Journal of Insect Physiology 133 (2021) 104274

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Microbial colonization promotes model cockroach gut tissue growth and development

Benjamin C. Jahnes [a], Keyshap Poudel [b], Amelia M. Staats [a], Zakee L. Sabree [a,b,*]

[a] Department of Microbiology, Ohio State University, Columbus, OH, USA
[b] Department of Evolution, Ecology and Organismal Biology, Ohio State University, Columbus, OH, USA

ARTICLE INFO

Keywords:
Periplaneta americana
Gut development
Microbiome
Cockroach
Gut epithelium
Germ free

ABSTRACT

Background: Digestive tissues are essential for diet processing and nutrient accessibility, especially in omnivores, and these functions occur despite and in collaboration with dynamic microbial communities that reside within and upon these tissues. Prolonged host development and reduced digestive tissue sizes have been observed in germ-free animals, and normal host phenotypes were recovered following the re-introduction of typical gut microbiomes via coprophagy.

Results: High-resolution histological analyses of *Periplaneta americana* cockroach digestive tissues revealed that total prevention of microbial colonization of the gut had severe impacts on the growth and development of gut tissues, especially the posterior midgut and anterior hindgut subcompartments that are expected to be colonized and inhabited by the greatest number of bacteria. Juveniles that were briefly exposed to normal gut microbiota exhibited a partial gut morphological recovery, suggesting that a single inoculation was insufficient. These data highlight gut microbiota as integral to normal growth and development of tissues they are in direct contact with and, more broadly, the organism in which they reside.

Conclusions: We draw on these data, host life history traits (i.e. multigenerational cohousing, molting, and filial coprophagy and exuvia feeding), and previous studies to suggest a host developmental model in which gut tissues reflect a conflict-collaboration dynamic where 1) nutrient-absorptive anterior midgut tissues are in competition with transient and resident bacteria for easily assimilable dietary nutrients and whose growth is least-affected by the presence of gut bacteria and 2) posterior midgut, anterior hindgut, and to a lesser degree, posterior hindgut tissues are significantly impacted by gut bacterial presence because they are occupied by the greatest number of bacteria and the host is relying upon, and thus collaborating with, them to assist with complex polysaccharide catabolism processing and nutrient provisioning (i.e. short-chain fatty acids).

1. Introduction

Host organisms and their gut microbiota meet at the gut lumen-epithelium interface where they are in close proximity and intimately engaged in the exchange of metabolites that include nutrients (Colombani et al., 2003; Macfarlane et al., 2005; Martens et al., 2008; Storelli et al., 2011; Tailford et al., 2015; Zheng et al., 2017), signaling molecules (Capo et al., 2016; Lee et al., 2015), and virulence factors (Ashida et al., 2012; Juge, 2012). Microbial stimulation of gut tissue development has been documented in numerous animal systems and attributed to nutrient provisioning and stress response (Bracke et al., 1978; Donohoe et al., 2011; Jahnes et al., 2019; Jones et al., 2013; Patel et al., 2013). Animal models, especially those amenable to germ-free rearing, are essential for linking host phenotypes to endemic and synthetic microbial communities. Germ-free *Periplaneta americana,* an emerging host-microbiome experimental platform, were conventionalized with feces from wild-type *P. americana* and subsequently colonized by the microbes therein and as a result hindgut tissues in fifth instar insects exhibited near wild-type level growth compared to fifth instar germ-free insects (Jahnes et al., 2019). Gut microbial mutualists are hypothesized to promote gut development through assistance with diet accessibility (Cruden and Markovetz, 1979), provisioning of essential amino acids (Wong et al., 2014), vitamins and short-chain fatty acids (Donohoe et al., 2011; Zurek and Keddie, 1996), and by being directly digested by the host (Yamada et al., 2015). For example, normal organismal growth rates in *Drosophila* reared under nutrient-limited conditions could be

* Corresponding author at: 318 W. 12th Avenue, Room 300, Columbus, Ohio 43210, USA.
E-mail address: sabree.8@osu.edu (Z.L. Sabree).

https://doi.org/10.1016/j.jinsphys.2021.104274
Received 5 March 2021; Received in revised form 22 June 2021; Accepted 22 June 2021
Available online 1 July 2021

rescued by dietary supplementation with the *Issatchenkia orientalis* fungal commensal that promoted dietary amino acid uptake (Yamada et al., 2015), or the *Lactobacillus plantarum* bacterial commensal that promoted growth by activating amino acid-sensing cascades (Storelli et al., 2011). Similarly, acetic acid production by the *Acetobacter pomorum* bacterial commensal has been shown to be essential for host organismal growth promotion (Shin et al., 2011).

The germ-free condition in animals may result in undernutrition if the host's net nutrient harvest is less than when in the presence of a normal gut microbiota. Starvation and undernutrition can decrease intestinal stem cell division, DNA replication and increase apoptosis within midgut nidi (sites of rapid stem cell division and differentiation) (Park et al., 2009; Park and Takeda, 2008), which may explain reduced midgut lengths, and reduced hindgut turgidity, lateral infolds, and muscularity in germ-free *P. americana* (Jahnes et al., 2019). Some gut microbiota may compete with the host for assimilable nutrients, effectively reducing net host nutrient harvest, in which case germ-free insects would experience increased growth and development when compared to wild-type, yet previous work suggest that growth and development are positively affected by colonization with microbes typically associated with the host (Bracke et al., 1978; Jahnes et al., 2019; Jones et al., 2013; Shin et al., 2011; Storelli et al., 2011; Yamada et al., 2015). Cockroaches emerge from the oothecum with only the intracellular symbiont *Blattabacterium* sp. embedded in its fat body tissues, while further microbial colonization of the cockroach gut requires exposure to microbes through coprophagy, exuvia consumption, necrophagy, and through the diet (Nalepa and Bignell, 2001). Coprophagy is common across all life stages, with it occurring most frequently during first instar (Kopanic et al., 2001; Kopanic and Schal, 1999) and the major constituents of the gut community composition are thought to be present by second instar (Carrasco, 2014). This suggests that inoculation of the gut at early instars may be sufficient for establishing a diverse gut microbial community, yet it is unknown how a single inoculation event at an early instar influences long term development of the gut.

