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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 *Xanthamonadaceae*, 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

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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 H_2O, 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 (ddH_2O), 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 5×10^7 total CFU ml^{-1}, 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 microbiota composition of flies, total genomic DNA was extracted from five pooled male flies as described previously (23, 24). 16S rRNA amplicons 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′-TGCTGCCTCCCGTAGGAGT-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 Quant-iT PicoGreen, and normalized to 10^9 molecules per μl. Emulsion PCR was conducted at 1.5 copies per bead using only "A" beads for unidirectional 454 GS-FLX pyrosequencing with standard titanium chemistry. Pyrosequencing flowgrams were analyzed by the procedure of Wong et al. (16) using QIIME 1.7.0 (25), except that reads from two half plates were separately demultiplexed. A total of 669,705 reads were obtained. Following quality filtering and removal of chimeras, the reads were clustered at 97% identity (see Data Set S1A in the supplemental material). After removal of reads representing *Wolbachia* sequences, all samples were rarefied to 501 reads, and single reads were discarded (Data Set S1B). Assignments to taxonomic ranks were performed in QIIME using the Greengenes database (26) (class, order, family, genus, species). Operational taxonomic units (OTUs) were binned based on QIIME taxonomic assignments during correlation analysis. Every OTU in the reagent-only controls had <10 reads, ensuring minimal contamination of experimental samples.

Assays of fly weight and nutritional indices. Flies were lightly anesthetized on CO_2, and males were sorted for downstream assays. For dry weight measurements, flies were flash-frozen on dry ice and desiccated over 7 days at 55°C prior to weighing. To quantify nutritional indices, triplicate samples of five flies each were homogenized in 125 μl TET buffer (10 mM Tris [pH 8], 1 mM EDTA, 0.1% Triton X-100) with 1.4-mm ceramic beads (MP Biomedicals) in a FastPrep-24 instrument (MP Biomedicals) for 30 s. Twenty microliters of homogenate was frozen at −80°C, and 40 μl was heat treated at 72°C for 15 min prior to freezing at −80°C. As described previously (12), commercial kits were used to assay protein content (catalog no. 500-0111; Bio-Rad) in directly frozen samples, and glucose and glycogen (catalog no. GAGO20-1KT; Sigma-Aldrich) and triglyceride (catalog no. TR0100-1KT; Sigma-Aldrich) content in the heat-treated samples. All nutritional indices were normalized to dry weight.

Bacterial culture and monoassociation experiments. For total bacterial counts, pools of five flies were homogenized in 125 μl phosphate-buffered saline (PBS), and the homogenate was directly plated onto mMRS plates using a spiral plater (WASP-2 instrument; Microbiology International). To measure *Lactobacillus* abundance, a replicate plate from the same homogenate was plated onto mMRS medium and incubated in a CO_2-flooded airtight container. To obtain selective growth of *A. tropicalis* from gnotobiotic flies containing both *A. tropicalis* and *A. pomorum*, the flies were homogenized in TET buffer, which disrupted growth of the *A. pomorum* cells. Bacterial colonies were read using a Protocol 3 colony counter (Microbiology International).

Microbiota abundance correlations with nutritional indices. Spearman's rank correlations performed in R (27) identified significant correlations between nutritional indices and microbiota abundance (OTU abundance at every taxonomic level assigned in QIIME OTU table [class, order, family, genus, species, OTU [97%] with Bonferroni's correction]).

Genome-wide association. The DGRP lines used for genome-wide association (GWA) (see Table S2A in the supplemental material) were raised with *A. pomorum*, *A. tropicalis*, *L. brevis*, *L. fructivorans*, and *L. plantarum*. Male flies were sorted and homogenized in TET buffer (as described above), and *A. tropicalis* load was quantified in three triplicate pools of 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 nlme 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 S2B 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 (sqrt) 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,

