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Current Biology

Domestication through clandestine cultivation constrained genetic diversity in magic mushrooms relative to naturalized populations

Highlights

- Magic mushrooms (*Psilocybe cubensis*) have genetic signatures of domestication
- Cultivars are closely related and have low allelic diversity and low heterozygosity
- Naturalized populations in Australia have comparatively higher levels of diversity
- *Psilocybe cubensis* has a tetrapolar mating-compatibility system

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In brief

McTaggart et al. compare impacts of domestication and naturalization in magic mushrooms (*Psilocybe cubensis*). Domesticated cultivars have few founders, highly homozygous genomes, and low allelic diversity caused by inbreeding. Naturalized populations in Australia maintained genetic diversity and have unique alleles for psilocybin production.



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Domestication through clandestine cultivation constrained genetic diversity in magic mushrooms relative to naturalized populations

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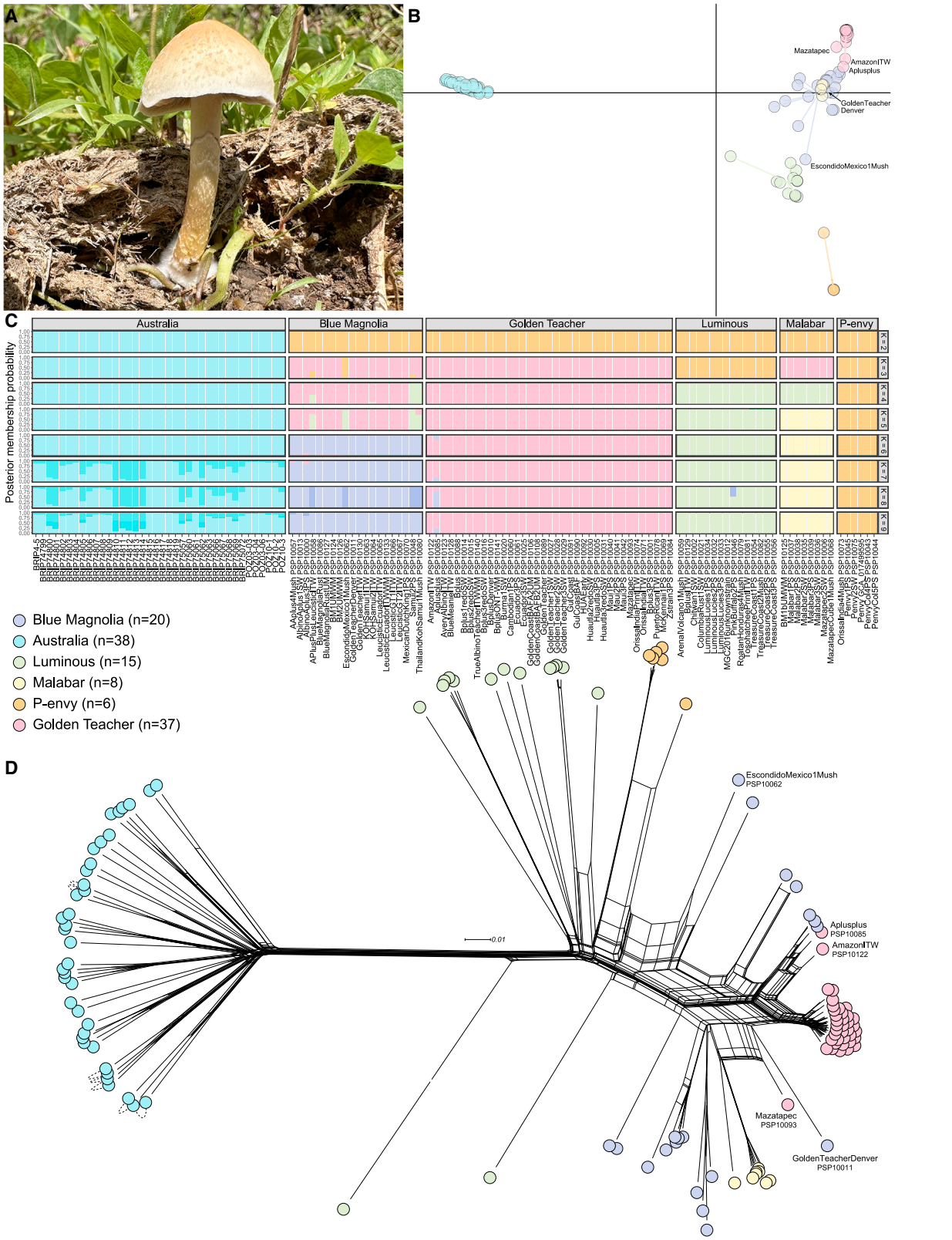
SUMMARY

Fungi that are edible or fermentative were domesticated through selective cultivation of their desired traits. Domestication is often associated with inbreeding or selfing, which may fix traits other than those under selection, and causes an overall decrease in heterozygosity. A hallucinogenic mushroom, *Psilocybe cubensis*, was domesticated from its niche in livestock dung for production of psilocybin. It has caused accidental poisonings since the 1940s in Australia, which is a population hypothesized to be introduced from an unknown center of origin. We sequenced genomes of 38 isolates from Australia and compared them with 86 genomes of commercially available cultivars to determine (1) whether *P. cubensis* was introduced to Australia, and (2) how domestication has impacted commercial cultivars. Our analyses of genome-wide SNPs and single-copy orthologs showed that the Australian population is naturalized, having recovered its effective population size after a bottleneck when it was introduced, and it has maintained relatively high genetic diversity based on measures of nucleotide and allelic diversity. In contrast, domesticated cultivars generally have low effective population sizes and hallmarks of selfing and clonal propagation, including low genetic diversity, low heterozygosity, high linkage disequilibrium, and low allelic diversity of mating-compatibility genes. Analyses of kinship show that most cultivars are founded from related populations. Alleles in the psilocybin gene cluster are identical across most cultivars of *P. cubensis* with low diversity across coding sequence; however, unique allelic diversity in Australia and some cultivars may translate to differences in biosynthesis of psilocybin and its analogs.

INTRODUCTION

Domestication in agriculture has advanced human development and cultural practice.[1] Domestication artificially selects for desired traits by causing genetic bottlenecks that may leave a footprint across the genome through fixation of favored alleles.[2] Fungi have been domesticated for baking,[3] brewing,[4] cheese-making,[5] and soybean fermentation,[6] and yeasts, molds, and mushrooms were selected for efficiency at metabolic loci or reduced production of metabolites toxic to humans. Clonality may favor co-selection of multiple desired traits but can fix unselected traits, such as the fixation of a single mating-type allele along with reduced toxin production and desirable color in the cheese mold, *Penicillium camemberti*,[5] and alleles that increase survival along with those that reduce competency for sexual reproduction in brewing yeasts.[7] Desirable traits have been fixed by inbreeding in outcrossing fungi, such as edible mushrooms.[8] However, inbreeding in long-term outcrossing fungi leads to extended regions of homozygosity and inbreeding depression that cause strain degeneration and require outcrossing for genetic enhancement by breeding. Understanding the genetic outcomes of domestication and selfing in fungi is essential for sustainable and efficient agriculture and biotechnology.

A community of recreational mushroom growers has innovated and shared techniques to domesticate mushrooms that produce the psychoactive compound psilocybin since protocols were published in the 1970s.[9,10] This community effort and long-term knowledge from cultural use of magic mushrooms may ultimately benefit a commercial industry poised to flourish with changes to scheduling of psilocybin, which is a breakthrough



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compound for treatment of mental health disorders. The Oregon Psilocybin Advisory Board recommended that *Psilocybe cubensis* (Earle) Singer should be the sole taxon grown to commercially produce psilocybin, given the extensive knowledge held by the community.[11]

Psilocybe cubensis is propagated from spore prints and dikaryotic cultures shared by growers who have established cultivars that produce unique and desirable fruiting phenotypes. Historical collecting and breeding of magic mushrooms was clandestine, and the provenance and pedigrees of different cultivars exist in lore but are not authenticated. Commercially available cultivars of *P. cubensis*, such as Golden Teacher, P-envy, B+, Treasure Coast, and McKennaii, anecdotally do not vary in their effects experienced by users. The origin of genetic differences among cultivars is unknown but must either stem from unique collections from wild populations or by crossing different phenotypes. The phenotypic stability of cultivars may suggest their genomes have low heterozygosity, which is potentially an outcome of either unintentional or deliberate selfing to fix traits, such as color and shape.

McKernan et al.[12] sequenced and assembled genomes from commercially available spore prints that covered a range of popular cultivars and determined that cultivars sold under similar names were not genetically similar among different US-based spore providers. The degree of inbreeding and intercrossing among these cultivars is untested, as is the extent to which genetic differences among cultivars impact the production of psilocybin and its analogs.[13–15]

Mating compatibility in *P. cubensis* may be controlled by a tetrapolar system, which is the ancestral state in Basidiomycota[16,17] and present in a sister species, *P. subaeruginosa*.[18] Mating-type genes in mushrooms are highly polymorphic[16,19,20] and can act as markers to track population history, in particular clones and half-sibs. For example, in populations of the wild mushroom *Coprinellus disseminatus*, nearly each strain has a unique mating type.[21] The number of mating-type alleles recovered in a cultivated population should give an indication of numbers of dikaryotic founders. Knowledge of mate compatibility, clonality, and mating-type allele diversity among cultivars will benefit breeding of *P. cubensis*, for which haploid germ plasm can be kept indefinitely and crossed to optimize traits, such as growth rate and production of psychoactive metabolites.