Gut development may be influenced by the size and makeup of the microbial population in a given gut compartment. Previous cockroach gut microbial community profiling along the digestive tract suggests that the lowest microbial abundance and Shannon diversity exists in the proximal gut (crop: $2.2x10^6$ mg^{-1}, H = 0.51; midgut: $2.0x10^6$ mg^{-1}, H = 0.48), with abundance and diversity peaking in the hindgut ($2.2x10^7$ mg^{-1}, H = 1.55) and dropping somewhat in the rectum ($1.5x10^7$ mg^{-1}, H = 1.13) (Schauer et al., 2012). Correspondingly, a cultivation-based study revealed greater microbial abundance in the posterior midgut (10^8 ml^{-1}) compared to the anterior midgut (10^7 ml^{-1}) and three to eight-fold greater abundance within the anterior hindgut (10^8 ml^{-1}) than the posterior hindgut (Bignell, 1977).

The degree to which microorganisms affect gut development may depend on developmental programs unique to the gut compartment. Foregut and hindgut tissues develop from embryonic ectoderm, while the midgut develops from embryonic endoderm leading to different physical characteristics and maintenance regimes for these tissues (Hartenstein et al., 1985; Takashima and Hartenstein, 2012). The foregut and hindgut are composed exclusively of enterocytes and bears a chitinous lining that offers the epithelial cells some protection from the gut contents (Bell and Adiyodi, 1982; Bignell, 1977). The foregut and hindgut don't continuously regenerate, as the midgut, and regeneration is primarily responsive to cell damage in these compartments (Fox and Spradling, 2009; Takashima et al., 2008). The normal function of the midgut and its responses to commensals and pathogens depends on continued renewal of the midgut epithelial surface (Jiang et al., 2009; Takashima and Hartenstein, 2012). To accomplish continuous midgut renewal, intestinal stem cells divide to form two daughter cells, one which retains pluripotency and another which begins differentiation as an enteroblast (Guo et al., 2016; Osman et al., 2012; Takashima and Hartenstein, 2012). Enteroblasts have two fates, one with differentiation into an enterocyte, and another with differentiation into an enteroendocrine cell (Royet, 2011). As enteroendocrine cells and enterocytes suffer damage or age they undergo apoptosis and slough off into the gut lumen (Day and Powning, 1949; Hakim et al., 2010). The speed of cell turnover is responsive to conditions within the gut, such as the presence of pathogens, commensals, food availability, and chemical and physical stressors (Buchon et al., 2013a; Hakim et al., 2010; Jones et al., 2013; Park et al., 2009; Park and Takeda, 2008; Petkau et al., 2014; Xiao et al., 2017), with cell turnover rates increasing with stressors such as pathogens, commensals, toxins, and physical damage, and cell turnover rates decreasing with lack of food, undernutrition, or under a germ free condition. Insect gut epithelial cells cycle on the order of one week (Ohlstein and Spradling, 2006) and the rate of cell division recovers to a basal level after the removal of a stressor (Park and Takeda, 2008), yet it is unclear how temporary changes in cell cycle propagate to tissue level morphological changes over the accumulation of time. This study scrutinizes anterior and posterior regions of the cockroach mid- and hind-gut tissues to identify sites where decreased proliferative activity in the absence of microbial colonization may have led to observed attenuated midgut and hindgut phenotypes.

2. Materials and methods

2.1. Insects

P. americana nymphs, adults and ootheca were obtained from a live collection maintained in the insectary in the Ohio State University Biological Sciences Greenhouse (Columbus, Ohio).

2.2. Ootheca treatment

Ootheca were surface-sterilized and germ-free nymphs were hatched as previously described (Jahnes et al., 2019). First instar nymphs from each oothecum were divided into cohorts of 3 or 4 insects divided across treatments as follows. Diagnostic PCR and cultivation-based quality control measures were employed as previously described (Jahnes et al., 2019) to monitor potential contamination.

2.3. Germ-free P. americana

Germ-free (GF) first instar nymphs were aseptically-transferred to sterile rearing chambers stocked with sterilized rat chow and aseptic 1% agar, sufficient for 60 + days of feeding, and chambers were ventilated with air filtered through a 0.22 μm membrane.

2.4. Conventionalized P. americana

To introduce bacteria native to *P. americana*, GF first instars were allowed to feed *ad libitum* for seven days on a 10:1 mix of sterilized rat food and frass taken from a lab-maintained colony of nonsterile or 'wild-type' (WT) *P. americana*. These conventionalized (Con) first instar nymphs were housed in sterilized rearing chambers to limit their access and exposure to microbiota to only those they obtained in the initial oral inoculation by feeding. Fresh sterilized rat chow was provided on a weekly basis as required to limit growth of spoilage organisms. As each generation of *P. americana* typically acquires their gut microbial community through conspecific coprophagy (Bell et al., 2007; Nalepa et al., 2001), the conventionalization approach reflects this route of gut microbe acquisition, while maintaining insects in habitats equivalent to those of GF insects.

