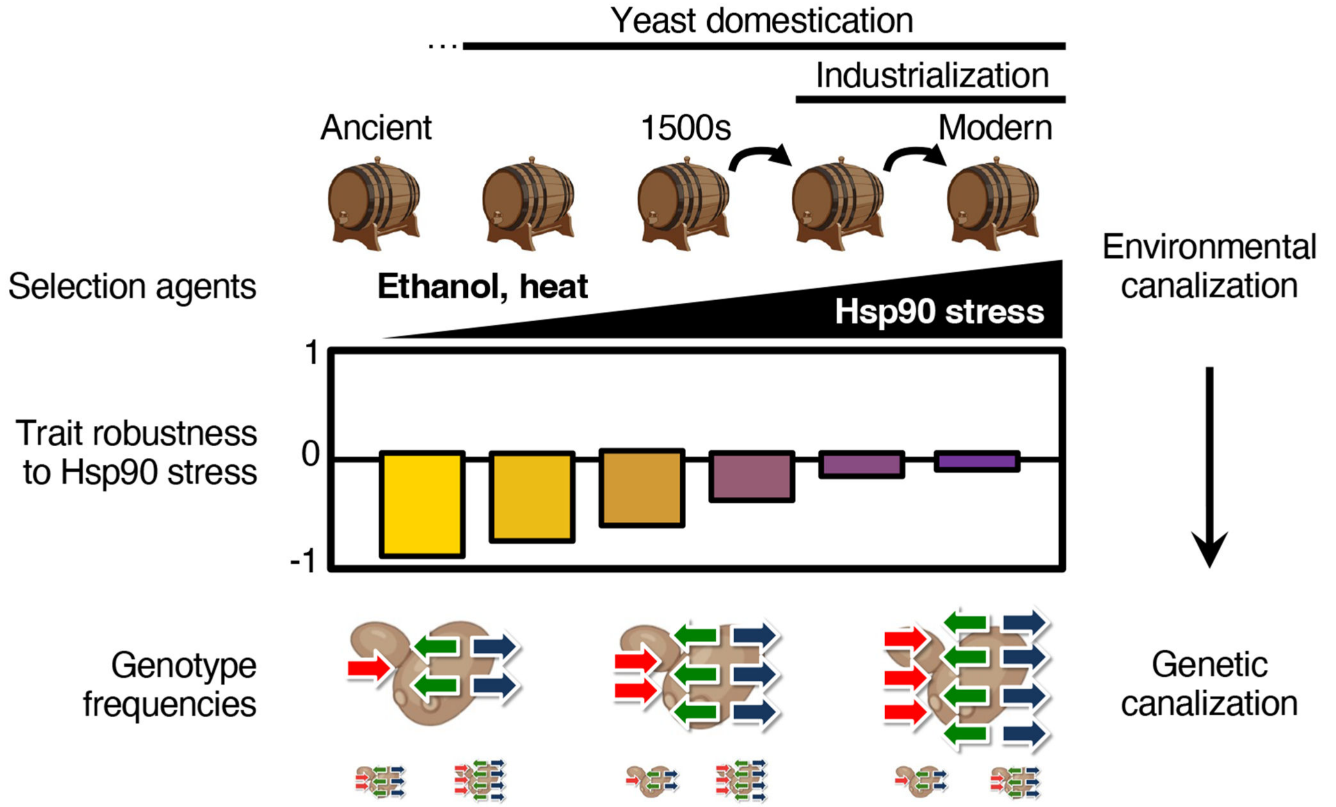

Fig. 6. Canalization of industrial traits against niche-related Hsp90 stress enables yeast domestication.

Model of environmental canalization and congruent genetic canalization of Hsp90-dependent industrial traits. Diverse metabolic traits in yeast are inherently sensitive to Hsp90 stress. Industrial traits maltose and maltotriose metabolism evolved robustness to Hsp90 stress across diverse domesticated yeasts without losing their inherent Hsp90-dependence. This canalization was driven by gains in redundancy. Redundant copies of metabolic *MAL* genes confer strong fitness advantages to industrial yeast in wort and sourdough only in the presence of Hsp90 stressors such as ethanol. Ethanol impairs a central Hsp90-dependent regulator of maltose and maltotriose metabolism; redundant *MAL* genes make up for ethanol's proteotoxic effects on these traits. Through the practice of backslopping, ancient *MAL* gene duplications that preexisted within pre- and early domestication yeast communities were strongly selected because they canalize maltose and maltotriose metabolism against ethanol stress and other niche-related Hsp90 stressors (i.e., heat), thus yielding the modern genomic signatures of yeast domestication. Size of the yeast genotype schematic indicates proposed average relative *MAL* gene copy number between ancient, medieval, and modern yeast niches associated with humans.