The center of origin of *P. cubensis* is unknown. Its epithet indicates "from Cuba"; however, ethnobotanists and taxonomists hypothesize it is not native to South and Central America, where there is no record of cultural practice.[22] *Psilocybe cubensis* has a tropical and subtropical distribution and fruits prolifically from cow manure in eastern Australia (Figure 1A), mistakenly eaten as a field mushroom since at least the 1940s, or used recreationally.[23] Low[24] put forward two explanations for the presence of *P. cubensis* in Australia: either it is native to Australia, colonizing the dung of macrofauna prior to their extinction, or it was recently introduced along with its niche. Cleland[25] and Margot and Watling[26] hypothesized that fungi in niches of ruminant dung were introduced, and Allen et al.[23] reviewed their potential pathways of introduction and distribution through livestock that came to Australia from South Africa, India, and the United States, as long ago as 1788.

We used population genomics to test two hypotheses: first, that populations of *P. cubensis* are naturalized in Australia and will have the hallmarks of an introduced taxon, such as low allelic diversity and bottlenecks in effective population size. Second, that cultivars of *P. cubensis* were founded from limited source populations and domestication has further decreased genetic diversity. We examined rates of heterozygosity and allelic diversity at mitochondria, psilocybin, and mate-compatibility loci in *P. cubensis* to compare natural versus domesticated populations and determine how mate compatibility is controlled. The comparison of natural and cultivated populations of an emerging commercial fungus shows the short-term genetic impacts of invasion and domestication, provides a foundation to further explore the center of origin and diversity of *P. cubensis*, and provides new directions for genetic improvement through breeding.

RESULTS

Genome assemblies, annotations, and SNP calling

We assembled 38 haploid genomes of *P. cubensis* collected from wild populations in Australia that were 37.4–44.8 Mb, with an estimated sequencing depth between 51 and 116 (mean 99.7) (Table S1). Genomes of 86 cultivars sequenced and assembled by McKernan et al.[12] were 34.6–46.58 Mb. The Australian sequence data differ from McKernan et al.,[12] who sequenced the equivalent of dikaryotic genomes by pooling spores from one parent. The number of genes annotated by FunAnnotate with a protein model from the reference genome of *P. cubensis* was 10,478–17,507 (mean 11,707) per genome across the Australian and cultivated populations.

We called SNPs from 124 assembled genomes to assess population structure and measure inbreeding, kinship, and effective population size through tests described below. After correction of sites under linkage disequilibrium (LD) the entire SNP dataset had 301,524 SNPs (Table 1).

Figure 1. Analyses of SNPs called from 124 genomes of *Psilocybe cubensis* to determine ancestry of populations in Australia and under cultivation

(A) *Psilocybe cubensis* growing in cow manure, Tallebudgera, Queensland, Australia.

(B) Two-dimensional plot of discriminant analysis of principle components (DAPC) at K = 6 based on 39,696 SNPs with indels and sites under linkage disequilibrium (LD) removed from the dataset. Individuals have been labeled if their assigned cluster is ambiguous based on network analyses in 1D.

(C) Barplot of shared ancestry using DAPC exploring K-values 2–9 based on 39,696 SNPs with indels and sites under LD removed from the dataset. Facets reflect clustered populations at K = 6.

(B) and (C) produced by R packages adegenet, vcfR, and ggplot2.

(D) SplitsTree network based on 301,524 LD-corrected SNPs, including indels, with all genomes treated as haploids. Individuals are colored by groups defined in DAPC analysis at K = 6. Edge length reflects genetic difference and reticulation may indicate recombination. Individuals connected by dashed lines in the Australian lineage are sibling haplotypes sampled from the same pileus.

See also Figure S1.

Table 1. Number of individuals, SNPs, measures of linkage disequilibrium (index of association), mean heterozygosity, measures of inbreeding, and allelic diversity at mating-compatibility genes in populations of *Psilocybe cubensis* defined by DAPC

Population	Genomes	No. called SNPs	LD corrected SNPs and indels removed	rbarD[a]	Mean heterozygosity	Mean F_{IS}	HD allele diversity	PR allele diversity
Australia	38	224,423	29,212	0.0126	N/A	N/A	13	9
Blue Magnolia	20	219,304	48,429	0.0635	0.0015	0.647	5	7
Golden Teacher	37	166,792	38,760	0.5221	0.0008	0.784	3	5
Luminous	15	265,470	51,025	0.0566	0.0016	0.508	10	8
Malabar	8	89,092	21,867	0.3993	0.0013	0.571	2	5
P-envy	6	84,400	54,782	0.7710	0.0012	0.811	2	5
All	124	311,362	39,696	0.0380	0.0012[b]	0.677[b]	25	10

See also Figure 1A. N/A, not applicable as Australian genomes are haploid.
[a]rbarD was calculated from SNPs corrected for linkage disequilibrium and with indels removed. It approaches 0 in randomly recombining populations and 1 in clonal/selfing populations.
[b]Cultivated populations.

Psilocybe cubensis was not introduced to Australia from cultivated lineages

Using discriminant analysis of principle components (DAPC), based on 39,696 LD-corrected SNPs excluding indels, we identified approximately six groups among the data (Figures 1B and 1C). Beyond K = 6, substructure, or mixed ancestry, appeared in the Australian population based on posterior membership probability (Figure 1C). DAPC analyses show the Australian population is most different from those under cultivation (Figures 1B and 1C; K = 2) and has no evidence of recent shared ancestry with a subset of the cultivar diversity.

We visualized relationships among genomes using a NeighborNet in SplitsTree, based on 301,524 SNPs including indels (Figure 1D). Combined with the groups defined by DAPC at K = 6, the populations of cultivars occurred in two clusters, one of which included the Luminous and P-envy populations, and the other that included Blue Magnolia, Golden Teacher, and Malabar. Population membership was uncertain for some cultivars, including the Malabar population clustering within Blue Magnolia in the 2D plot (Figure 1B), and four individuals not clearly placed in either Blue Magnolia or Golden Teacher (Figures 1B and 1D).

We examined single-copy orthologs to test whether genes and SNP data recovered the same relationships. OrthoFinder clustered 3,390 single-copy orthologs from 1,454,955 genes across 124 genomes. Relationships based on the core genome of single-copy orthologs (Figure S1) were comparable to SNP data.

We used the AJK statistic, which is a measure of relatedness based on the likelihood of sharing genetic similarity, in combination with known full-sibling relationships in the Australian population to determine whether any cultivars were propagated from the same parent (Figure 2). Isolates of P-envy, Treasure Coast, and cultivars from Thailand had evidence of sibling relationships because their AJK likelihood values were $\geq$1.1. These cultivars may have been established from a single mushroom parent or were propagated clonally. Higher values for the AJK statistic showed kinship among cultivars and supported relationships among populations that clustered in DAPC analyses.

Structure in populations of non-domesticated *P. cubensis* from Australia was determined with a DAPC analysis (Figure S2), which recovered six groups from 31,403 SNPs. Populations were substructured by geography, with the exception of a potential long-distance dispersal event between northern Queensland (Cairns) and southern Queensland (Gold and Sunshine Coasts). Populations from the Scenic Rim and New South Wales were the most different (at K = 2, 3, and 4). Known sibling haplotypes in the Australian population that were sampled from the same pileus (linked by dashed lines in Figure 1D) may suggest that other grouped haplotypes come from related parental dikaryons.

Cultivars of *P. cubensis* have low heterozygosity

We measured heterozygosity and the inbreeding coefficient (F_{IS}) as an indication of selfing within the defined populations (Figure 3A; Tables 1 and S1), as F_{IS} provides a measure of inbreeding relative to a random mating population. Mean heterozygosity of the cultivar genomes (n = 86) was 0.0011 and mean F_{IS} was 0.677, which indicates low levels of heterozygosity and high relatedness among cultivars as F_{IS} approaches 1 in completely inbred populations. Golden Teacher and P-envy were the least heterozygous populations (with individuals between 0.0003 and 0.0021 heterozygosity and 0.713–0.978 F_{IS}), relative to Luminous (0.0003–0.0044 heterozygosity and 0.002–0.817 F_{IS}), for which some cultivars are potentially sourced from outcrossed or natural populations (Figure 3A; Tables 1 and S1). Heterozygosity and F_{IS} could not be measured in the Australian genomes that were sequenced from haploid cultures.

We plotted density of homozygous and heterozygous positions for the two most and one least heterozygous individuals of each cultivated population across the 13 largest scaffolds of the reference genome (Figure S3). The least and most heterozygous genomes of Golden Teacher did not differ from each other (Tables 1 and S1), which shows a similar level of heterozygosity across the population. Aside from OrissaIndia4Mush, the P-envy cultivars were homozygous across most chromosomes, which suggests they have been highly inbred.