2.5. Normal P. americana

One day old first instar insects from 10 surface-sterilized ootheca were deposited in an aquarium containing 10 adult male cockroaches from a nonsterile mixed generation colony maintained in the lab and provided with sterilized rat chow and access to sterile Milli-Q water *ad*

libitum; nymphs from this colony were designated 'normal' (WT) as they experienced a life history largely indistinct from other lab-reared insects. WT hatchlings were free to interact with adult cockroaches and their frass, which encourages normal coprophagic behavior and acquisition of normal gut microbiota. Late-stage nymphs from the nonsterile colony were sacrificed and deposited in the WT colony, as cannibalism and necrophagy of deceased nestmates is also a putative mechanism for gut microbiota acquisition.

2.6. Histology and imaging

Five insects were dissected and sectioned for each treatment, for a total of 15 insects used in this study. Insects were dissected in sterile PBS to extract full-length digestive tissues, which were fixed and ten-micron transverse sections were prepared from the posterior anal cavity to proventriculus (foregut-midgut interface) and affixed to positively-charged slides. Each slide had 4–8 sections from the same gut sample and region, with about 20–30 slides per gut being prepared. Sectioned tissues were stained according to Heidenhain's Azan Trichrome method (Schmid, 1989) and mounted and cover-slipped with Cytoseal 60. Five cross-sections were imaged per gut subcompartment per individual (n = 5) for a total of 25 measurements per treatment per gut region for cross-sectional area and perimeter measurements. For visceral muscle measurements, five transverse sections per gut subcompartment per individual (n = 5) were imaged at higher magnification (400x), capturing four micrographs per section for a total of 200 images per treatment per gut region. Micrographs containing digestate and bacterial biomass directly adjacent to the gut lumen required manual demarcation of the luminal boundary to ensure accurate thresholding. Images were converted to masks of total gut cross-sectional area and luminal area and the difference of these constituted the epithelial cross-sectional area (CSA) (Fig. 1). Luminal (LPR) and gut (GPR) perimeter measurements were obtained from the luminal and total cross-sectional area masks, respectively (Fig. 1). For visceral muscle thickness (VMT) measurements, grid overlays were projected over micrographs to direct measurements in a random fashion to avoid measurement bias, as muscle thickness within a sample was often heterogeneous, and two measurements were taken per image. Additionally, midgut and hindgut tissue thin sections (three per gut region) were DAPI stained to detect and image microbes present amongst the biomass at 200X and 1,000X magnification. Detailed description of histological and imaging methods can be found in the Supplemental Materials.

2.7. Statistics

As multiple measures per individual were not independent, pseudoreplication was avoided by using the average of measures from each individual within a treatment and gut region and the averaged data was evaluated using a conventional ANOVA. Linear mixed model ANOVA was conducted in R using the nlme package based on the model lme(y ~ trt, random = ~1 | ind) to account for variation due to multiple measures of each individual insect.

3. Results

3.1. Gut subcompartment colonization

Normal (WT) and conventionalized (Con) midgut and hindgut tissues exhibited biomass emitting fluorescence that, when inspected at higher magnification (1,000X), was linked to cellular morphologies consistent with bacteria (Figure S1 and S2), indicating that bacteria were present in these tissues. Only autofluorescent angular and amorphous dietary particles were observed in tissues taken from germ-free (GF) insects (Figure S1 and S2).

