(*31*). Our bd oxidase structure does not indicate the presence of such voluminous pathways; instead, molecular oxygen may access heme d laterally from the alkyl chain interface with the membrane over a short distance (Fig. 2B). Hence, oxygen dissolved in the membrane could rapidly bind to heme d without traveling through any tunnel-like protein cavity.

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SUPPLEMENTARY MATERIALS

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MICROBIOME

Durable coexistence of donor and recipient strains after fecal microbiota transplantation

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Fecal microbiota transplantation (FMT) has shown efficacy in treating recurrent *Clostridium difficile* infection and is increasingly being applied to other gastrointestinal disorders, yet the fate of native and introduced microbial strains remains largely unknown. To quantify the extent of donor microbiota colonization, we monitored strain populations in fecal samples from a recent FMT study on metabolic syndrome patients using single-nucleotide variants in metagenomes. We found extensive coexistence of donor and recipient strains, persisting 3 months after treatment. Colonization success was greater for conspecific strains than for new species, the latter falling within fluctuation levels observed in healthy individuals over a similar time frame. Furthermore, same-donor recipients displayed varying degrees of microbiota transfer, indicating individual patterns of microbiome resistance and donor-recipient compatibilities.

Fecal microbiota transplantation (FMT), which entails the transfer of a microbial community from a healthy donor to a patient (often after bowel lavage), has emerged as a promising treatment option for a range of chronic disorders (*1*–*3*). However, despite having a success rate of over 90% for recurrent *Clostridium difficile* infection (R-CDI) (*1*), there are indications that the therapy as currently performed is not as effective in treating other diseases (*4*). This, in addition to inherent risks (*5*), has created a need to characterize and understand the full effects of FMT on the microbiome of the recipient, across different diseases.

Recent studies have indicated that establishment of donor-specific species is possible alongside resident microbiota of the recipient (*6*–*9*) and that they can still be detected 70 days after FMT (*7*). However, the definitive origin of these newly observed species—whether they are indeed from the donor or from other sources (for example, diet or environment) or had simply been below detection levels in the recipient—is uncertain. Moreover, in the majority of these studies, the FMT procedure was preceded by a course of antibiotics, which is known to alter the gut flora (*10*, *11*) and may or may not facilitate implantation of exogenous microorganisms.

The full extent to which donor microbiota colonize in a recipient host has not yet been measured, as studies to date have been restricted to genus- and species-level comparisons and have not distinguished or elucidated the fate of donor and recipient strains of the same species (*6*–*9*, *12*). Genetic variants of bacterial species are known to coexist in the gut (*13*), and strain-level differences have recently been shown to have functional and clinically relevant consequences (*13*–*15*). However, other studies in humans and model systems have found that newly introduced nonpathogenic strains are unable to persist in an established gut ecosystem—especially if the species was already present—be they probiotics (*16*), xenomicrobiota (*17*), or even modified strains isolated from the same healthy individual (*18*).

Here, we quantify and describe the extent of changes to the population structure of the gut microbiome after FMT, at both species and strain level, using shotgun metagenomic data. Donor strains established extensively in the recipient and persisted over the 3-month observational period, either replacing or, more strikingly, existing alongside indigenous strains. Outcomes varied across donor-recipient pairs, indicating that microbiome compatibility (the likelihood that donor strain populations are able to coexist or even replace microbial strains in the recipient) is a factor that could provide a rationale for more targeted microbiota-based therapies.