Measures of clonality and effective population size show lineages of *P. cubensis* were bottlenecked in recent generations

We used the index of association (IA) as a measure of clonality or selfing in each of the lineages (Table 1). Values of rbarD that approach 1.0 have fewer random pairwise associations of

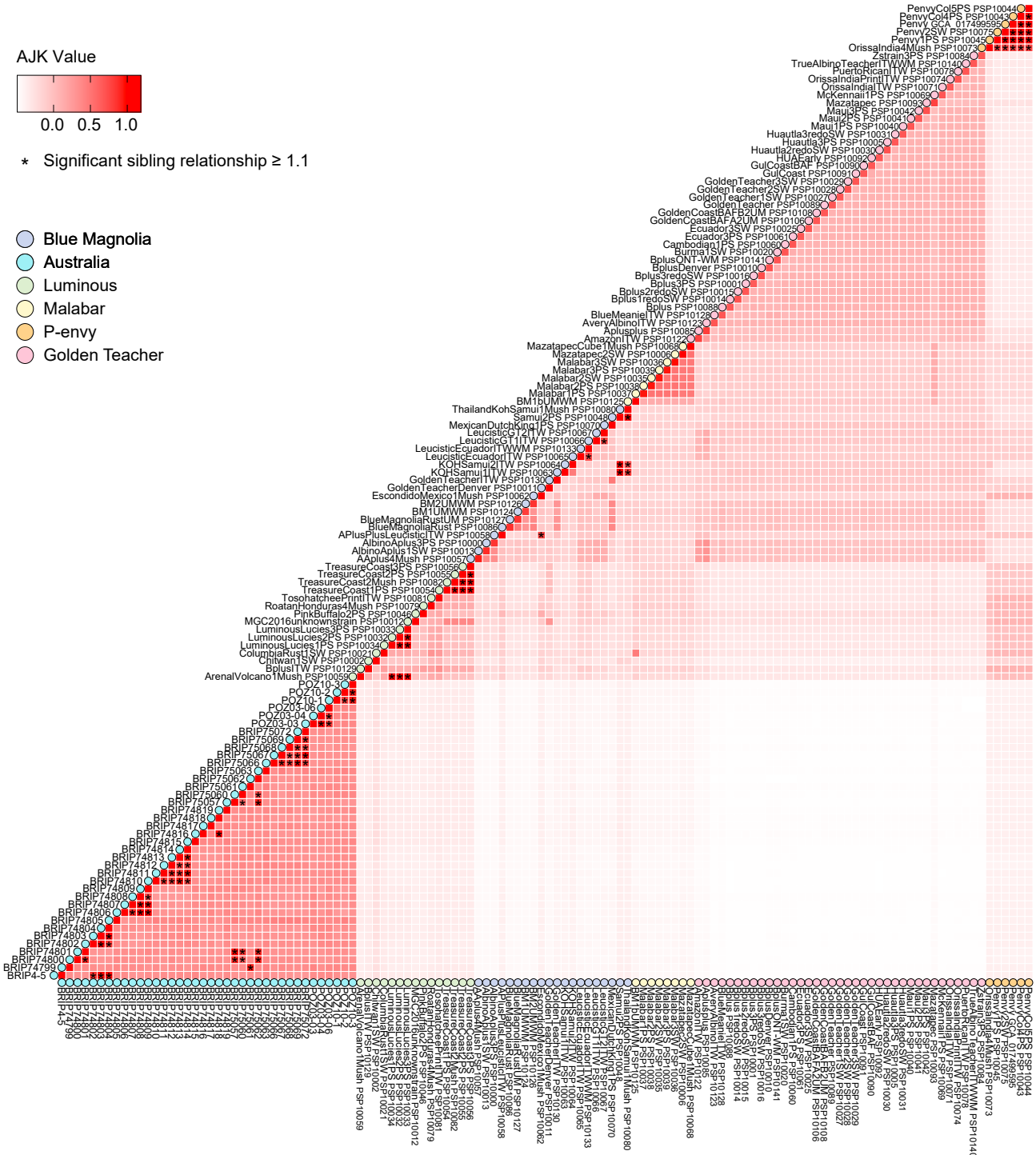


Figure 2. Heatmap of sibling relationships based on pairwise comparisons of the AJK statistic

Known siblings in the Australian population had an AJK likelihood value of 1.1, and pairwise relationships ≥1.1 are considered support that isolates are siblings. All cultivated populations have a level of kinship that is not shared with the Australian population based on the AJK statistic.

SNPs, which may be caused by clonal reproduction or selfing. The Golden Teacher, Malabar, and P-envy populations have likely been propagated clonally or selfed in their domestication and commercialization, with IA values ≥0.1.

We modeled effective population size (Ne) of the defined populations based on LD and recombination rate between pairs of SNPs across a genome in GONE (Figure 3B). The Australian population experienced a sharp decline in Ne approximately 75

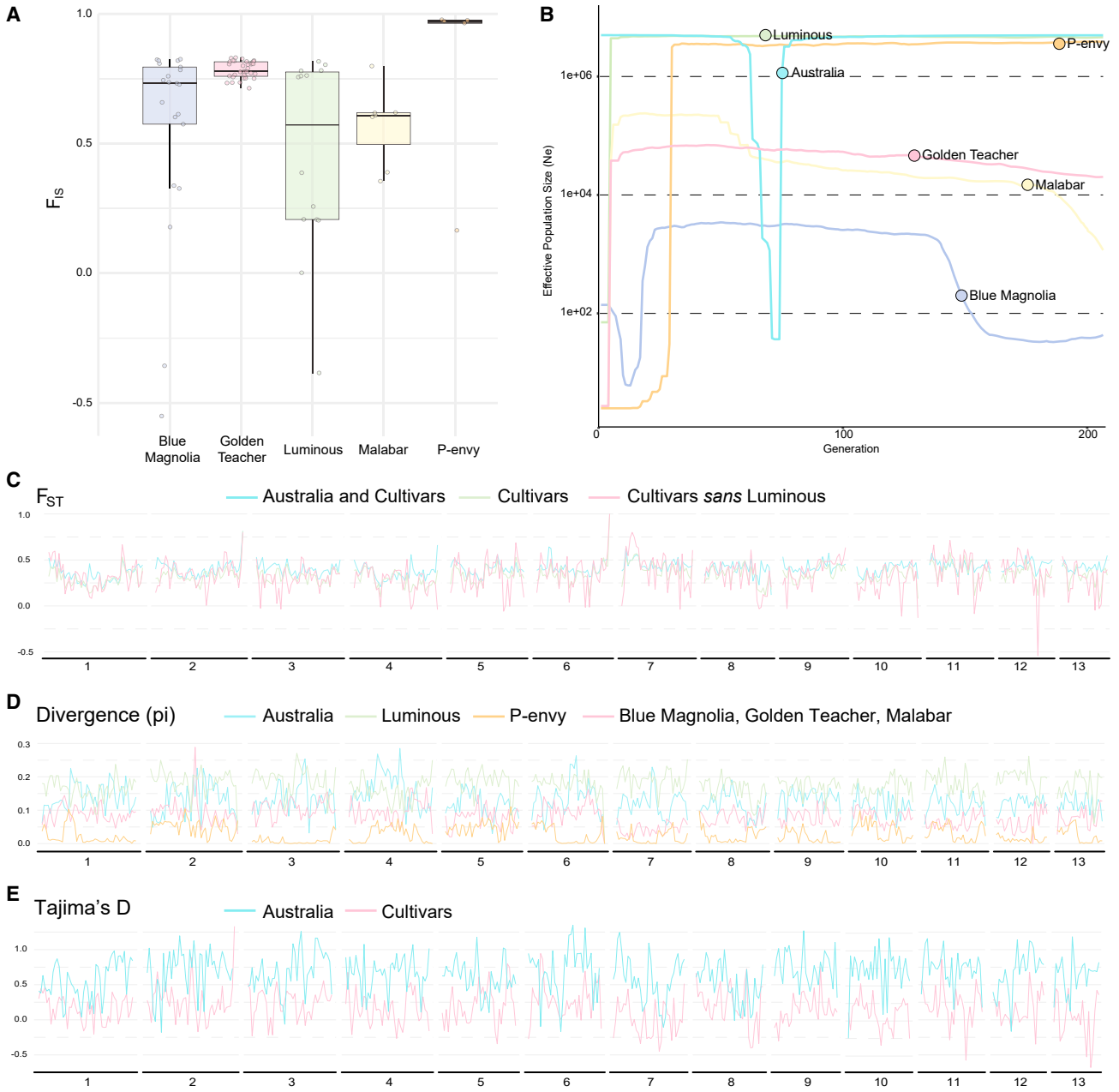


Figure 3. Measures of genetic diversity among populations of *Psilocybe cubensis*

(A) Box and scatterplots of the inbreeding coefficient (F_{IS}) for cultivated populations of *P. cubensis*. F_{IS} values are higher (approach 1.0) in inbred populations, such as Golden Teacher and P-envy.

(B) Estimates of effective population (Ne) size based on LD using GONE. Populations with a high starting Ne (in older generations) are likely reflective of the center of origin. Declines in Ne may be caused by population bottlenecks, such as introduction to Australia, or domestication in cultivated lineages.

(C) F_{ST} plotted in 100,000 base pair windows across chromosomes of *P. cubensis* as a measure of genetic differentiation among populations. F_{ST} decreases when the Australian population is removed from comparisons and further decreases when cultivars in the Luminous population are removed. There is little genetic differentiation among cultivars based on F_{ST}.

(D) Nucleotide diversity (pi) plotted across chromosomes of *P. cubensis* as a measure of diversity within populations defined by DAPC. pi approaches 0 in populations with low genetic diversity and is low overall across sampled populations of *P. cubensis* (<0.2), with the Australian and Luminous populations the most diverse.

(E) Tajima's D index plotted in 100,000 base pair windows across chromosomes of *P. cubensis* as a measure of selection between domesticated (cultivars) and natural (Australia) populations. Positive and negative values of Tajima's D are departures from neutral evolution. The positive values in the Australian population may be a signature of population bottlenecks or balancing selection. Neutral and negative values in the populations under cultivation may be signatures of directional selection through breeding or decreased effective population sizes, both of which decrease genetic diversity.