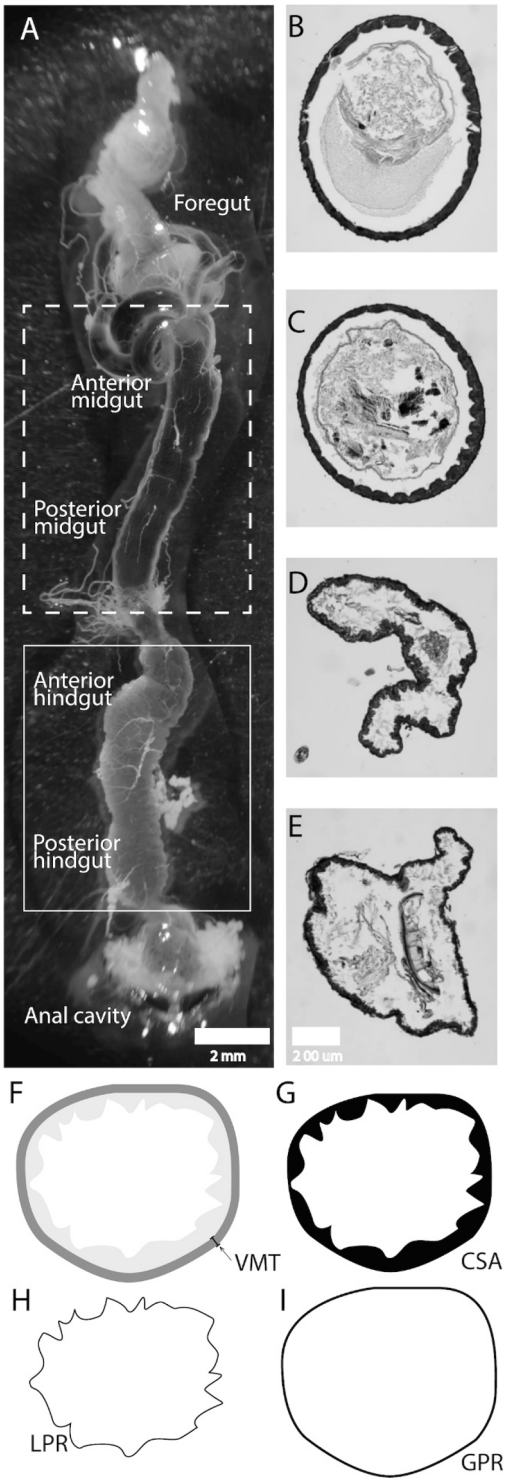

Fig. 1. *P. americana* digestive tract and transverse section measurement schematic. Dissection of complete digestive tract (A) from fifth instar *P. americana* with the midgut (dashed box) and subcompartments and hindgut (solid box) subcompartments labeled. Exemplars of stained and imaged (100X magnification) transverse sections from lab-reared *P. americana* anterior midgut (B), posterior midgut (C), anterior hindgut (D) and posterior hindgut (E). Diagram depicts the visceral muscle (dark gray), epithelial tissue (light gray) and lumen (white) in a stylized simplification of a transverse section (100x total magnification) of *P. americana* gut tissues (F). Masks (black) were generated from processed and imaged thin-sections and used to collect visceral muscle thickness (VMT; F), epithelial cross-sectional area (CSA; G), luminal perimeter (LPR; H), and gut perimeter (GPR; I) measurements.

3.2. Epithelial cross-sectional area

Cross-sectional area (CSA) included visceral-muscle and epithelial biomass of the gut tissues, and, in general, WT CSA was the greatest in all four subcompartments and GF CSA was the least. No significant differences were observed across anterior midgut CSA treatments (Fig. 2A). Posterior midgut CSA was greatest in WT (mean 958 μm^2, SD 303), which was greater than GF CSA (mean 518 μm^2, SD 69.2; ANOVA: WT-GF $p = 0.010$), with Con CSA being intermediate to WT and GF (mean 741 μm^2, SD 76.7) (Fig. 2B). Similarly, anterior hindgut CSA in WT tissues was the greatest (mean 1128 μm^2, SD 344) and GF was the least (mean 526 μm^2, SD 182; ANOVA: WT-GF $p = 0.018$), with Con CSA being intermediate (828 μm^2, SD 325) to GF and WT AHG CSA (Fig. 2C). Finally, posterior hindgut CSA (mean 691 μm^2, SD 81.7) was significantly greater than both Con CSA (mean 473 μm^2, SD 150) and GF CSA (mean 353 μm^2, SD 124; ANOVA: WT-Con $p = 0.038$, WT-GF $p = 0.002$) (Fig. 2D).

3.3. Gut perimeter

The exterior perimeter of the cockroach gut (GPR) reflects the degree of lateral expansion of the gut and is proportional to the overall gut diameter. Average GPR of thin sections from gut epithelial tissue were compared across all bacterial treatments and gut subcompartments. Significant GPR length differences were observed in the posterior midgut (Fig. 2F) and anterior hindgut (Fig. 2G) tissues. Posterior midgut GPR was greater in WT (mean 238 μm, SD 15.0) insects compared to GF (mean 190 μm, SD 19.2) insects (ANOVA: WT-GF $p = 0.004$). Similarly, anterior hindgut GPR was greater in WT (mean 342 μm, SD 50.3) insects compared to GF (mean 199 μm, SD 41.2) insects and greater in Con (mean 317 μm, SD 55.9) insects compared to GF insects (ANOVA: WT-GF $p = 0.002$, Con-GF $p = 0.007$). Although differences between treatments in the anterior midgut (ANOVA: $p = 0.143$; Fig. 2E) and posterior hindgut (ANOVA: PHG - $p = 0.344$; Fig. 2H) tissues were observed, they were not significant.

3.4. Luminal perimeter

The length of the interior luminal perimeter (LPR) of thin sections from gut epithelial tissue were different across all treatments, but these differences were significant only in the posterior midgut and anterior hindgut tissues (Fig. 2, I-L). LPR posterior midgut was greatest in WT (mean 355 μm, SD 76.6) insects when compared to GF (mean 208 μm, SD 24.4) insects (ANOVA: WT-GF $p = 0.002$). The luminal perimeter of the anterior hindgut was greater in Con (mean 483 μm, SD 117) insects compared to GF (mean 332 μm, SD 75.3) insects (ANOVA: Con-GF $p = 0.040$) but overlapped.

3.5. Ratio of gut perimeter to luminal perimeter

The ratio (RPR) of gut perimeter to luminal perimeter (RPR = GPR/LPR) represents the degree to which the luminal epithelium was invaginated and thus reflected the presence and depth of infoldings associated with increased surface area for digestive processes (Fig. 2M–P).

Although the WT anterior midgut RPR was the least (mean 0.71, SD 0.25) when compared to Con and GF, the differences were not significant (ANOVA: $p = 0.24$). WT posterior midgut RPR were relatively broadly distributed, but overall were lower in WT (mean 0.70, SD 0.141) insects compared to GF (mean 0.92, SD 0.075) insects (ANOVA: WT-GF

