Host Genetic Control of the Microbiota Mediates the Drosophila Nutritional Phenotype

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A wealth of studies has demonstrated that resident microorganisms (microbiota) influence the pattern of nutrient allocation to animal protein and energy stores, but it is unclear how the effects of the microbiota interact with other determinants of animal nutrition, including animal genetic factors and diet. Here, we demonstrate that members of the gut microbiota in Drosophila melanogaster mediate the effect of certain animal genetic determinants on an important nutritional trait, triglyceride (lipid) content. Parallel analysis of the taxonomic composition of the associated bacterial community and host nutritional indices (glucose, glycogen, triglyceride, and protein contents) in multiple Drosophila genotypes revealed significant associations between the abundance of certain microbial taxa, especially Acetobacteraceae and Xanthomonadaceae, and host nutritional phenotype. By a genome-wide association study of Drosophila lines colonized with a defined microbiota, multiple host genes were statistically associated with the abundance of one bacterium, Acetobacter tropicalis. Experiments using mutant Drosophila validated the genetic association evidence and reveal that host genetic control of microbiota abundance affects the nutritional status of the flies. These data indicate that the abundance of the resident microbiota is influenced by host genotype, with consequent effects on nutrient allocation patterns, demonstrating that host genetic control of the microbiome contributes to the genotype-phenotype relationship of the animal host.

The recognition that animals are routinely colonized by dense and often diverse communities of microorganisms is driving a major reassessment of fundamental aspects of animal biology (1). Notably, there is accumulating evidence that resident microorganisms influence the nutritional status of animals in multiple ways, including competition for ingested nutrients, providing supplementary nutrients (e.g., vitamins, short-chain fatty acids, essential amino acids), and by modulating the nutrient signaling circuits that regulate nutrient allocation (2–5). These discoveries demonstrate the inadequacy of traditional explanations of animal nutrition in terms of nutritional inputs (amount and composition of food ingested) and outputs (animal nutritional demand for activity, growth, reproduction, etc.) and highlight our ignorance of how microbial effects on animal nutrition interact with other factors, especially animal genotype (6–10).

The focus of this study is the impact of interactions between the gut microbiota and host genotype on animal nutrition. The nutritional consequence of the microbiota is known to vary with the abundance and composition of the microorganisms (7, 10–13). For example, comparison of nutritional phenotypes in Drosophila melanogaster raised with or without gut bacteria revealed that the microbiome can influence penetrance of host mutations (12). To the extent that the abundance and composition of the microbiota are determined by host genotype, the impact of animal genetic variation on nutrition may be mediated via effects on the microbiota, and host genotype-independent differences in the microbiota among individual animals may also make an appreciable contribution to the nutritional phenotype of animals (10). These issues are immediately relevant to the promise of microbial therapies and microbiologically informed dietary therapies for nutritional health (i.e., probiotics and prebiotics). The rational application of these therapies will require an understanding of how the effects of the microbiota and host genotype interact to shape animal nutrition.

The gut microbiota in the fruit fly Drosophila melanogaster is an excellent system to investigate the fundamentals of interactions between resident microorganisms and host genotype on animal nutrition. A nutritionally important component of the Drosophila microbiota is the gut-inhabiting bacteria, including members of the Acetobacteraceae (alphaproteobacteria), Lactobacillales, and gammaproteobacteria (14–16), which contribute to the B vitamin and protein nutrition of the host, and can reduce energy storage as triglyceride (TAG) and glycogen (9, 17–19). The Drosophila model is also supported by a wealth of genetic and genomic resources, including the Drosophila Genetic Reference Panel (DGRP) comprising multiple inbred lines with sequenced genomes used in this study (20, 21).

In this study, we investigate the relationship between the composition of the microbiota and nutritional phenotype of Drosophila, quantified as a set of nutritional indices (protein, TAG, glycogen, and glucose contents). Using the DGRP, we demonstrate that the composition of the microbiota varies in a Drosophila population and identify microbial species with previously unappreciated...
influence of host nutrition whose abundance correlates with different nutritional indices. We also identify candidate host genes that influence the abundance of one bacterium, Acetobacter tropicalis, with nutritional consequences. These results demonstrate the significance of host genetic control of the microbiota on animal nutrition.