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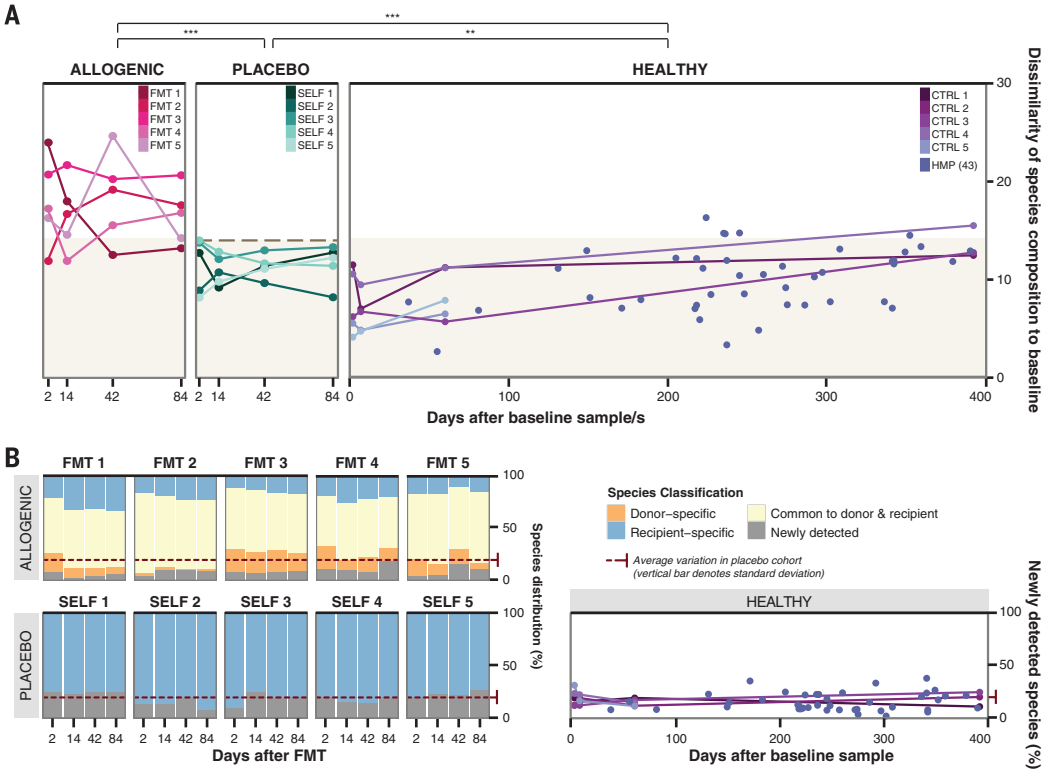

Fig. 1. Microbial communities undergo larger compositional changes after allogenic FMT compared with placebo-treated and healthy individuals but contain similar proportions of newly acquired species. (**A**) Post-allogenic FMT microbiomes display species composition changes above the range of temporal variation observed in placebo (beige) and healthy controls, persisting over 84 days (Wilcoxon rank-sum test; ***$P < 0.0002$; **$P < 0.004$; Euclidean distance) (*22*). Lines connect time point samples from the same individual. (**B**) Proportion of acquired donor species in allogenic FMT subjects is within, or close to, the level of variation observed in placebo (20.1 ± 5.0%) (dotted line) and healthy individuals for up to 400 days after initial sampling. These species thus may not necessarily have originated from the donor but may have been acquired from other sources or had previously been undetected. A majority of species is common to both donor and recipient (yellow). Same-donor FMT recipients are grouped by a black bar below the subject identifier.