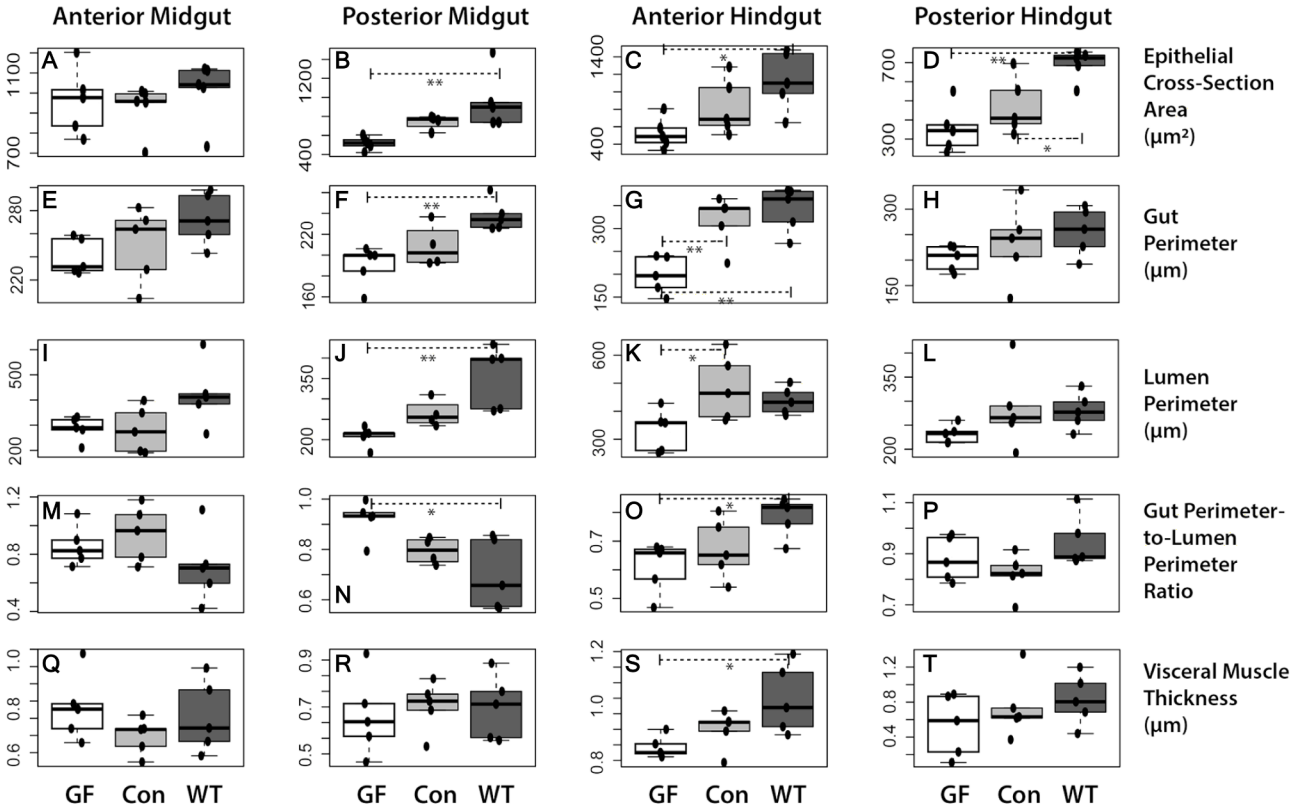

Fig. 2. Exposure to normal gut bacterial community impacts gut subcompartment growth and development across several gut physiological measures. ANOVA was conducted on the average value of epithelial cross-sectional area (CSA), gut perimeter (GPR), luminal perimeter (LPR), gut perimeter-to-luminal perimeter ratio (a measure of luminal crypt depth) measurements and visceral muscle thickness (VMT) measurements from each insect. Boxplots represent first and third quartiles, median, minimum and maximum values. Germ-free insects (GF, open fill), Single exposure to conspecific feces (Con, light grey fill); *ad libitum* access to conspecific feces and cohousing with nonsanitized insects under nonsterile conditions (WT, dark grey fill).

p = 0.013), and Con RPR was, on average, intermediate to WT and GF RPR. WT anterior hindgut RPR was higher (mean 0.79, SD 0.070) than GF (mean 0.61, SD 0.091) insects (ANOVA: WT-GF p = 0.023), with Con RPR values being intermediate. Finally, posterior hindgut RPR values were evenly distributed and generally similar, and thus the differences were not significant (ANOVA: p = 0.13).

3.6. Visceral muscle thickness

The thickness of visceral muscle (VMT) was measured at eight randomly-selected positions around the periphery of each gut section and averages from each individual were compared across all treatments and gut subcompartments (Fig. 2, Q-T). Significant differences between treatments were only observed in the anterior hindgut VMT where WT (mean 1.07 μm, SD 0.15) tissues were significantly greater than GF (mean 0.86 μm, SD 0.05) tissues (ANOVA: WT-GF p = 0.020) and muscle thickness in Con (mean 0.93 μm, SD 0.08) insects was intermediate to WT and GF, but not significantly different. Muscle thickness in the posterior hindgut followed the same trend of increasing thickness from GF (mean 0.76 μm, SD 0.24) to Con (mean 0.90 μm, SD 0.24) and WT (mean 0.96 μm, SD 0.20). Although slight treatment effects in the anterior midgut and posterior midgut VMT were observed, they were not significant.

3.7. Averaged measures vs linear mixed model

Multiple measures from the same insect and gut compartment were either averaged before statistical analysis or a linear mixed model was applied to compensate for multiple measures. Though p-values were lower using the linear mixed model, only two changes in significance were observed between the two methods (Table 1). In the posterior midgut gut perimeter analysis the WT – Con comparison becomes significantly different (LMM-ANOVA: WT-Con p = 0.043), as well as the posterior midgut lumen perimeter WT-Con comparison (LMM-ANOVA: WT-Con p = 0.021).