MATERIALS AND METHODS

Drosophila stock cultures and manipulations. The Drosophila melanogaster lines (see Table S2 in the supplemental material) were cultured at 25°C on a light-dark cycle (12 h light, 12 h dark). The Drosophila lines were fed a yeast-glucose diet (1 liter H₂O, 100 g inactive brewer’s yeast [catalog no. 903312; MP Biomedicals], 100 g glucose [catalog no. 158968; Sigma], 1.2% agar [catalog no. 66-103; Apex], 0.84% propionic acid, 0.08% phosphoric acid). Routine cultures were maintained on cooked, but not autoclaved, food, and experimental cultures were reared on sterile yeast-glucose diet prepared by autoclaving the diet, then aseptically adding acid preservatives, and transferring 7.5-mL aliquots into sterile 50-mL Falcon tubes. For experiments using Drosophila with unmanipulated microbiota (conventional Drosophila), eggs (<22 h old) were collected from grape juice agar plates, rinsed gently with double-distilled water (ddH₂O), and transferred in groups of 30 to 50 to sterile diet. To prepare gnotobiotic flies (containing defined microbiota), eggs were collected as described above, then surface sterilized by two 2.5-min washes in 0.6% hypochlorite, rinsed three times with sterile water, and aseptically transferred to sterile diet in a biosafety cabinet. Bacterial inocula, comprising Acetobacter pomorum DmCS_004, Acetobacter tropicalis DmCS_006, Lactobacillus brevis DmCS_003, Lactobacillus fructivorans DmCS_002, and Lactobacillus plantarum DmCS_001 (22), were prepared from cells grown at 30°C to stationary-phase culture in mMRS medium (11), normalized to 5 × 10⁸ total CFU/mL, mixed in equal proportions, and added directly to the food surface in 50 μL within 3 h of egg transfer. All fly assays were performed on male flies, 5 to 8 days posteclosion, and 5 to 8 h into the daily light cycle.

Identification of microbiota community composition. To assess the composition of the microbiota populations, total genomic DNA was extracted from five pooled male flies as described previously (23, 24). 16S rRNA amplifications of the V2 region were prepared by triplicate PCRs (23), using the 16S rRNA gene primers 27F (F stands for forward) (5′-AGAGTTTGATCMTGGCTCAG-3′) and 338R (R stands for reverse) (5′-GTCTCCTCCGTTAGAGT-3′), tagged with different molecular identifiers (MIDs) (see Table S3 in the supplemental material). Equal amounts of the triplicate products per sample were mixed, purified by Sephadex filtration, quantified by growth after homogenization in TET buffer, and transferred in groups of triplicate pools (five flies each). Dry weights of three pools of five flies were also collected, as described above. A. tropicalis was distinguished from A. pomorum by growth after homogenization in TET buffer (A. pomorum does not grow), and from Lactobacillus species by color (11). Each DGRP line was tested in one of five experimental blocks.

The GWA was conducted with custom R scripts and the nlmfit R package (28), with single nucleotide polymorphism (SNP) data from the Freeze 1.0 DGRP release. Log-1-transformed A. tropicalis CFU number per fly, measured in triplicate and expressed as a fraction of the total number of CFU, was the response variable, SNP identity was the fixed effect, and experimental block and DGRP genotype were random effects. Following established procedures (29), the most frequent (major) and second-most frequent (minor) SNP identities were retained at each locus; all other alleles were omitted from the analysis, and any SNP with the minor allele present in three or fewer lines was discarded. In total, 2,027,631 SNPs were tested, and significance values for SNP effect are reported. Genetic contributions to variance were calculated as the square of the standard deviation for the random effect of DGRP genotype in the model.