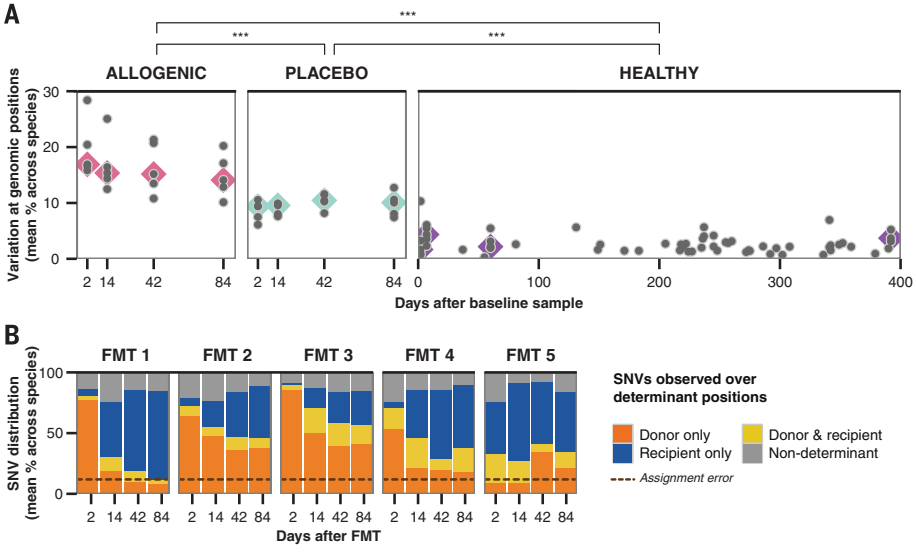

Fig. 2. Allogenic FMT instigates more variation at the strain level compared with placebo-treated and healthy individuals, most likely due to donor microbiota transfer. (**A**) Fraction of single-site allelic variation (compared to pre-FMT samples), averaged across species, is significantly higher in the allogenic FMT group than placebo-treated and healthy individuals (Wilcoxon rank-sum test; ***$P <$ 0.0001). Median values across individuals are highlighted by the diamond shape. (**B**) Average fraction of donor- and recipient-specific SNVs detected across 42 prevalent species in allogenic FMT recipients. Genomic positions containing both donor- and recipient-specific SNVs are shown in yellow; non-determinant positions (SNVs observed in none or both donor and recipient before FMT) are in gray. Presence of donor- and recipient-specific SNVs indicate durable coexistence of donor and recipient strains; decline of donor strains between 42 and 84 days after FMT is negligible. Retention of donor-specific SNVs is notably higher than the assignment error rate of 11.9% (dotted line) (*22*).

Using shotgun metagenomics, we sequenced 55 fecal samples from a recent FMT study of metabolic syndrome patients (*2*). In contrast to previous investigations, subjects in this study had not used antibiotics or other medication for at least 3 months before treatment, nor did they do so during treatment, thus minimizing associated confounding effects (*10*, *11*, *19*). The cohort of 10 subjects—half of whom received a single allogenic FMT (from one of three lean donors unrelated to the recipients) and the other half, an autologous FMT (i.e., a placebo equivalent)—had samples collected at consistent time points (once before treatment and four times after treatment). To contextualize our findings, we compared the cohort to 109 metagenomes of 48 healthy, FMT-naïve individuals from published longitudinal studies including the Human Microbiome Project (*20*–*22*). Changes after FMT were monitored over multiple time points, using pre-FMT samples of each donor-recipient pair as a baseline for allogenic FMT and placebo-treated recipients and initial time point (day 0) samples for healthy control subjects (fig. S1).

Using a taxonomic marker gene-based approach that accounts for both known and putative species (*22*, *23*), we observed compositional shifts in both allogenic and placebo-treated FMT recipients. These were above the range of natural temporal variation in healthy individuals (Wilcoxon rank-sum test, $P = 2.20 \times 10^{-6}$ and 3.86×10^{-3}) (Fig. 1A and fig. S2), suggestive of an additive effect of bowel lavage and microbiota transfer on the gut microbiome.

Compared with the other two groups, allogenic FMT microbiomes underwent considerably larger changes ($P = 7.49 \times 10^{-8}$) regardless of clinical outcome (table S1), which can thus be attributed to the introduction of donor microbiota and reflects previous reporting of increased microbial diversity in these subjects (*2*). These changes persisted for at least 3 months after treatment, even though recipient microbiomes gradually lost similarity to their respective donors (fig. S3), in contrast to findings in R-CDI patients (*7*, *12*).

Species putatively assigned as donor-specific varied across recipients and were observed throughout the 3 months of the study (Fig. 1B and fig. S4A). Retention of these new species positively correlated to the number of donor-specific species identified in pre-FMT samples ($R^2 = 0.83$) (fig. S4B). However, in four of five cases, the proportion of donor-specific species was either below or not significantly different from the fluctuations of new species detected over time in the placebo-treated group (20.8 ± 5.5%) and healthy group (17.7 ± 10.9%) (Fig. 1B and table S2), which was also observed using 16S ribosomal RNA gene-based profiling (fig. S5). We therefore propose that the detection of presumably donor-specific species in recipients after FMT is by itself not sufficient proof of colonization and in future studies should be contextualized by background variation.