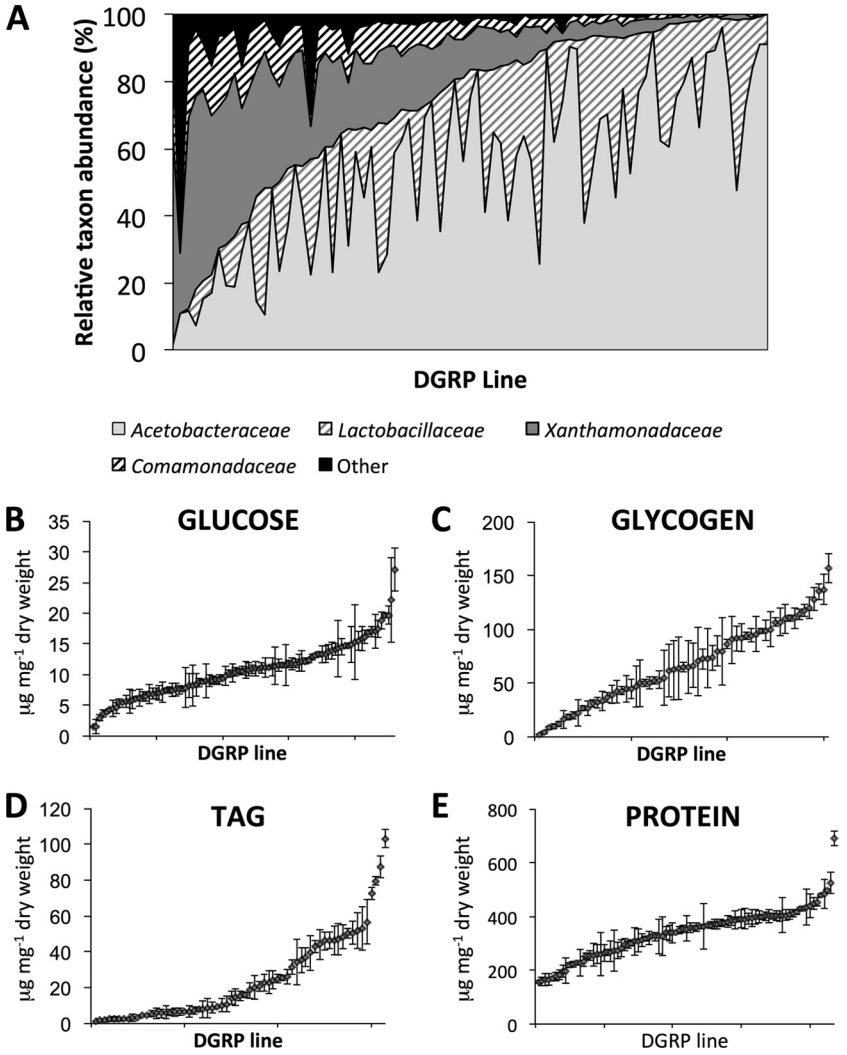

FIG 1 Bacterial communities and phenotypic traits of 79 *Drosophila* lines from DGRP. (A) Microbiota composition was assessed by pyrosequencing with OTUs called at 97% sequence identity (see also Data Set S1 in the supplemental material). (B to E) Nutritional indices (in micrograms per milligram [dry weight]), with data represented as means ± standard errors of the means (SEMs) (error bars). In each panel, *Drosophila* lines are ordered by the sum of *Acetobacter* and *Lactobacillus* species (A) or by mean nutritional index value (B to E).

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 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

TABLE 1 Correlation between *Drosophila* nutritional indices and bacterial taxa in DGRP lines

Drosophila nutritional index and bacterial taxon[a]	No. of lines	Spearman rank order correlation		
		rho	*P* value	Critical probability (no. of samples)[b]
TAG content				
Komagataeibacter	10	−0.42	0.003	0.0055 (9)
Acetobacteraceae	49	−0.43	0.002	0.006 (8)
Lactobacillus OTU7	21	+0.44	0.002	0.002 (25)
Glucose content				
Komagataeibacter OTU5	14	−0.37	0.002	0.0017 (29)
Komagataeibacter	14	−0.38	0.001	0.0055 (9)
Komagataeibacter OTU115	11	−0.42	<0.001	0.0017 (29)
Glycogen content				
Comamonas	40	+0.40	0.005	0.007 (7)
Comamonadaceae	40	+0.40	0.005	0.008 (6)
Achromobacter	29	+0.42	0.003	0.007 (7)
Alcaligenaceae	29	+0.42	0.003	0.007 (7)
Xanthomonadaceae OTU160	45	+0.45	0.002	0.0022 (23)
Achromobacter OTU10	29	+0.45	0.001	0.0022 (23)
Xanthomonadaceae	45	+0.45	0.001	0.008 (6)
Acetobacteraceae	47	−0.54	<0.001	0.008 (6)

[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).

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 *Gluconobacter* and *Komagataeibacter*, as well as the previously documented *Acetobacter*, 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 *Xanthamonadaceae.*

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 103 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 neural 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