GWA validation. Drosophila mutants (see Table SB in the supplemental material) corresponding to genes containing or near GWA-identified SNPs and background stocks were obtained from the Bloomington Drosophila Stock Center and used directly in subsequent assays, following the experimental design of our previous work and others (12, 29–31). Gnotobiotic flies were raised with the five-species microbiota, as in the GWA and A. tropicalis abundance and nutritional indices were determined. Data were collected in triplicate for each of three separate experiments, and significant differences between mutant and background lines were identified by a linear mixed model (28), with the vial nested within the experiment as a random effect. Data were normalized by log or square root (sqr) transformation, whichever gave the highest significance value above 0.05 in a Shapiro test. Significance values were assigned using multcomp (32) (critical threshold P < 0.05). Mutant flies and their backgrounds were also raised with a three-species microbiota of L. brevis, L. fructivorans, and L. plantarum and analyzed exactly as described above.
except that the experimental replicate was the only random effect used in the models.

RESULTS

Relationship between the microbiota and nutritional traits in the DGRP. Pyrosequencing of 16S rRNA gene amplicons in 5- or 6-day-old male flies from 79 DGRP lines yielded 177 bacterial OTUs at 97% sequence identity (see also Data Set S1A in the supplemental material). Consistent with the previously reported dominance of Acetobacteraceae and Lactobacillaceae in laboratory cultures of Drosophila (14–16, 23, 33–35), all the DGRP lines yielded reads assigned to Acetobacteraceae, and all but eight of the lines also bore Lactobacillaceae; these bacteria accounted for 54 and 19%, respectively, of the reads after removal of sequences assigned to Wolbachia and rarefaction (Fig. 1A; Data Set S1B). The Acetobacteraceae included representation of three genera, Acetobacter, Gluconobacter, and Komagataeibacter (Gluconacetobacter [36]), but all the Lactobacillaceae were members of one genus, Lactobacillus, of which 76% of the reads could be assigned to a single species, L. brevis. In addition, the Xanthomonadaceae and Comamonadaceae were highly prevalent (present in all but two and eight DGRP lines, respectively), representing 17% and 6% of the total reads. As found in previous analyses (14,16), a core microbiota (i.e., OTUs present in all lines) was not detected at 97% sequence identity. The most prevalent OTU (OTU160 in Xanthomonadaceae; Data Set S1B) was present in 77 of 79 lines, but further analysis revealed nine sequence variants within this OTU, including two (OTU4 and OTU131 in Data Set S1C) with nearly mutually exclusive distributions (detected in 53 and 28 DGRP lines, respectively). These OTUs were both most similar to Stenotrophomonas species.

A parallel analysis of nutritional indices in the DGRP flies revealed wide between-line variation (Fig. 1B to D) that correlated with the relative abundance of associated microbes (Table 1; see Data Set S2 in the supplemental material). The results are congruent with published studies on the nutritional traits of Drosophila in...
monoassociation (11, 22, 37) or polyassociation (12) with different bacteria. A previous analysis of the same nutritional indices in an overlapping subset of the DGRP raised under axenic or five-species gnotobiotic conditions demonstrated contributions of both host genotype and presence of bacteria to variation in nutritional indices (12). The current analysis extends these published data by demonstrating that the microbial influence is sufficient to yield significant correlations between microbial abundance and nutritional traits identified even across genetically distinct hosts. Specifically, Acetobacteraceae of the genera Glucobacter and Komagataeibacter, as well as the previously documented Achromobacter, are associated with reduced energy storage (11, 17, 18, 37), while a single Lactobacillus OTU is predicted to influence TAG content, and Xanthomonadaceae and Achromobacter (Betaproteobacteria) are associated with high glycogen content. In principle, the correlations between abundance of specific bacterial taxa and host nutritional traits can be attributed to bacterium-mediated modulation of Drosophila nutritional indices or the suitability of hosts with different nutritional phenotypes for different bacteria. A causal role of Acetobacteraceae is indicated by published demonstrations of significantly reduced energy storage indices in Drosophila monoassociated with these bacteria (11, 18, 19, 22, 37). Multiple attempts to culture Xanthomonadaceae from the DGRP were unsuccessful, although non-Xanthomonadaceae were readily isolated, and the causal basis of both the Xanthomonadaceae and Achromobacter on glycogen content remains to be investigated. In summary, correlations obtained in this study between microbial composition and nutritional traits in genetically distinct conventional Drosophila can identify taxa with causal effects on host nutrition demonstrated previously through monoassociation, and suggest putative roles for novel taxa in the Achromobacter and Xanthomonadaceae.