A large fraction of species in allogenic FMT recipients was also present in their donor (64.5 ± 6.0%) (Fig. 1B). Determining the fate of these bacteria—that is, whether donor microbes are able to colonize and whether they replace or coexist with recipient microbiota—requires analytical resolution at the strain level. To this end, we used metagenomic single-nucleotide variant (SNV) analysis (*24*) to distinguish and trace donor- and recipient-specific strain populations, because the full genomic reconstruction of uncharacterized strains from a complex community is not yet available (*25*).

We extended the approach by Schloissnig *et al.* (*24*) to account for rare and less abundant strains and applied it to the 164 fecal metagenomes from the three study groups (*22*). This provided a starting set of 11.8 million variant positions across the genomes of 1105 detected prokaryotic species (table S3).

To compare the extent of strain-level changes among the study groups, SNVs identified in baseline samples were monitored over multiple time points (Fig. 2A and fig. S1). We observed a higher level of single-site allelic variation in allogenic FMT recipients than in the placebo and healthy groups

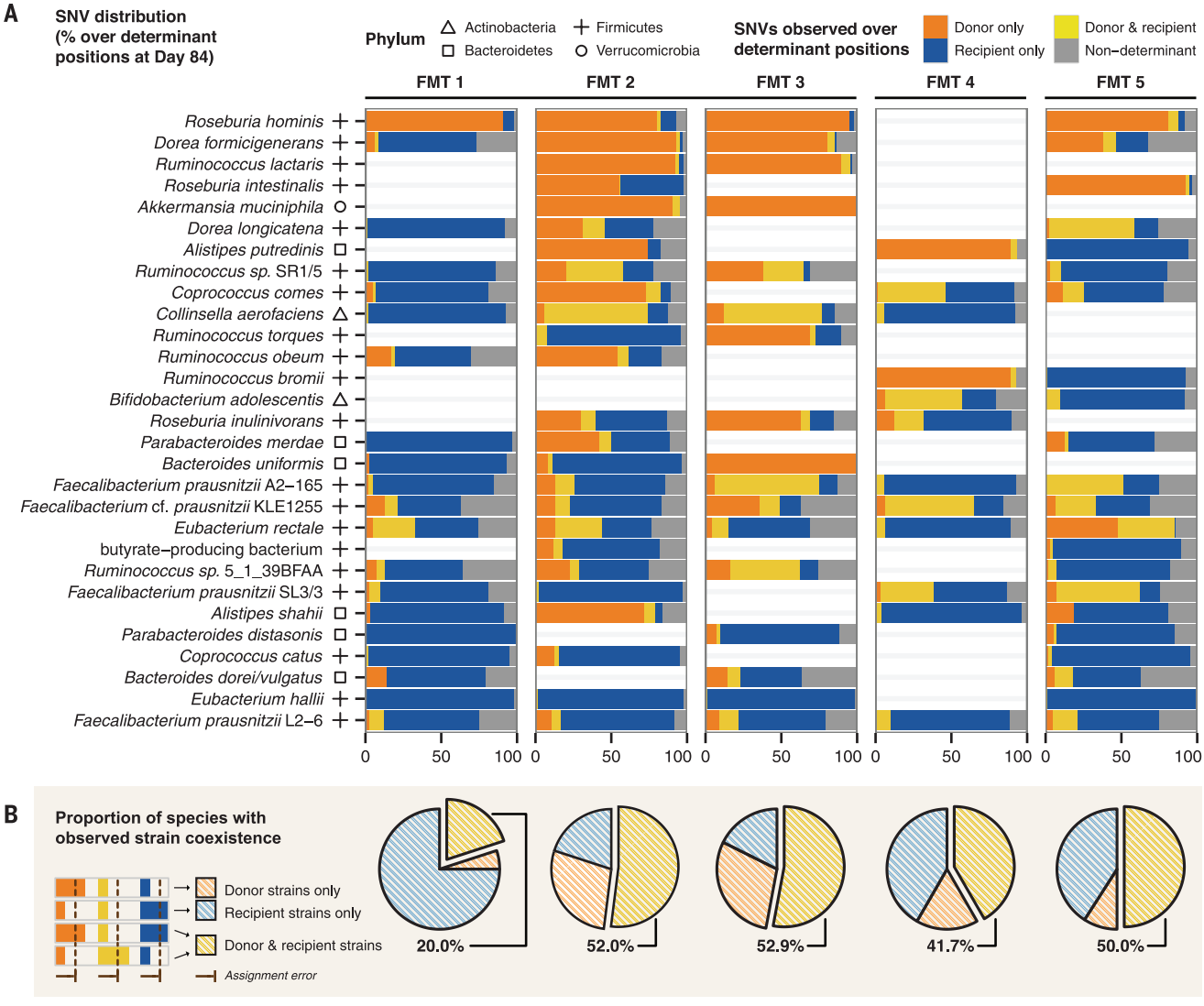

Fig. 3. Coexistence of donor and recipient strains across species, 84 days after FMT. (**A**) Distribution of donor- and recipient-specific SNVs for species detected in more than one recipient at day 84. Species are ranked by the likelihood of observing consistent donor (top) to recipient (bottom) strain dominance patterns (fig. S9). For clarity, full strain names are used in cases where species names were ambiguous (table S4). Blanks signify species that were not detected at day 84 or before FMT in donor and/or recipient. Coexistence of donor- and recipient-specific strains can be observed across species and recipients. The colonization success of donor strains does not appear to be associated with taxonomic affiliation of the species. (**B**) Extent of strain coexistence (yellow) in each recipient, summarized for detected species. Species with observed dominance of donor (orange) or recipient (blue) strains comprised a smaller proportion.

($P = 1.35 \times 10^{-5}$ and 1.50×10^{-6}, respectively), which indicated the presence of additional strains (*22*).