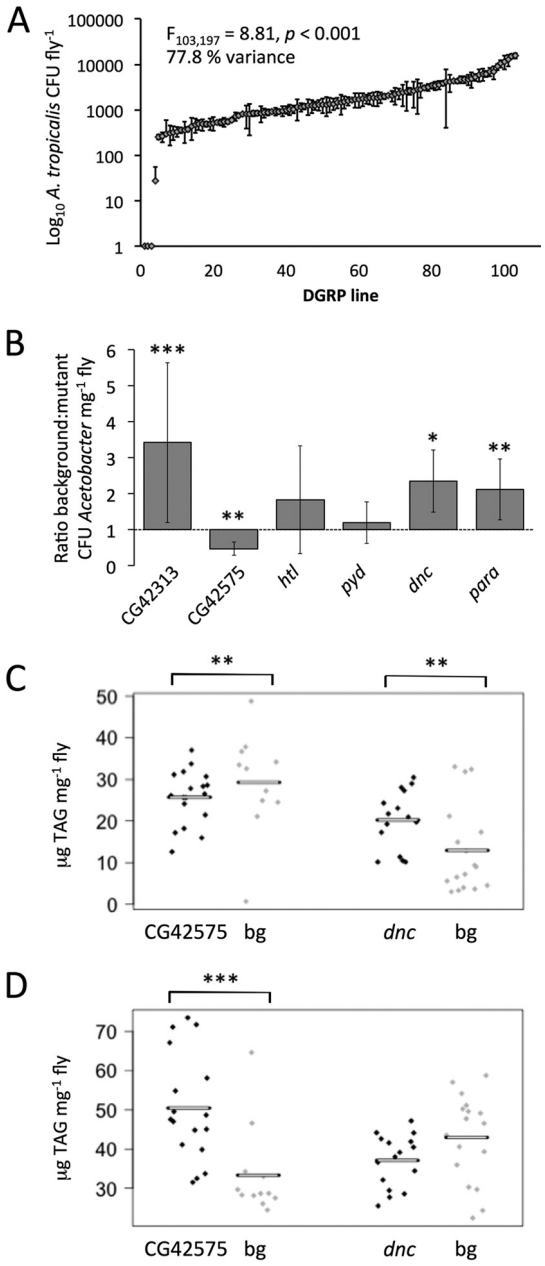

FIG 2 Host genetic control of the microbiota. (A) CFU counts per fly of *A. tropicalis* in five-species gnotobiotic DGRP lines (colonized with *Acetobacter pomorum* DmCS_004, *A. tropicalis* DmCS_006, *Lactobacillus brevis* DmCS_003, *L. fructivorans* DmCS_002, and *L. plantarum* DmCS_001), ranked by CFU abundance, and expressed as $\log_{10}$(number of CFUs +1). The contribution of genotype to the total variance (77.8%) was calculated as the square of the standard deviation when DGRP genotype was included as a random effect in the linear mixed model (LMM). (B) Validation of *A. tropicalis* abundance GWA. Differences between mutant and control lines each raised with the defined five-species microbiota were 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: *, $P < 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. bg, background.

gnotobiotic conditions. Four of the six mutations, including mutations in the well-characterized *dnc* and *para* genes, had significant effects on *A. tropicalis* abundance relative to background controls (Fig. 2B; statistics in Table S1A in the supplemental material). *dnc* is a classical learning gene in *Drosophila* (39), encoding a cyclic AMP (cAMP) phosphodiesterase (40), and has homology to human genes associated with disease (e.g., schizophrenia [41]). *para* encodes a sodium channel that, when disrupted, leads to paralytic phenotypes at elevated temperatures (42) or to olfactory dysfunction (43), and its human homologs are associated with epilepsy (44). In summary, these findings identify specific host genetic factors with homology to human genes that influence the abundance of associated microorganisms in *Drosophila*.

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

Gene name	FlyBase ID[a]	*P* value	SNP rank(s)[b]	Mean CFU of *A. tropicalis* fly^{-1}[c]		No. of fly lines with the following allele type:		Function	Human homolog[d]	
				Major	Minor	Major	Minor		No. of homologs	Function
Polychaetoid	FBgn 0262614	1.3e−10	1,5,9	2,839	406	81	8	PDZ domain-containing	3	Tight junction proteins 1 to 3
Paralytic	FBgn 0264255	3.1e−10	2	2,713	332	87	6	Sodium channel	9	Sodium channels
Calnexin 14D	FBgn 0264077	3.1e−10	2	2,713	332	87	6	Ca^{2+} binding	4	Including Ca^{2+} binding
Defensin	FBgn 0010385	8.6e−10	3	2,649	146	88	4	Antimicrobial peptide	NA	NA
Heartless	FBgn 0010389	1.0e−09	4	2,572	150	85	5	Fibroblast growth factor receptor (FGFR 1)	4	Including FGFR 1 to 4
Dunce	FBgn 0000479	3.1e−09	6	2,868	379	81	11	cAMP phosphodiesterase (PDE)	4	PDE4 (A to D)
CG42575	FBgn 0260795	1.5e−07	17	2,568	157	84	4	Unknown	2	Phosphate transporters
CG42313	FBgn 0259213	2.7e−07	26	2,708	1,010	80	10	Unknown	NA	NA

[a] FlyBase ID, FlyBase identifier.
[b] Rank 1 has the lowest probability.
[c] *A. tropicalis* with the major or minor allele.
[d] NA, not available.

cillus, 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 interactions may be evolutionarily conserved and driven by a common molecular processes.

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