**Host genetic determinants of the abundance of the bacterium A. tropicalis.** We hypothesized that host genetic factors may contribute to the variation in the bacterial communities among the DGRP. As a test of this hypothesis, we took a discovery-based approach, using genome-wide association (GWA) to identify Drosophila mutations that influence microbial abundance. The test flies were colonized with a defined, five-species microbiota used previously (9, 11, 22) to ensure uniform access to bacterial species, and the DGRP lines comprised 61 lines used for pyrosequencing (Fig. 1A) (18 of the pyrosequenced lines were unsuitable for gnotobiotic culture, and 42 lines were added to increase sampling of genetic variation). We focused on the abundance of one bacterial species, A. tropicalis, which displayed high between-line variation in abundance, ranging from undetected (in three lines) to 20,000 CFU mg^-1 fly (Fig. 2A). Overall, most of this variation (78%) could be attributed to host genotype. Then, as a surrogate for a functional screen for Drosophila genes that contribute to variation in A. tropicalis abundance, we conducted a genome-wide association of the abundance of A. tropicalis CFU with SNP identity. As in other Drosophila GWA studies (GWAS), P value distributions displayed bias (12, 38) (see Text S1, Fig. S1, and Fig. S2 in the supplemental material). Subsequent analysis was restricted to genes near SNPs with P values below a nominal threshold of 2 × 10^-8. Seven SNPs associated with six unique genes fit these criteria (Table 2; all results shown in Data Set S3). Loss-of-function mutants derived by P- and Minos-elements or chemical mutagenesis were readily available for four of six genes: polychaetoid (pyd), paralytic (para), heartless (htl), and dunce (dnc) (Table S2B). The two other SNP-neighboring genes were Calnexin 14D (Cnx14D) and defensin (def), which have known functions in neuronal function and antimicrobial response, respectively.

To validate genes that affect A. tropicalis abundance, we used Drosophila lines bearing mutations in the four genes in the preceding paragraph and, to identify roles for genes with uncharacterized functions, two genes of unknown function with the lowest P values for which mutants were readily available (CG42575 and CG42313 [Table 2]). As in previous experiments, eggs from each Drosophila line were raised under axenic or five-bacterial-species...

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### Table 1: Correlation between Drosophila nutritional indices and bacterial taxa in DGRP lines

<table>
<thead>
<tr>
<th>Drosophila nutritional index and bacterial taxon</th>
<th>No. of lines</th>
<th>Spearman rank order correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>rho</td>
</tr>
<tr>
<td><strong>TAG content</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Komagataeibacter</td>
<td>10</td>
<td>-0.42</td>
</tr>
<tr>
<td>Acetobacteraceae</td>
<td>49</td>
<td>-0.43</td>
</tr>
<tr>
<td>Lactobacillus OTU7</td>
<td>21</td>
<td>+0.44</td>
</tr>
<tr>
<td><strong>Glucose content</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Komagataeibacter OTU5</td>
<td>14</td>
<td>-0.37</td>
</tr>
<tr>
<td>Komagataeibacter</td>
<td>14</td>
<td>-0.38</td>
</tr>
<tr>
<td>Komagataeibacter OTU115</td>
<td>11</td>
<td>-0.42</td>
</tr>
<tr>
<td><strong>Glycogen content</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comamonas</td>
<td>40</td>
<td>+0.40</td>
</tr>
<tr>
<td>Comamonadaceae</td>
<td>40</td>
<td>+0.40</td>
</tr>
<tr>
<td>Achromobacter</td>
<td>29</td>
<td>+0.42</td>
</tr>
<tr>
<td>Alcaligenaceae</td>
<td>29</td>
<td>+0.42</td>
</tr>
<tr>
<td>Xanthomonadaceae OTU160</td>
<td>45</td>
<td>+0.45</td>
</tr>
<tr>
<td>Achromobacter OTU10</td>
<td>29</td>
<td>+0.45</td>
</tr>
<tr>
<td>Xanthomonadaceae</td>
<td>45</td>
<td>+0.45</td>
</tr>
<tr>
<td>Acetobacteraceae</td>
<td>47</td>
<td>-0.54</td>
</tr>
</tbody>
</table>