To investigate whether the increased variation was due to the transfer and establishment of donor microbiota, we defined a set of determinant genomic positions (containing both donor- and recipient-specific SNVs), which we monitored over time (fig. S1 and table S3) (*22*). For confident SNV detection and tracking, we focused on species that were consistently detected with sufficient abundance in at least one donor-recipient pair, resulting in 42 species across five phyla (table S4). Determinant positions for these species, which are prevalent in the general population (*24*), ranged from 66 to 161,800 (median 15,550) and were located throughout their respective genomes (fig. S6).

Across recipients, we observed transfer of donor strains (Fig. 2B and fig. S7). Donor-specific SNVs were most highly retained 2 days after FMT (69.3 ± 21.8% of determinant positions across recipients) and were still present 3 months later (37.6 ± 16.6%) (Fig. 2B and fig. S7). This contrasts with much lower rates of variation observed at equivalent time points in placebo-treated (day 84) and healthy (days 60 to 84) individuals (9.5 ± 1.8% and 2.9 ± 1.3%, respectively) (Fig. 2A) and shows that our findings result from donor-strain transfer and not only temporal variability or abundance variation beyond detection thresholds. Furthermore, it indicates that donor strains can colonize in the human gut after bowel lavage [which causes a short-term decrease in microbial diversity (*20*)] and without prior antibiotic treatment.

Marked differences in colonization success were observed between allogenic recipients who shared a donor (subjects FMT1, 2, and 3). Three months after treatment, FMT2 and 3 retained a higher amount of donor-specific SNVs compared with FMT1 (46.1%, 56.6%, and 12.0%, respectively) (Fig. 2B). The perceived resistance to incoming strains in FMT1 was also reflected in the overall microbiome composition of this subject, which reverted to the pre-FMT state, whereas FMT2 and 3 displayed more persistent compositional changes (Fig. 1A). Responses to FMT treatment may thus be idiosyncratic and depend on the compatibility of the donor and recipient microbiomes and/or the disease state involved. A "one-stool-fits-all" model currently supported by standardized donor stool banks may not be clinically appropriate (*26*).