See also Figure S3.

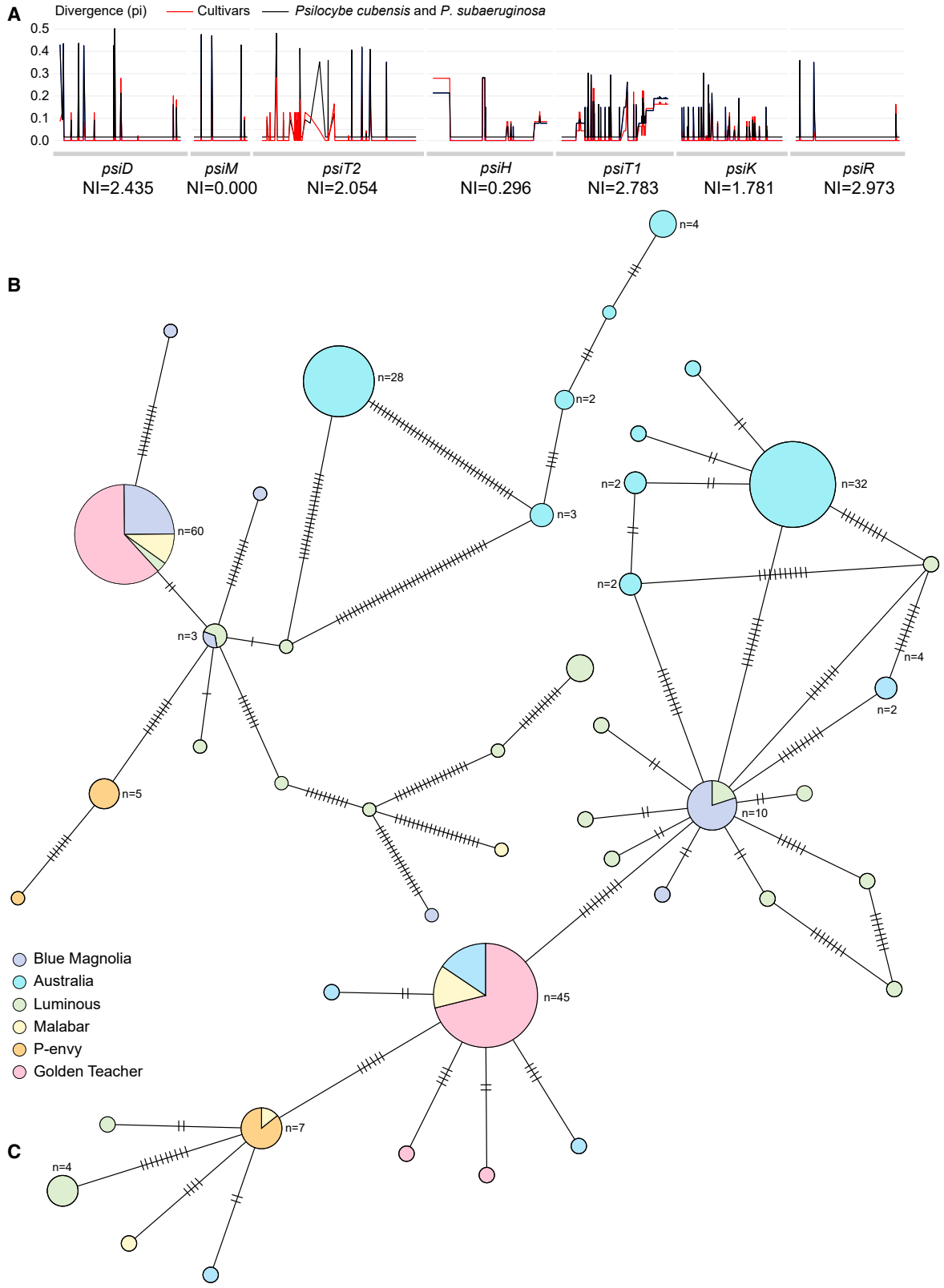


Figure 4. Analyses of genetic diversity at the psilocybin locus and mitochondrial genome of *Psilocybe cubensis*

(A) Nucleotide diversity (pi) of *P. cubensis* compared to *P. subaeruginosa* (black line), and within cultivars of *P. cubensis* (red line) across coding sequence (CDS) of *psiD*, *psiM*, *psiT2*, *psiH*, *psiT1*, *psiK*, and *psiR*. There is little diversity in cultivars of *P. cubensis* (pi = 0.0 in most genes). Neutrality index (NI) values from the

(legend continued on next page)

generations ago and recovered in approximately 20 generations, which may correspond to the original introduction in Australia, followed by naturalization. Most of the cultivated populations experienced a recent decline in effective population size, from which they have not recovered.

We measured F_{ST} to compare genetic differentiation of natural populations to domesticated cultivars (Figure 3C), with lower F_{ST} values indicating gene flow or common ancestry among populations. F_{ST} decreased when the Australian population was removed from the cultivated population set and decreased further when the Luminous population was removed from the comparison of cultivars. This outcome may be due to high relatedness among cultivars caused by domestication from one or a few founding populations, or that more recent breeding has reduced genetic differentiation among cultivars.

We compared nucleotide diversity (pi) in Australian, Luminous, P-envy, and combined Blue Magnolia, Golden Teacher, and Malabar populations to determine whether genetic diversity decreased during domestication (Figure 3D). Diversity was low (<0.3) in all populations. The Australian and Luminous populations had relatively higher levels of nucleotide diversity than P-envy and the combined Blue Magnolia, Golden Teacher, and Malabar populations.

We used Tajima's D statistic, which compares mean pairwise sequence diversity to expected diversity based on numbers of segregating sites, to infer differences in selection and population history between natural and domesticated populations (Figure 3E). The Australian population generally had positive values, which is consistent with either a recent strong bottleneck or balancing selection. Cultivars were generally neutral to negative, which may indicate domestication has caused a recent selective sweep across the genome due to selection for desired traits. The different results on population bottlenecks from GONE and Tajima's D for the Australia population are likely method specific, and recovery of Ne in the Australian population detected by GONE may have been too recent for the appearance of new rare alleles from mutation.

Diversity of genes controlling psilocybin production

We identified variation across 8,191 aligned base pairs of coding sequences in the psilocybin gene cluster, including *psiD*, *psiM*, *psiT2*, *psiH*, *psiT1*, *psiK*, and *psiR*, to examine whether there may be differences in psilocybin production among cultivars and their divergence from a sister taxon, *P. subaeruginosa* (Figure 4A). Most genomes (n = 60) did not differ by any parsimony informative sites across the coding sequence; this included cultivars in the Blue Magnolia, Golden Teacher, Luminous, and Malabar populations (Figure 4B). The Australian, Blue Magnolia, Luminous, and P-envy populations had multiple alleles for psilocybin production (Figure 4B).

We tested whether genes in the psilocybin locus were under selection by comparing non-synonymous and synonymous polymorphisms versus substitutions in *P. cubensis* and *P. subaeruginosa* using the McDonald-Kreitman test (MKT)[27] and determined nucleotide diversity comparing cultivars to *P. subaeruginosa* (Figure 4A). There was no evidence of positive selection in genes at the psilocybin locus based on the MKT contingency test ($p > 0.05$), and there was low polymorphism in these genes compared to divergence from *P. subaeruginosa*. There was low nucleotide diversity within cultivars across coding sequences of the psilocybin locus, with *psiT1* and *psiK* the most diverse genes (Figure 4A).

Limited genetic diversity in mitochondrial genomes of cultivars

We examined diversity in mitochondrial genomes, from 1,050 homologous SNPs called to the 191,520 base pair reference genome, as an indication of founding events in the Australian and cultivated populations (Figure 4C). There was one dominant mitochondrial genotype in 32 genomes of the Australian population. Diversity of mitochondrial genotypes within the Australian population was limited to 2–4 parsimonious SNPs different from the dominant genotype. Cultivars in the Luminous population had the highest mitochondrial diversity with 11 different genotypes, congruent with analyses from genomic data. Most other cultivars had one of three dominant mitochondrial genotypes.

Low mating-type allele diversity in cultivated populations compared to the Australian population

We identified the homeodomain (HD) and pheromone receptor (PR) mating-type loci to infer how mate compatibility is controlled in *P. cubensis*. The HD locus (Figure 5A) is composed of two divergently transcribed genes, *HD1* and *HD2*, and is adjacent to a mitochondrial intermediate peptidase (MIP) gene on the largest chromosome of the reference assembly of *P. cubensis* (CM038998)[28] as observed in other Agaricales.[16,17]

The cultivated lineages of *P. cubensis* had low allelic diversity of HD genes, specifically *HD2* (Figure 5B; Table 1), of which 12 of the 25 alleles occurred in the cultivars, supporting a hypothesis of low founding genetic diversity, as outcrossing selects for variation at HD genes and diversity is expected to be high in natural populations. Luminous, Malabar, P-envy, and some members of the Blue Magnolia populations had different alleles for *HD2* (Figure 5B) compared to other cultivars. The Australian lineage had comparatively high diversity at the HD locus (13 alleles), with different alleles in each of the geographically structured populations. Higher diversity of HD genes in the Australian and Luminous populations may indicate they are naturalized or sourced from naturalized populations.