a. The bacterial taxa in DGRP lines were family, genus, or OTU as indicated.
b. Adjusted with Bonferroni’s correction for number of comparisons at each taxonomic level (see Data Set S2 in the supplemental material).
Host genetic control of the microbiota. (A) CFU counts per fly of A. tropicalis in five-species gnotobiotic DGRP lines (colonized with Acetobacter pomorum DmCS_004, A. tropicalis DmCS_006, Lactobacillus brevis DmCS_003, L. fructivorans DmCS_002, and L. plantarum DmCS_001), ranked by CFU abundance, and expressed as log_{10}(number of CFUs) relative to background controls (bg). (D) Host genetic control of the microbiota was identified using a linear mixed model. Data are represented as means ± SEMs. (C) Mutants with altered microbiota composition have altered nutritional status. TAG content was measured in five-species gnotobiotic fly lines with mutations in genes that exert genetic control over the microbiota (CG42575, dnc) relative to background controls (bg). (D) Host genetic control of the microbiota mediates fly leanness. TAG content was measured in three-species (Lactobacillus brevis, L. fructivorans, and L. plantarum) gnotobiotic mutant fly lines (omission of Acetobacter species relieves host genetic control of Acetobacter-mediated TAG effects). In panels C and D, each symbol represents the value for an individual fly, and the horizontal bar shows the mean for the group. The data were evaluated by LMM and analysis of variance (ANOVA), with all statistical results shown in Table S1 in the supplemental material. Values that are significantly different are indicated by asterisks as follows: *, P < 0.05; ** P ≤ 0.01; *** P ≤ 0.001.

Relationship between D. melanogaster genotype and A. tropicalis-dependent nutrition. Our demonstration that host nutritional phenotype, first, varies with microbiota composition (Table 1) and, second, is associated with certain host genes (Table 2 and Fig. 2B) suggests that host genetic control of microbiota abundance may contribute to the effect of host genotype on nutritional indices. To test this hypothesis, our analysis focused on TAG, following the published demonstration of negative correlations between Acetobacter abundance and TAG content in one Drosophila line, Canton S (11). The first experiments colonized mutant flies and background control flies with five-species associations of Acetobacter and Lactobacillus species. As predicted, the dnc mutant (with reduced A. tropicalis load) displayed significantly elevated TAG levels, and the CG42575 mutant (with increased A. tropicalis load) had reduced TAG levels (Fig. 2C; statistics in Table S1B in the supplemental material). The TAG contents of para and CG42313 mutants were not significantly altered compared to background (P > 0.05; data not shown), perhaps because of genotype-specific effects in these mutants, and were omitted from subsequent analysis.

As a test that the interactions between host genotype and TAG content are mediated by Acetobacter, the mutant flies were reared with the three Lactobacillus species from the five-species microbiota used in the previous experiment. These associations were predicted not to replicate the effect of Acetobacter on TAG content, since Lactobacillus species do not reduce fly TAG content relative to axenic flies (11) (Table 1). Consistent with the expectation, the TAG content was not significantly affected by dnc mutation in Lactobacillus-colonized flies (Fig. 2D; statistics in Table S1C in the supplemental material) and was significantly increased by a Lactobacillus-only microbiota in the CG42575 mutant, the reverse of the effect in Acetobacter-colonized flies. These data demonstrate that the effects of the dnc and CG42575 mutations on host TAG content are congruent with their effects on the abundance of TAG-reducing Acetobacter and abolished in flies lacking Acetobacter. Taken together, the most parsimonious explanation is that some host genetic factors do not influence the metabolic determinants of TAG content directly but by their effect on the abundance of associated microorganisms.