Apart from the one case of microbiome resistance, we observed extensive coexistence of donor and recipient strains (in 50.7 ± 10.1% of shared species) in all other recipients, which persisted for at least 3 months (Fig. 2B). This suggests that novel strains can colonize in the gut without replacing the indigenous strain population of the recipient. It appears that introduced strains are more likely to establish in a new environment if the species is already present. The decline of donor-strain populations detected between 1.5 and 3 months after FMT was negligible, implying that potentially long-term strain coexistence might occur.

We sought to determine the extent of donor and recipient strain coexistence across species. Focusing on the 29 species that could be detected in more than one recipient at the end of the study, a pattern of donor strains establishing alongside indigenous strains of the recipient was seen (Fig. 3A). Whereas 39 ± 23% of species showed resistance to introduced strains, coexistence of donor and recipient strains was observed in 44 ± 14% of species detected in recipients (Fig. 3B). Durability of donor strains varied widely for most species, ranging from complete replacement of recipient strains to extinction (for example, *Alistipes putredinis* and *Ruminococcus bromii* fall at the extremes in FMT4 and 5). Most strikingly, however, donor strains of *Roseburia hominis*, *Ruminococcus lactaris*, and *Akkermansia muciniphila* appeared to dominate recipient strains, with almost complete substitution, even in the case of FMT1, who rejected most donor strains and species over time (Figs. 1B and 3A and fig. S8). By contrast, recipient strains of *Eubacterium hallii* and *Parabacteroides distasonis* showed resistance to donor strains by day 84 (fig. S8) (*22*). Based on these observations, we used a random permutation approach to rank species that showed consistent patterns of donor or recipient strain dominance (fig. S9) (*22*). Overall, colonization success and subsequent dominance or rejection of donor strains did not seem to depend on taxonomic affiliation of the species nor on differences in relative abundance between donor and recipient species (Fig. 3A and fig. S10), in contrast to previous findings in R-CDI patients (*6*, *9*). It was also not restricted to particular strains, genome size, phenotypic features (such as Gram stain), known functional capacities of a species, or clinical parameters (tables S1 and S5). Given these results, we propose that aspects such as strain and species fitness (*27*) and colonization resistance (*28*) should be studied in the context of microbiome resilience and immune responses of the recipient. Differences in the colonization success of donor strains across recipients also highlight the need for resolution beyond species level to understand the mechanisms (for example, how nonindigenous strains can elude host immune defense responses) and ecological effects underlying microbiota-based therapies.

Taken together, we have used intraspecies genetic variation to monitor strain populations within gut microbial communities. Applied to FMT, we observed an extensive and durable coexistence of donor and recipient strains of the same species, with a considerable amount of strain replacement (compared with a negligible uptake of donor species) that was not associated with clinical outcome. This suggests that introduced bacterial strains can be readily accepted and persist in an established gut microbial community, even when the species is already present. This contrasts with previous findings on isolated strains in non-FMT settings (*16*–*18*) and potentially extends to a wider context, such as the infant gut during development (*13*). The complete replacement of indigenous strains also introduces the possibility for the use of a consortia of characterized and/or customized strains to modulate the microbiome by, for example, outcompeting undesirable strains (*29*), hence encouraging the development of more defined microbiota-based precision treatments.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
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Tables S1 to S7
References (*30*–*46*)

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Editor's Summary

Persistence of fecal transplants

Fecal microbiota transplantation is a successful way of treating the distressing symptoms of irritable bowel disease or *Clostridium difficile* infection. The procedure is done by administering a concentrate of colonic bacteria from a healthy donor. Li *et al.* used metagenomic data to look at single-nucleotide variants after transplants in humans. Donor and recipient strains coexisted for at least 3 months. Some donor strains replaced related strains of the same species, but totally novel species from a donor were unlikely to thrive in a recipient. Rational design of personalized fecal transplant "cocktails" will therefore rely on resolution beyond the species level.

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