There were several copies of *STE3* at different genomic locations examined in the Australian lineage. The likely candidate

McDonald-Kreitman test may indicate that the psilocybin locus has been under positive selection with high rates of non-synonymous substitutions between *P. cubensis* and *P. subaeruginosa*. *psiM* (NI = 0) is the shortest gene in the psilocybin pathway and may be under purifying selection to conserve its function, or be under neutral or weak selection, with few non-synonymous or synonymous changes between the species.

(B) Haplotype network of CDS at the psilocybin locus, including CDS of *psiD*, *psiM*, *psiT2*, *psiH*, *psiT1*, *psiK*, and *psiR*. Dashes indicate parsimony informative sites among haplotypes. There are 71 non-synonymous substitutions across 4,407 amino acids of psilocybin genes based on translations of the CDS alignment.
(C) Haplotype network of 1,050 SNPs called across the mitochondrial genome that shows low mitochondrial diversity across the Australian and cultivated lineages.

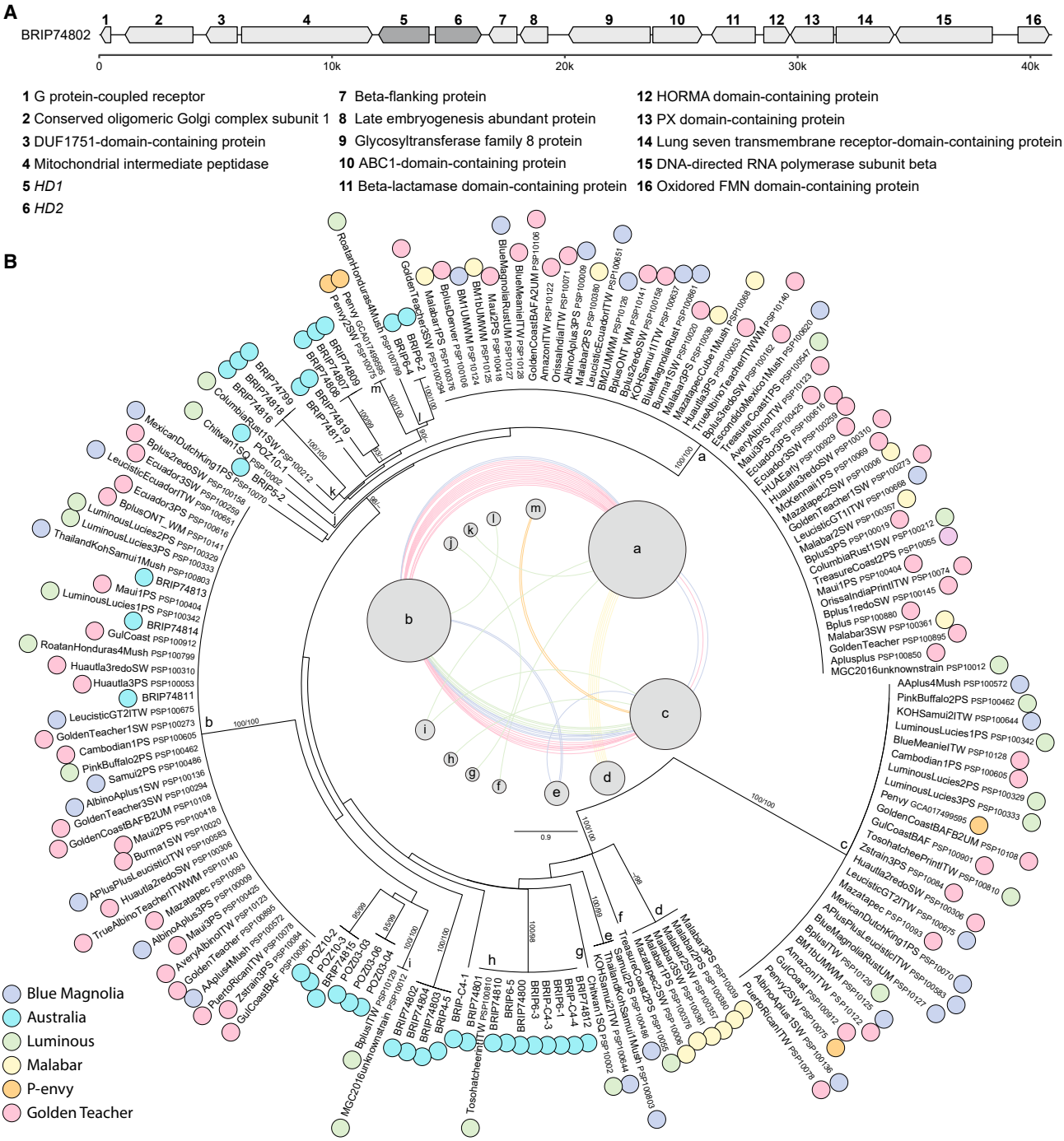


Figure 5. Gene order and diversity of the homeodomain (HD) mating locus in *Psilocybe cubensis*

(A) Gene map of the HD locus plotted using the R pack gggenomes.

(B) Phylogram of relationships of alleles at *HD2*. aLRT and ultrafast bootstrap values from 10,000 replicates are labeled above branches. The inset network shows links between heterozygous genomes in each clade. Cultivars have low allelic diversity of HD genes, and the majority of cultivars are heterozygous for the same two alleles (*HD1* available in Figure S3). Naturalized populations in Australia have relatively high allelic diversity of HD genes among examined haploid genomes. See also Figures S4 and S5.

to control mate recognition contains the most *STE3* genes and an MFS domain-containing gene, similar to PR loci in *P. subaeruginosa* (Figure S4A). This locus occurred on chromosome 10 of the reference assembly of *P. cubensis* (CM039008).[28] *Psilocybe cubensis* has a multi-allelic PR system, with high diversity at one homolog of *STE3* (Figure S4B, clade 1). The unlabeled clades in Figure S4B are examples of *STE3* homologs that do not occur at the PR locus.

We crossed haploid isolates and visualized clamp connections to determine the boundaries of recombination based on predicted protein distance at PR loci (Figure S4B). The compatible and incompatible crosses among different alleles of *STE3* in clade one illustrate that small genetic differences of these alleles govern compatibility. We further demonstrated that the PR locus controls mate compatibility as it was mostly heterozygous in the cultivars (Figure S5). Not all *STE3* alleles were annotated in our pipeline, and we excluded alleles from the cultivars if their location could not be confirmed at the PR locus. This may, in part, explain the low diversity at the PR locus (10 alleles) across all populations.

DISCUSSION

Historically, *P. cubensis* is entwined with humans as it is associated with livestock dung. It was domesticated from its niche of manure over the last 50 years for production of psilocybin and has been incidentally moved with cattle. We compared a naturalized population with numerous cultivars to examine how domestication has impacted genetic diversity of magic mushrooms. The results presented here support a hypothesis that *P. cubensis* is an introduced taxon in Australia that has naturalized separately from domestication of magic mushrooms. Considered with the fact that it has an introduced niche in Australia, support for the naturalization hypothesis comes from a bottleneck in modeled effective population size and low diversity of mitochondrial genotypes. Compared to cultivars, the population of *P. cubensis* in Australia has recovered in effective population size, has higher genetic diversity measured by pi, has evidence of random mating, and has high allelic diversity of HD mate-compatibility genes maintained in the wild and absent in domesticated cultivars.

Domesticated cultivars were composed of five admixed populations. Cultivars showed a higher relatedness between Blue Magnolia, Golden Teacher, and Malabar populations and higher relatedness between Luminous and P-envy populations. Kinship among cultivars may either stem from a shared founding genotype, such as sourcing genetic material from the same invasive populations, or crossing within and among populations during domestication. Cultivars, especially those in Golden Teacher, Malabar, and P-envy populations, have low levels of heterozygosity, which may be an outcome of selfing, whether intentionally to fix traits for cultivation or through lack of source genetic diversity under cultivation.

Psilocybe cubensis occurred in Australia prior to the 1950s based on evidence of accidental ingestion before they were cultivated for recreational use.[23] Our analyses of effective population size support that *P. cubensis* was introduced to Australia as the population experienced a sharp bottleneck approximately 75 generations ago, although it is difficult to interpret how many years ago this took place, given there could be multiple generations in one season and survival of clones from season to season. Our analyses of structure in the Australian lineage support that *P. cubensis* is transported with cattle as related genotypes are spread over long distances. Allen et al.[23] summarized the origins of cattle in Australia over the last two centuries, and knowledge of the center of origin of *P. cubensis* may pinpoint the period they were co-introduced, whether via South Africa or India.

The Australian lineage of *P. cubensis* is genetically distinct from commercial cultivars based on differences among SNPs and genes, F_{ST}, and diversity of alleles at the psilocybin locus and mating-type loci. *P. cubensis* was not introduced to Australia by recreational users, and there is no evidence of cultivars outcrossing with naturalized populations. Spores of cultivars have been imported to Australia, based on phenotypes available in the Australian market. This finding raises a question of why domesticated genotypes have not infiltrated natural areas. Domestication involving selfing may have selected traits amenable to lab cultivation, and cultivars may lack fitness to survive in natural ecosystems in competition with other microorganisms, such as through reduced expression of anti-microbial secondary metabolites; however, this hypothesis requires testing.

The cultivars of *P. cubensis* investigated in this study have low genetic diversity compared to the naturalized population in Australia. There is less variation within cultivated populations, bar Luminous, than within the Australian population, evidenced by low allelic diversity at psilocybin and mating-type loci, and low levels of nucleotide diversity across genomes. The network, DAPC, and kinship analyses indicate the diversity of cultivars, with the exception of those in the Luminous population, was founded from related sources of genetic material.