4. Discussion

All four gut subcompartments exhibited some degree of tissue growth and development following exposure to gut microbiota, and the posterior midgut and anterior hindgut regions were most influenced by microbial colonization and significant treatment effects across all examined histological features were consistently observed (Table 1). Measures from conventionalized (Con) tissues were typically intermediate to those from germ-free (GF) and normal (WT) tissues, which was also observed in *P. americana* gut morphology following similar treatments (Jahnes et al., 2019). Taken together, these data suggest a relationship between microbial gut community presence and composition and host gut tissue growth and development (Fig. 2). The GF, Con, and WT treatments reflect increasing exposure to a diverse residential bacterial community. GF insects lack bacteria (and other microbes) except for the vertically-inherited, organelle-like obligate endosymbiont, *Blattabacterium* (Sabree et al., 2009), that is incarcerated within fat body tissues of all cockroaches and thus present in all treatments. Conventionalization was conducted by allowing GF insects to feed upon, and domicile with, sterile diet infused with frass from lab-reared conspecifics for a single, seven-day period. WT insects were GF hatchlings that were housed under nonsterile conditions with lab-reared adult male conspecifics and had *ad libitum* access to their frass and the same diet used in other treatments for the entire duration of their development. Therefore, it was expected that WT insects had a better chance to comprehensively sample the diversity of typical gut bacteria than Con-reared insects. Additionally, the bacterial community in conventionalized individuals frequently led to elevated tissue development intermediate to that of WT individuals and GF individuals, which suggested that a microbial community capable of stimulating development was present in the Con treatment. The inability of the Con treatment to fully recover normal development could have been due to the initial inoculum being insufficiently representative of normal gut microbiota. Additionally, taxa may have been progressively lost during molting events that remove the fore- and hind-gut tissues and their associated microbiota, and expose them to the external atmosphere, which would negatively impact many oxygen-sensitive taxa from groups typically abundant in the low-to-anoxic hindgut (i.e. Bacteroidetes, Firmicutes, Fusobacteria)

Table 1
p-values associated with conventional (A) and linear mixed model (B) analysis of variance (ANOVA) results from comparisons of measurements across treatments within each gut subcompartment. Instances where none of the treatment-based comparisons were significantly different, the lowest p-value observed was recorded. Significant p-values are italicized. Treatments: GF – germ-free, Con – conventionalized, WT –normal lab-rearing.

A

	Anterior Midgut			**Posterior Midgut**			**Anterior Hindgut**				**Posterior Hindgut**	
Treatment Comparison	GF-Con	GF-WT	Con-WT	GF-Con	GF-WT	Con-WT	GF-Con	GF-WT	Con-WT	GF-Con	GF-WT	Con-WT
Cross Sectional Area	0.697	0.238	**0.01**	0.253	0.269	***0.018***	0.277	0.303	***0.002***	*0.038*		
Gut Perimeter	0.143	0.321	**0.004**	0.083	***0.007***	***0.002***	0.702	0.344				
Lumen Perimeter	0.065	0.283	**0.002**	0.053	***0.040***	0.166	0.683	0.268				
Ratio Gut/Lumen Perimeter	0.235	0.197	**0.013**	0.358	0.524	***0.023***	0.158	0.127				
Muscle Thickness	0.350	0.892	0.497	***0.020***	0.152	0.408						

B

	Anterior Midgut			**Posterior Midgut**			**Anterior Hindgut**			**Posterior Hindgut**		
Treatment Comparison	GF-Con	GF-WT	Con-WT	GF-Con	GF-WT	Con-WT	GF-Con	GF-WT	Con-WT	GF-Con	GF-WT	Con-WT
Cross Sectional Area	0.696	0.194	**8.61E-04**	0.21	0.233	**0.003**	0.234	0.267	**<0.001**	0.013		
Gut Perimeter	0.143	0.283	**<0.001**	**0.0431**	**4.96E-04**	**<1E-04**	0.691	0.344				
Lumen Perimeter	0.065	0.241	**<0.001**	**0.0211**	**0.014**	0.119	0.685	0.267				
Ratio Gut/Lumen Perimeter	0.235	0.151	**0.001**	0.323	0.488	**0.005**	0.122	0.256				
Muscle Thickness	0.350	0.892	0.370	**2.43E-03**	0.106	0.412						

(Takashima and Hartenstein, 2012). Cockroaches typically consume their exuvia following molting (Takashima et al., 2008), which may facilitate reseeding with typical gut commensals, but oxygen-sensitive taxa may not survive the *ex vivo* period prior to exuvia feeding. Since Con treatment individuals received a single exposure to the normal gut microbial community and were denied subsequent access to them, stochastic and/or oxygen-related taxon losses would magnify with each molting event resulting in a diminished microbial community potentially lacking taxa necessary for normal development. WT-treated insects had regular access to fresh frass from the adult conspecifics so that any losses of gut bacteria during molting could be recovered via coprophagy. Examining microbial taxa common to the Con and WT treatments may uncover candidates responsible for stimulating host epithelial development. Likewise, identifying and examining microbiota at specific sites of enhanced epithelial expansion, such as in the anterior hindgut and posterior midgut, and taxa common to both sites may help uncover host/microbial mutualisms. Profiling the gut microbial community composition across the three treatments over the development period would detail the degree to which the conventionalization method reflects or diverges from the normal means for acquiring gut bacteria, as modeled in the WT treatment. In light of periodic molting events, it is likely that Con insects could have lost substantial gut microbial diversity as a result of molting, especially removal of the hindgut, and denied access to frass from normally reared insects.

5. Conclusions

This study evaluated the degree to which the presence and composition of gut microbiota impacted the development of invertebrate gut tissues, revealing a strong relationship between gut development and gut microbial community status. We report that gut subcompartments that are typically occupied by high abundances of bacteria exhibited pronounced treatment-dependent responses, suggesting heterogeneous responses to gut bacteria within these tissues. Recent transcriptomic and histological analyses of *Drosophila* midguts suggest a highly structured midgut (Buchon et al., 2013b) and support this hypothesis, yet few studies of invertebrates and vertebrates resolve host gut phenotypes at this scale. The diversity of animal host-gut microbiome model systems currently available enables high-resolution mapping of microbe-mediated digestive tissue function towards illustrating how gut microbiota are integrated in host digestive health, growth and development.