DISCUSSION
This study investigated microbiota effects on the relationship between host genotype and phenotype, with respect to Drosophila nutritional traits. It extends previous research demonstrating strong statistical associations between genotype and multiple nutritional indices in the DGRP (45) to reveal that members of the microbiota are correlated with certain host nutritional indices. Multiple lines of evidence indicate that the bacteria are the causal basis of these correlations, at least with respect to the Acetobacteraceae: flies bearing Acetobacteraceae, but not Lactoba-
**TABLE 2 Genes with associated SNPs that had P values < 10^{-9} in GWAS or were tested for an effect on microbiota composition**

<table>
<thead>
<tr>
<th>Gene name</th>
<th>FlyBase ID*</th>
<th>P value</th>
<th>SNP rank(s)*</th>
<th>Mean CFU of A. tropicalis fly^{12}</th>
<th>No. of fly lines with the following allele type:</th>
<th>Human homolog^d</th>
<th>No. of homologs</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polychaetoid</td>
<td>FBgn 0262164</td>
<td>1.3e-10</td>
<td>1,5,9</td>
<td>2,839 406</td>
<td>Major 81 5</td>
<td>Sodium channel</td>
<td>PDZ domain-containing</td>
<td>3</td>
</tr>
<tr>
<td>Paralytic</td>
<td>FBgn 0264255</td>
<td>3.1e-10</td>
<td>2</td>
<td>2,713 332</td>
<td>Minor 87 6</td>
<td>Ca^2+ binding</td>
<td>Sodium channel</td>
<td>9</td>
</tr>
<tr>
<td>Calnexin 14D</td>
<td>FBgn 026077</td>
<td>3.1e-10</td>
<td>2</td>
<td>2,713 332</td>
<td>Minor 87 6</td>
<td>Antimicrobial peptide</td>
<td>Ca^2+ binding</td>
<td>9</td>
</tr>
<tr>
<td>Defensin</td>
<td>FBgn 0010385</td>
<td>8.6e-10</td>
<td>3</td>
<td>2,649 146</td>
<td>Major 88 4</td>
<td>Fibroblast growth factor receptor (FGFR 1)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Heartless</td>
<td>FBgn 0010389</td>
<td>1.0e-09</td>
<td>4</td>
<td>2,572 150</td>
<td>Minor 85 5</td>
<td>Na+ K+ ATPase</td>
<td>Including FGFR 1 to 4</td>
<td></td>
</tr>
<tr>
<td>Dunce</td>
<td>FBgn 0000479</td>
<td>3.1e-09</td>
<td>6</td>
<td>2,868 379</td>
<td>Minor 81 11</td>
<td>cAMP phosphodiesterase</td>
<td>Phosphate transporters</td>
<td>4</td>
</tr>
<tr>
<td>CG42575</td>
<td>FBgn 0260795</td>
<td>1.5e-07</td>
<td>17</td>
<td>2,568 157</td>
<td>Major 84 4</td>
<td>Antimicrobial peptide</td>
<td>Unknown</td>
<td>2</td>
</tr>
<tr>
<td>CG42313</td>
<td>FBgn 0259213</td>
<td>2.7e-07</td>
<td>26</td>
<td>2,708 1,010</td>
<td>Minor 80 10</td>
<td>Antimicrobial peptide</td>
<td>Unknown</td>
<td>NA</td>
</tr>
</tbody>
</table>

^a FlyBase ID. FlyBase identifier.
^b SNP rank having major or minor allele.
^c A. tropicalis with the major or minor allele.
^d NA, not available.

cilia, display the predicted reduction in TAG and glycogen contents (18, 37; this study), likely driven by bacterially mediated competition for dietary carbohydrate (37) and stimulation of insulin signaling in the Drosophila host (18). Variation in the contributions of distinct bacterial taxa to different traits (Table 1; see Data Set S2 in the supplemental material) is consistent with previous demonstrations of strain-specific effects in Drosophila that are not limited to high-level taxonomic classifications (19, 22, 37).