Cultivars of the Luminous population defined by DAPC were non-monophyletic in networks based on SNPs and single-copy orthologs and may be an eclectic mix of genotypes from diverse source populations. Cultivars of the Luminous population have high nucleotide diversity among individuals and high mitochondrial diversity, are more heterozygous and less clonal than other cultivated lineages, and have high allelic diversity at mate-compatibility and psilocybin loci. For example, the Chitwan cultivar has high heterozygosity (0.004), low evidence of inbreeding (0.0023 F_{IS}), and unique alleles compared to other cultivars at all examined loci. Cultivars of the Luminous population may have been established through multiple collections from invasive or native source populations outside of Australia. The Australian population and cultivars of the Luminous population have the highest allelic diversity of mating-compatibility genes, which may reflect higher founding genetic diversity.

Many cultivars (15/43) of *P. cubensis* are close relatives in the Golden Teacher population, based on analyses that supported clonality/selfing and a crash in modeled effective population size. Cultivar names that may indicate provenance in Golden Teacher and Blue Magnolia populations, such as Amazon, Burma, Cambodia, Ecuador, Escondido (Mexico), Maui (Hawaii), Puerto Rico, and Thailand, suggest they were originally collected from several natural populations. Based on the relatedness of these populations across a wide geographic range, they were founded from closely related genotypes, have since lost genetic diversity through domestication, or the names do not indicate provenance.

Strong evidence of LD in the Golden Teacher, Malabar, and P-envy populations suggests clonal propagation and selfing in the history of cultivation. Inbreeding and restricted recombination in the button mushroom (*Agaricus bisporus*) have linked traits and prevented genetic gain when crossing.[8] Given the impact of inbreeding in the button mushroom industry, breeding of *P. cubensis* for commercial production of psilocybin may benefit from outcrossing in the long term. Breeding efforts can

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focus on our knowledge of the mating system. We confirmed mate compatibility is tetrapolar in *P. cubensis*, as HD and PR loci are located on two separate chromosomes[16] and segregate in naturalized Australian populations and to some extent in the cultivars. The cultivars have low diversity of alleles at the HD locus, with 2–5 alleles in Blue Magnolia, Golden Teacher, Malabar, and P-envy populations. Low diversity at HD loci indicates that cultivars, save Luminous, are not supplemented by diversity from native or naturalized populations, or they came from limited founding populations as most species of mushroom have dozens to hundreds of alleles at each mating-compatibility locus.[20]

Anecdotally, different cultivars of *P. cubensis* are not reported to vary in their psychedelic effects; "a cube is a cube" was coined by recreational users to describe the similar experiences among different cultivars. The psychedelic effects of diversity at the psilocybin locus are unknown, whether that diversity is caused from a horizontal gene transfer, such as between *Psilocybe* and *Panaeolus*[15]; diversity in psilocybin gene copy number among species[18,29]; allelic differences at the psilocybin cluster observed in the present study; or accessory metabolites from undetermined pathways.[30] Future clinical research directions are needed to determine whether different amounts of psilocybin and its analogs, putatively caused by diversity at the psilocybin locus among populations of *P. cubensis* or different species of *Psilocybe*, impact the psilocybin experience and offer varying treatment options for a range of mental health conditions.

Domestication of *P. cubensis* has selected for magic mushrooms that can be grown with ease in basements, bathrooms, or under beds, or that have produced characteristic phenotypes from limited underlying genetic diversity. Whether these cultivars can now survive in natural conditions is unknown. Industries that produce psilocybin from *P. cubensis* will ultimately benefit from incorporation of new genetic diversity to bring new traits into cultivation. A remaining knowledge gap is the center of origin of *P. cubensis*, which has implications for diversifying phenotypes, resistance to diseases, and benefit sharing in the psilocybin industry under the Nagoya protocol. This will require more dedicated sampling campaigns from putative source populations in the tropics and sub-tropics.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2023.10.059.

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AUTHOR CONTRIBUTIONS

Conceptualization, A.R.M., J.C.S., and T.Y.J.; methodology, A.R.M.; software, A.R.M.; investigation, A.R.M., S.M., K.M., and T.L.B.; resources, C.A., M.W., and L.S.S.; writing – original draft, A.R.M.; writing – review & editing, S.M., J.C.S., K.M., C.B., C.A., T.L.B., M.W., L.N.W., L.S.S., A.D., and T.Y.J.; supervision, A.D. and T.Y.J.

DECLARATION OF INTERESTS

A.R.M. and C.A. are owners of Funky Fungus, a company that grows magic mushrooms to produce medicinal psilocybin. K.M. is the founder of Medicinal Genomics.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used Open AI's ChatGPT to generate R code for production of Figures 3 and S3. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Culture of *Psilocybe cubensis*	This manuscript	BRIP74799
Culture of *Psilocybe cubensis*	This manuscript	BRIP74800
Culture of *Psilocybe cubensis*	This manuscript	BRIP74801
Culture of *Psilocybe cubensis*	This manuscript	BRIP74802
Culture of *Psilocybe cubensis*	This manuscript	BRIP74803
Culture of *Psilocybe cubensis*	This manuscript	BRIP74804
Culture of *Psilocybe cubensis*	This manuscript	BRIP74805
Culture of *Psilocybe cubensis*	This manuscript	BRIP74806
Culture of *Psilocybe cubensis*	This manuscript	BRIP74807
Culture of *Psilocybe cubensis*	This manuscript	BRIP74808
Culture of *Psilocybe cubensis*	This manuscript	BRIP74809
Culture of *Psilocybe cubensis*	This manuscript	BRIP74810
Culture of *Psilocybe cubensis*	This manuscript	BRIP74811
Culture of *Psilocybe cubensis*	This manuscript	BRIP74812
Culture of *Psilocybe cubensis*	This manuscript	BRIP74813
Culture of *Psilocybe cubensis*	This manuscript	BRIP74814
Culture of *Psilocybe cubensis*	This manuscript	BRIP74815
Culture of *Psilocybe cubensis*	This manuscript	BRIP74816
Culture of *Psilocybe cubensis*	This manuscript	BRIP74817
Culture of *Psilocybe cubensis*	This manuscript	BRIP74818
Culture of *Psilocybe cubensis*	This manuscript	BRIP74819
Culture of *Psilocybe cubensis*	This manuscript	BRIP75057
Culture of *Psilocybe cubensis*	This manuscript	BRIP75060
Culture of *Psilocybe cubensis*	This manuscript	BRIP75061
Culture of *Psilocybe cubensis*	This manuscript	BRIP75062
Culture of *Psilocybe cubensis*	This manuscript	BRIP75063
Culture of *Psilocybe cubensis*	This manuscript	BRIP75066
Culture of *Psilocybe cubensis*	This manuscript	BRIP75067
Culture of *Psilocybe cubensis*	This manuscript	BRIP75068
Culture of *Psilocybe cubensis*	This manuscript	BRIP75069
Culture of *Psilocybe cubensis*	This manuscript	BRIP75072
Culture of *Psilocybe cubensis*	This manuscript	BRIP74799
Culture of *Psilocybe cubensis*	This manuscript	BRIP74800
Culture of *Psilocybe cubensis*	This manuscript	BRIP74801
Chemicals, peptides, and recombinant proteins		
Potato Dextrose Agar	Merck	Cat# P6685
Deposited data		
Assembled genome of BRIP4-5	This manuscript	GenBank: JARAEW000000000
Assembled genome of BRIP74799	This manuscript	GenBank: JARAEV000000000
Assembled genome of BRIP74800	This manuscript	GenBank: JARAEU000000000
Assembled genome of BRIP74801	This manuscript	GenBank: JARAET000000000
Assembled genome of BRIP74802	This manuscript	GenBank: JARAES000000000
Assembled genome of BRIP74803	This manuscript	GenBank: JARAER000000000