6. Ethics approval and consent to participate

Insects were treated and euthanized respectfully.

7. Consent for publication

All authors consent to the publication of these data.

8. Availability of data and material

All histological samples, images and associated measurements not present in supplemental materials are available from the corresponding author upon reasonable request.

Funding

BCJ and ZLS received support from the US National Science Foundation (IOS1656786) and KP received support through the Ohio State University.

CRediT authorship contribution statement

Benjamin C. Jahnes: Conceptualization, Formal analysis, Investigation, Writing - original draft. **Keyshap Poudel:** Investigation. **Amelia M. Staats:** Investigation. **Zakee L. Sabree:** Conceptualization, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors acknowledge George Keeney of the OSU Insectary for supplying insects used in these studies. Thank you to John Freudenstein and Marymegan Daly at the OSU Museum for Biological Diversity for kindly providing access to histology prep instrumentation and related advice.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jinsphys.2021.104274.

References

Ashida, H., Ogawa, M., Kim, M., Mimuro, H., Sasakawa, C., 2012. Bacteria and host interactions in the gut epithelial barrier. Nat. Chem. Biol. 8 (1), 36–45. https://doi.org/10.1038/nchembio.741.

Bell, W.J., Adiyodi, K.G., 1982. The American cockroach. Physiol. Entomol. 7, 117–118. https://doi.org/10.1111/j.1365-3032.1982.tb00674.x.

Bell, W.J., Roth, L.M., Nalepa, C.A., 2007. Cockroaches: ecology, behavior, and natural history.

Bignell, D.E., 1977. Some observations on the distribution of gut flora in the American cockroach, *Periplaneta americana*. J. Invertebr. Pathol. 29 (3), 338–343. https://doi.org/10.1016/S0022-2011(77)80040-2.

Bracke, J.W., Cruden, D.L., Markovetz, A.J., 1978. Effect of metronidazole on the intestinal microflora of the American cockroach Periplaneta americana l. Antimicrob. Agents Chemother. 13 (1), 115–120.

Buchon, N., Broderick, N.A., Lemaitre, B., 2013a. Gut homeostasis in a microbial world: insights from *Drosophila melanogaster*. Nat. Rev. Microbiol. 11 (9), 615–626. https://doi.org/10.1038/nrmicro3074.

Buchon, N., Osman, D., David, F.A., Yu Fang, H., Boquete, J.-P., Deplancke, B., Lemaitre, B., 2013b. Resource morphological and molecular characterization of adult midgut compartmentalization in *Drosophila*. Cell Reports 3 (5), 1725–1738. https://doi.org/10.1016/j.celrep.2013.04.001.

Capo, F., Charroux, B., Royet, J., 2016. Bacteria sensing mechanisms in *Drosophila* gut: Local and systemic consequences. Dev. Comp. Immunol. 64, 11–21. https://doi.org/10.1016/j.dci.2016.01.001.

Carrasco, P., 2014. Succession of the gut microbiota in the cockroach Blattella germanica. Int. Microbiol. 99–109 https://doi.org/10.2436/20.1501.01.212.

Colombani, J., Raisin, S., Pantalacci, S., Radimerski, T., Montagne, J., Léopold, P., 2003. A nutrient sensor mechanism controls *Drosophila* growth. Cell 114 (6), 739–749. https://doi.org/10.1016/S0092-8674(03)00713-X.

Cruden, D.L., Markovetz, A.J., 1979. Carboxymethyl cellulose decomposition by intestinal bacteria of cockroaches. Appl. Environ. Microbiol. 38 (3), 369–372.

Day, M.F., Powning, R.F., 1949. A study of the processes of digestion in certain insects. Aust. J. Biol. Sci. 2, 175–215. https://doi.org/10.1071/BI9490175.

Donohoe, D., Garge, N., Zhang, X., Sun, W., O'Connell, T., Bunger, M., Bultman, S., 2011. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. Cell Metab. 13 (5), 517–526. https://doi.org/10.1016/j.cmet.2011.02.018.

Fox, D.T., Spradling, A.C., 2009. The *Drosophila* hindgut lacks constitutively active adult stem cells but proliferates in response to tissue damage. Cell Stem Cell 5 (3), 290–297. https://doi.org/10.1016/j.stem.2009.06.003.

Guo, Z., Lucchetta, E., Rafel, N., Ohlstein, B., 2016. Maintenance of the adult *Drosophila* intestine: All roads lead to homeostasis. Curr. Opin. Genet. Dev. 40, 81–86. https://doi.org/10.1016/j.gde.2016.06.009.

Hakim, R.S., Baldwin, K., Smagghe, G., 2010. Regulation of midgut growth, development, and metamorphosis. Annu. Rev. Entomol. 55 (1), 593–608. https://doi.org/10.1146/annurev-ento-112408-085450.

Hartenstein, V., Technau, G.M., Campos-Ortega, J.A., 1985. Fate-mapping in wild-type *Drosophila melanogaster* - III. A fate map of the blastoderm. Wilhelm Roux's Arch. Dev. Biol. 194 (4), 213–216. https://doi.org/10.1007/BF00848248.

Jahnes, B.C., Herrmann, M., Sabree, Z.L., 2019. Conspecific coprophagy stimulates normal development in a germ-free model invertebrate. PeerJ 7, e6914. https://doi.org/10.7717/peerj.6914.