Correlations between microbiota composition and host lipid levels have been obtained previously for mouse mutants (Toll-like receptor 5 [TLR5], MyD88, and NOD2), with the microbiota identified as a causal factor by reproducing the deleterious metabolic phenotype via transplantation of gut microbiota from mutant to wild-type mice (46–48). Our study of Drosophila shows that the interactive effects of host genotype and microbiota on nutritional phenotype are not unique to mice (or mammals), and may be general to animals. Furthermore, the ease with which associations with different defined bacterial communities can be constructed in Drosophila enabled us to identify empirically the critical bacterial taxa. As our study illustrates, relatively small changes in the abundance of certain microbial taxa can have significant impacts on host phenotype, emphasizing the importance of attention to both microbial identity and abundance (e.g., reference 49).

Immediately relevant to the increasing evidence for microbial impacts on host nutritional phenotypes is the basis of the variation in microbiota composition. Multiple studies have shown that the microbiome in Drosophila and other animals, including mammals, is variable. Processes contributing to this variation include stochastic variation, such that each individual host samples only a subset of the total compatible microorganisms (43), positive and antagonistic interactions among microbial taxa that may be mediated directly (e.g., metabolic cross-feeding, toxin production) and indirectly via the host immune system (11, 50–54), and environmental factors, including diet (9, 14, 55–60). Other studies are revealing significant associations of certain microbial taxa in the mammalian gut microbiota with both host genotype (10, 61–63) and host phylogeny (64, 65). The GWA of A. tropicalis abundance in Drosophila in this study confirms the importance of host genetic factors as determinants of the microbiota and identifies candidate host genes contributing to this variation. We note that the percentage of variance in microbial community composition attributed to host genotype in this GWAS analysis is high (Fig. 2A), and this is likely a consequence of all fly lines being inoculated with the same set of microbes at the same starting density. Host genotype likely accounts for a lower proportion of the variation in conventional flies in laboratory culture and wild populations, where the gut microbiota community composition can be influenced by the availability of bacteria in the external environment (14–16). Full understanding of the determinants of gut microbiota composition will require integration of these multiple genetic, physiological, and ecological factors.

Strongly represented among the genes identified from the GWAS and associated validation in this study are genes with annotated roles in neural function or preferential expression in neural tissues. This is intriguing given the growing evidence linking the microbiota with neural function and behavior in mammals (66–68). Although further research is required to establish the mechanistic basis of the relationship between neural genes and microbiota in Drosophila, variation in neural function may, for example, alter the feeding response, and hence the amount of food bearing Acetobacter that the flies ingest (demonstrated, e.g., by reference 69), or the suitability of the host environment for the colonizing Acetobacter. Also of great interest is the relationship between the microbiota effects and other phenotypic consequences of variation in host genes. For example, to what extent is the role of the functional dnc gene in sustaining Acetobacter populations causally linked to its contribution to multiple other phenotypic traits, including associative learning (39, 70), life span (71), reproduction (71, 72), courtship (73), circadian rhythm (74), and locomotion (72)? The ready availability of mutants and natural variants in Drosophila, together with methods to manipu-
late the microbial complement of the flies, provides the opportunity to dissect whether and how the different phenotypic traits are linked.

In conclusion, this study reveals host genotype-specific effects on microbiota composition as a causal determinant of animal phenotype. The general relevance of the results on the Drosophila model system is indicated by both broad parallels with data obtained in microbiome studies of mammals, including humans (75), and the strong representation of genes with mammalian homologs among the genes associated with microbiota phenotypes (Table 2). These results indicate that the fundamentals of animal-microbiota-driven by a common molecular processes.

REFERENCES
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REFERENCES


64. Ochman H, Worobey M, Kuo CH, Ndjiangi JB, Peeters M, Hahn BH, Hugenholtz P. 2010. Evolutionary relationships of wild hominids reca-