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Assembled genome of BRIP74804	This manuscript	GenBank: JARAEQ000000000
Assembled genome of BRIP74805	This manuscript	GenBank: JARAEP000000000
Assembled genome of BRIP74806	This manuscript	GenBank: JARAEO000000000
Assembled genome of BRIP74807	This manuscript	GenBank: JARAEN000000000
Assembled genome of BRIP74808	This manuscript	GenBank: JARAEM000000000
Assembled genome of BRIP74809	This manuscript	GenBank: JARAEL000000000
Assembled genome of BRIP74810	This manuscript	GenBank: JARAEK000000000
Assembled genome of BRIP74811	This manuscript	GenBank: JARAEJ000000000
Assembled genome of BRIP74812	This manuscript	GenBank: JARAEI000000000
Assembled genome of BRIP74813	This manuscript	GenBank: JARAEH000000000
Assembled genome of BRIP74814	This manuscript	GenBank: JARAEG000000000
Assembled genome of BRIP74815	This manuscript	GenBank: JARAEF000000000
Assembled genome of BRIP74816	This manuscript	GenBank: JARAEE000000000
Assembled genome of BRIP74817	This manuscript	GenBank: JARAED000000000
Assembled genome of BRIP74818	This manuscript	GenBank: JARAEC000000000
Assembled genome of BRIP74819	This manuscript	GenBank: JARAEB000000000
Assembled genome of BRIP75057	This manuscript	GenBank: JARAEA000000000
Assembled genome of BRIP75060	This manuscript	GenBank: JARADZ000000000
Assembled genome of BRIP75061	This manuscript	GenBank: JARADY000000000
Assembled genome of BRIP75062	This manuscript	GenBank: JARADX000000000
Assembled genome of BRIP75063	This manuscript	GenBank: JARADW000000000
Assembled genome of BRIP75066	This manuscript	GenBank: JARADV000000000
Assembled genome of BRIP75067	This manuscript	GenBank: JARADU000000000
Assembled genome of BRIP75068	This manuscript	GenBank: JARADT000000000
Assembled genome of BRIP75069	This manuscript	GenBank: JARADS000000000
Assembled genome of BRIP75072	This manuscript	GenBank: JARADR000000000
Assembled genome of POZ03-3	This manuscript	GenBank: JARADO000000000
Assembled genome of POZ03-4	This manuscript	GenBank: JARADQ000000000
Assembled genome of POZ03-6	This manuscript	GenBank: JARADP000000000
Assembled genome of POZ10-1	This manuscript	GenBank: SAMN34393170
Assembled genome of POZ10-2	This manuscript	GenBank: SAMN34393171
Assembled genome of POZ10-3	This manuscript	GenBank: SAMN34393172
Software and algorithms		
Trimmomatic v.0.12	Bolger et al.[31]	https://github.com/usadellab/Trimmomatic/tree/V0.32
SPAdes v.3.12.0	Bankevich et al.[32]	https://github.com/ablab/spades
FunAnnotate	Palmer and Stajich[33]	https://doi.org/10.5281/zenodo.2604804
kSNP	Gardner et al.[34]	https://sourceforge.net/projects/ksnp/
PLINK v.1.9	Purcell et al.[35]	https://www.cog-genomics.org/plink/
SplitsTree v.4.14.8	Huson and Bryant[36]	https://software-ab.informatik.uni-tuebingen.de/download/splitstree/welcome.html
samtools v.1.9	Li et al.[37]	https://samtools.github.io
vcftools v.1.17	Daecek et al.[38]	http://vcftools.github.io/
R	R Core Team[39]	https://www.r-project.org
KaryoploteR	Gel and Serra[40]	https://github.com/bernatgel/karyoploteR
vcfR	Knaus and Grünwald[41]	https://github.com/knausb/vcfR
adegenet	Jombart[42]	https://adegenet.r-forge.r-project.org
GONE	Santiago et al.[43]	https://github.com/esrud/GONE/tree/master

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
OrthoFinder v.1.0.6	Emms and Kelly[44]	https://github.com/davidemms/OrthoFinder/tree/master
MAFFT	Katoh and Standley[45]	https://mafft.cbrc.jp/alignment/software/
FASconCAT-G	Kück and Longo[46]	https://frontiersinzoology.biomedcentral.com/articles/10.1186/s12983-014-0081-x
PopART	Leigh and Bryant[47]	https://datadryad.org/stash/dataset/doi:10.5061/dryad.4n4j1
BLAST	Altschul et al.[48]	https://blast.ncbi.nlm.nih.gov/doc/blast-help/downloadblastdata.html#downloadblastdata
parallel	Tange[49]	https://www.gnu.org/software/parallel/
gggenomes	Hackl and Ankenbrand[50]	https://thackl.github.io/gggenomes/index.html
IQTree v.1.7-beta7	Nguyen et al.[51]	http://www.iqtree.org
MKT-webserver	Egea et al.[52]	http://mkt.uab.es/mkt/help_mkt.asp
Other		
Assembled genome of AAplus4Mush	https://www.psilocydia.net/strains	PSP10057
Assembled genome of AlbinoAplus1SW	https://www.psilocydia.net/strains	PSP10013
Assembled genome of AlbinoAplus3PS	https://www.psilocydia.net/strains	PSP10000
Assembled genome of AmazonITW	https://www.psilocydia.net/strains	PSP10122
Assembled genome of Aplusplus	https://www.psilocydia.net/strains	PSP10085
Assembled genome of APlusPlusLeucisticITW	https://www.psilocydia.net/strains	PSP10058
Assembled genome of ArenalVolcano1Mush	https://www.psilocydia.net/strains	PSP10059
Assembled genome of AveryAlbinoITW	https://www.psilocydia.net/strains	PSP10123
Assembled genome of BlueMagnoliaRust	https://www.psilocydia.net/strains	PSP10086
Assembled genome of BlueMagnoliaRustUM	https://www.psilocydia.net/strains	PSP10127
Assembled genome of BlueMeanieITW	https://www.psilocydia.net/strains	PSP10128
Assembled genome of BM1bUMWM	https://www.psilocydia.net/strains	PSP10125
Assembled genome of BM1UMWM	https://www.psilocydia.net/strains	PSP10124
Assembled genome of BM2UMWM	https://www.psilocydia.net/strains	PSP10126
Assembled genome of Bplus	https://www.psilocydia.net/strains	PSP10088
Assembled genome of Bplus1redoSW	https://www.psilocydia.net/strains	PSP10014
Assembled genome of Bplus2redoSW	https://www.psilocydia.net/strains	PSP10015
Assembled genome of Bplus3PS	https://www.psilocydia.net/strains	PSP10001
Assembled genome of Bplus3redoSW	https://www.psilocydia.net/strains	PSP10016
Assembled genome of BplusDenver	https://www.psilocydia.net/strains	PSP10010
Assembled genome of BplusITW	https://www.psilocydia.net/strains	PSP10129
Assembled genome of BplusONT_WM	https://www.psilocydia.net/strains	PSP10141
Assembled genome of Burma1SW	https://www.psilocydia.net/strains	PSP10020
Assembled genome of Cambodian1PS	https://www.psilocydia.net/strains	PSP10060
Assembled genome of Chitwan1SW	https://www.psilocydia.net/strains	PSP10002
Assembled genome of Chromosome_Penvy	https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_017499595.1/	GCA_017499595
Assembled genome of ColumbiaRust1SW	https://www.psilocydia.net/strains	PSP10021
Assembled genome of Ecuador3PS	https://www.psilocydia.net/strains	PSP10061
Assembled genome of Ecuador3SW	https://www.psilocydia.net/strains	PSP10025
Assembled genome of EscondidoMexico1Mush	https://www.psilocydia.net/strains	PSP10062
Assembled genome of GoldenCoastBAFA2UM	https://www.psilocydia.net/strains	PSP10106

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Assembled genome of GoldenCoastBAFB2UM	https://www.psilocydia.net/strains	PSP10108
Assembled genome of GoldenTeacher	https://www.psilocydia.net/strains	PSP10089
Assembled genome of GoldenTeacher1SW	https://www.psilocydia.net/strains	PSP10027
Assembled genome of GoldenTeacher2SW	https://www.psilocydia.net/strains	PSP10028
Assembled genome of GoldenTeacher3SW	https://www.psilocydia.net/strains	PSP10029
Assembled genome of GoldenTeacherDenver	https://www.psilocydia.net/strains	PSP10011
Assembled genome of GoldenTeacherITW	https://www.psilocydia.net/strains	PSP10130
Assembled genome of GulCoast	https://www.psilocydia.net/strains	PSP10091
Assembled genome of GulCoastBAF	https://www.psilocydia.net/strains	PSP10090
Assembled genome of HUAEarly	https://www.psilocydia.net/strains	PSP10092
Assembled genome of Huautla2redoSW	https://www.psilocydia.net/strains	PSP10030
Assembled genome of Huautla3PS	https://www.psilocydia.net/strains	PSP10005
Assembled genome of Huautla3redoSW	https://www.psilocydia.net/strains	PSP10031
Assembled genome of KOHSamui1ITW	https://www.psilocydia.net/strains	PSP10063
Assembled genome of KOHSamui2ITW	https://www.psilocydia.net/strains	PSP10064
Assembled genome of LeucisticEcuadorITW	https://www.psilocydia.net/strains	PSP10065
Assembled genome of LeucisticEcuadorITWWM	https://www.psilocydia.net/strains	PSP10133
Assembled genome of LeucisticGT1ITW	https://www.psilocydia.net/strains	PSP10066
Assembled genome of LeucisticGT2ITW	https://www.psilocydia.net/strains	PSP10067
Assembled genome of LuminousLucies1PS	https://www.psilocydia.net/strains	PSP10034
Assembled genome of LuminousLucies2PS	https://www.psilocydia.net/strains	PSP10032
Assembled genome of LuminousLucies3PS	https://www.psilocydia.net/strains	PSP10033
Assembled genome of Malabar1PS	https://www.psilocydia.net/strains	PSP10037
Assembled genome of Malabar2PS	https://www.psilocydia.net/strains	PSP10038
Assembled genome of Malabar2SW	https://www.psilocydia.net/strains	PSP10035
Assembled genome of Malabar3PS	https://www.psilocydia.net/strains	PSP10039
Assembled genome of Malabar3SW	https://www.psilocydia.net/strains	PSP10036
Assembled genome of Maui1PS	https://www.psilocydia.net/strains	PSP10040
Assembled genome of Maui2PS	https://www.psilocydia.net/strains	PSP10041
Assembled genome of Maui3PS	https://www.psilocydia.net/strains	PSP10042
Assembled genome of Mazatapec	https://www.psilocydia.net/strains	PSP10093
Assembled genome of Mazatapec2SW	https://www.psilocydia.net/strains	PSP10006
Assembled genome of MazatapecCube1Mush	https://www.psilocydia.net/strains	PSP10068
Assembled genome of McKennaii1PS	https://www.psilocydia.net/strains	PSP10069
Assembled genome of MexicanDutchKing1PS	https://www.psilocydia.net/strains	PSP10070
Assembled genome of MGC2016unknownstrain	https://www.psilocydia.net/strains	PSP10012
Assembled genome of OrissaIndia4Mush	https://www.psilocydia.net/strains	PSP10073
Assembled genome of OrissaIndiaITW	https://www.psilocydia.net/strains	PSP10071
Assembled genome of OrissaIndiaPrintITW	https://www.psilocydia.net/strains	PSP10074
Assembled genome of Penvy1PS	https://www.psilocydia.net/strains	PSP10045
Assembled genome of Penvy2SW	https://www.psilocydia.net/strains	PSP10075
Assembled genome of PenvyCol4PS	https://www.psilocydia.net/strains	PSP10043
Assembled genome of PenvyCol5PS	https://www.psilocydia.net/strains	PSP10044
Assembled genome of PinkBuffalo2PS	https://www.psilocydia.net/strains	PSP10046
Assembled genome of PuertoRicanITW	https://www.psilocydia.net/strains	PSP10078
Assembled genome of RoatanHonduras4Mush	https://www.psilocydia.net/strains	PSP10079

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Assembled genome of Samui2PS	https://www.psilocydia.net/strains	PSP10048
Assembled genome of ThailandKohSamui1Mush	https://www.psilocydia.net/strains	PSP10080
Assembled genome of TosohatcheePrintITW	https://www.psilocydia.net/strains	PSP10081
Assembled genome of TreasureCoast1PS	https://www.psilocydia.net/strains	PSP10054
Assembled genome of TreasureCoast2Mush	https://www.psilocydia.net/strains	PSP10082
Assembled genome of TreasureCoast2PS	https://www.psilocydia.net/strains	PSP10055
Assembled genome of TreasureCoast3PS	https://www.psilocydia.net/strains	PSP10056
Assembled genome of TrueAlbinoTeacherITWWM	https://www.psilocydia.net/strains	PSP10140
Assembled genome of Zstrain3PS	https://www.psilocydia.net/strains	PSP10084

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Alistair McTaggart (alistair.mctaggart@gmail.com).

Materials availability

Cultures generated in this study, listed in the key resources table (and Table S1), have been lodged in the Queensland Plant Pathology Herbarium (BRIP).

Data and code availability

- Genome assembly data generated in this study have been deposited in GenBank and are publicly available as of the date of publication. Accession numbers are listed in the key resources table (and Table S1). Raw sequencing data generated in this paper will be shared by the lead contact upon request. This paper analyzes existing, publicly available data. These accession numbers for the datasets are listed in the key resources table (and Table S1).
- This paper does not report original code.
- Any additional information required to re-analyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

We cultured single basidiospores on malt extract agar from spore prints of *P. cubensis* collected by citizen scientists from New South Wales and Queensland (Australia, Figure S2B). We streaked basidiospores of *P. cubensis* in sterile water on half-strength potato dextrose agar to germinate monokaryotic hyphae. Germinated basidiospores were isolated and grown on half-strength potato dextrose agar at 22°C.

METHOD DETAILS

Specimen collection, culturing, DNA sequencing

Monokaryotic cultures were transferred to half-strength potato dextrose broth, grown for three weeks, then sent to the Australian Genome Research Facility (AGRF, Brisbane, Australia) for DNA extraction and high-throughput sequencing. AGRF prepared a Nextera Flex 150PE library that was sequenced on an Illumina HiSeq.

Genome assembly and annotation

Raw sequencing reads were trimmed with Trimmomatic v.0.12[31] and assembled with SPAdes v.3.12.0.[32] Assembled genomes are accessioned in GenBank (Table S1). FunAnnotate[33] was used to annotate all examined genomes using protein models from the annotated *P. cubensis* reference assembly,[28] BUSCO models for Basidiomycota, and Augustus models from *Laccaria bicolor*.[53]

SNP calling

SNPs were called against the 13 longest scaffolds of a chromosome scale assembly of *P. cubensis*[28] using kSNP (k = 101, min frac = 1).[34] SNPs were called from the 38 Australian genomes and 86 genomes assembled by McKernan et al.[12] (Table S1). All genomes were treated as haploids for SNP calling by removing sites called as heterozygous. PLINK v.1.9[35] was used to remove SNP

sites under linkage disequilibrium (r^2 cutoff = 0.99). Relationships among SNPs were visualised with a neighbour net in SplitsTree v.4.14.8[36] (Figure 1D).

Orthogroup analyses

We used OrthoFinder v.1.0.6[44] with a Diamond search[54] to identify orthologous groups of genes. All single-copy orthologs were aligned with MAFFT,[45] concatenated with FASconCAT-G,[46] and their relationships visualised with a neighbour net in SplitsTree (Figure S1).

Mitochondrial diversity

Mitochondrial contigs for each genome were searched against the mitochondrion of the *P. cubensis* reference assembly (NW_025952838) using BLASTn. SNPs were called from all mitochondrial contigs using kSNP (k = 31, min frac = 1), and relationships were visualised with a haplotype network using PopART,[47] which groups identical or near identical genotypes and plots relationships based on genetic distance (Figure 4C).

Gene diversity at psilocybin and MAT loci

Psilocybin genes and MAT homologs were searched in gene annotations with BLASTp[48] using genes identified by Fricke et al.[14] and McKernan et al.[28] as queries. tBLASTn was used to identify contigs that contained the psilocybin gene cluster or mating genes. We used *parallel* to optimise computational resources in BLAST searches.[49] Annotated contigs were visualised with the R package ggge-nomes[50] (Figures 5A and S4A). The relationships among psilocybin loci were visualised with a haplotype network made with PopART (Figure 4B). The relationships among MAT alleles were visualised from alignments of the N-terminal regions with a phylogenetic hypothesis made using IQTree v.1.7-beta7[51] and a neighbour net in SplitsTree (Figures 5B, S4B, and S5).

Mating compatibility

Mating compatibility among haploid cultures of *P. cubensis* was tested on Potato Dextrose Agar (BD Micro, Bacto Laboratories Pty Ltd.) based on diversity at pheromone/receptor loci. All pairings were between strains that differed in alleles at the homeodomain locus (MAT-HD). Approximately 1 cm^2 pieces of culture from selected isolates were grown adjacently for two weeks. Hyphae were sampled from the zone of interaction and examined for clamp connections under a light microscope at 1000x magnification.

QUANTIFICATION AND STATISTICAL ANALYSIS

Measures of heterozygosity

Heterozygosity and F_{IS} were calculated for dikaryotic cultivars by mapping reads to their assemblies using samtools v.1.9[37] and the *het* flag in vcftools v.0.1.17[38] as described by McKernan et al.[12] The distribution of F_{IS} values in each of the cultivar populations was visualised with a boxplot with scattered points using *ggplot2* in R[40] (Figure 3A). Density of heterozygosity and homozygosity was visualised across the two most and one least heterozygous genomes (Table S1) from each population using 10,000 and 100,000 base pair windows plotted using the *kpPlotDensity* function in the R package KaryoploteR[39,40] (Figure S3).

Tests for population structure and relatedness

We used Discriminant Analysis of Principal Components (DAPC) and K-means clustering to determine whether there was underlying population structure in the Australian and domesticated genomes[55] (Figures 1B and 1C). The packages vcfR,[41] adegenet,[42] and ggplot2 implemented in R[40] were used to import an LD-corrected VCF file excluding indels, cluster lineages by k-means clustering and DAPC, and plot results. The index of association (IA) was estimated as a measure of clonality for all defined lineages using *bitwise.ia* in vcfR (Table 1).

We used the *relatedness* command in vcftools v.1.17,[38] which estimates relationships based on pairwise similarity of genetic markers between individuals due to shared genetic ancestry defined by the AJK statistic.[56] We plotted relatedness values of LD-corrected SNPs, including indels, in a pairwise heatmap using *ggplot2* in R[40] (Figure 2).

We compared genetic diversity and sites potentially under selection between natural and domesticated populations of *P. cubensis* using measures of F_{ST}, pi, and Tajima's D calculated in vcftools. F_{ST}, nucleotide diversity, and Tajima's D comparing the Australian population to cultivars were plotted across the 13 chromosomes of the reference assembly of *P. cubensis* using *ggplot2* in R (Figures 3C–3E).

Tests for effective population size

We used seven individuals from each of the defined populations, with the exception of P-envy (n = 6), to examine effective population size based on LD-corrected SNPs using GONE, which estimates generational changes in effective population size based on linkage disequilibrium and recombination rate between pairs of SNPs across a genome.[43] The parameters used for GONE were 200 generations, 200 bins, a maximum recombination rate (hc) of 0.05, and 40 replicates. Log values for effective population size were plotted in a line graph using *ggplot2* in R[40] (Figure 3B).

Nucleotide diversity at psilocybin loci

We aligned coding sequences of genes in the psilocybin cluster, *psiD, psiM, psiT1, psiH, psiT2, psiK,* and *psiR*, with orthologs of these genes from *P. subaeruginosa*, a sister taxon to *P. cubensis*. Diversity of nucleotide sites between *P. cubensis* and *P. subaeruginosa* was plotted using *ggplot2* in R calculated with the *site-pi* command in vcftools (Figure 4A). We further measured whether genes at the psilocybin locus were under positive selection using the McDonald-Krietman statistic on the MKT-webserver[52] (Figure 4A).