Jiang, H., Patel, P.H., Kohlmaier, A., Grenley, M.O., McEwen, D.G., Edgar, B.A., 2009. Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the *Drosophila* midgut. Cell 137 (7), 1343–1355. https://doi.org/10.1016/j.cell.2009.05.014.

Jones, R.M., Luo, L., Ardita, C.S., Richardson, A.N., Kwon, Y.M., Mercante, J.W., Alam, A., Gates, C.L., Wu, H., Swanson, P.A., Lambeth, J.D., Denning, P.W., Neish, A. S., 2013. Symbiotic lactobacilli stimulate gut epithelial proliferation via Nox-mediated generation of reactive oxygen species. EMBO J. 32, 3017–3028. https://doi.org/10.1038/emboj.2013.224.

Juge, N., 2012. Microbial adhesins to gastrointestinal mucus. Trends Microbiol. 20 (1), 30–39. https://doi.org/10.1016/j.tim.2011.10.001.

Kopanic, R.J., Schal, C., 1999. Coprophagy facilitates horizontal transmission of bait among cockroaches (Dictyoptera: Blattellidae). Environ. Entomol. 28 (3), 431–438. https://doi.org/10.1093/ee/28.3.431.

Kopanic, R.J., Holbrook, G.L., Sevala, V., Schal, C., 2001. An adaptive benefit of facultative coprophagy in the German cockroach *Blattella germanica*. Ecol. Entomol. 26, 154–162. https://doi.org/10.1046/j.1365-2311.2001.00316.x.

Lee, K.-A., Kim, B., Bhin, J., Kim, D., You, H., Kim, E.-K., Kim, S.-H., Ryu, J.-H., Hwang, D., Lee, W.-J., 2015. Bacterial uracil modulates *Drosophila* DUOX-dependent gut immunity via Hedgehog-induced signaling endosomes. Cell Host Microbe 17 (2), 191–204. https://doi.org/10.1016/j.chom.2014.12.012.

Macfarlane, S., Woodmansey, E.J., George, T., Macfarlane, G.T., 2005. Colonization of mucin by human intestinal bacteria and establishment of biofilm communities in a two-stage continuous culture system. Appl. Environ. Microbiol. 71, 7483–7492. https://doi.org/10.1128/AEM.71.11.7483.

Martens, E.C., Chiang, H.C., Gordon, J.I., 2008. Mucosal glycan foraging enhances fitness and transmission of a saccharolytic human gut bacterial symbiont. Cell Host Microbe 4 (5), 447–457. https://doi.org/10.1016/j.chom.2008.09.007.

Nalepa C.A., Bignell D.E., B.C., 2001. Detritivory, coprophagy, and the evolution of digestive mutualisms in Dictyoptera. Insectes Soc. 48, 194–201. https://doi.org/10.1007/PL00001767.

Ohlstein, B., Spradling, A., 2006. The adult Drosophila posterior midgut is maintained by pluripotent stem cells. Nature 439 (7075), 470–474. https://doi.org/10.1038/nature04333.

Osman, D., Buchon, N., Chakrabarti, S., Huang, Y.T., Su, W.C., Poidevin, M., Tsai, Y.C., Lemaitre, B., 2012. Autocrine and paracrine unpaired signaling regulate intestinal stem cell maintenance and division. J. Cell Sci. 125, 5944–5949. https://doi.org/10.1242/jcs.113100.

Park, M.S., Park, P., Takeda, M., 2009. Starvation induces apoptosis in the midgut nidi of Periplaneta americana: a histochemical and ultrastructural study 631–638. https://doi.org/10.1007/s00441-008-0737-y.

Park, M.S., Takeda, M., 2008. Starvation suppresses cell proliferation that rebounds after refeeding in the midgut of the American cockroach Periplaneta americana. J. Insect Physiol. 54 (2), 386–392. https://doi.org/10.1016/j.jinsphys.2007.10.011.

Patel, P.H., Maldera, J. a, Edgar, B. a, 2013. Stimulating crosstalk between commensal bacteria and intestinal stem cells. EMBO J. 32, 3009–10. https://doi.org/10.1038/emboj.2013.244.

Petkau, K., Parsons, B.D., Duggal, A., Foley, E., 2014. A deregulated intestinal cell cycle program disrupts tissue homeostasis without affecting longevity in *Drosophila*. J. Biol. Chem. 289 (41), 28719–28729. https://doi.org/10.1074/jbc.M114.578708.

Royet, J., 2011. Epithelial homeostasis and the underlying molecular mechanisms in the gut of the insect model *Drosophila melanogaster*. Cell. Mol. Life Sci. 68 (22), 3651–3660. https://doi.org/10.1007/s00018-011-0828-x.

Sabree, Z.L., Kambhampati, S., Moran, N.A., 2009. Nitrogen recycling and nutritional provisioning by *Blattabacterium,* the cockroach endosymbiont. Proc. Natl. Acad. Sci. 106 (46), 19521–19526.

Schauer, C., Thompson, C.L., Brune, A., 2012. The bacterial community in the gut of the cockroach Shelfordella lateralis reflects the close evolutionary relatedness of cockroaches and termites. Appl. Environ. Microbiol. 78 (8), 2758–2767. https://doi.org/10.1128/AEM.07788-11.

Schmid, A., 1989. How to use Heidenhains AZAN staining in insects. Neurosci. Lett. 101 (1), 35–38. https://doi.org/10.1016/0304-3940(89)90436-9.

Shin, S.C., Kim, S., You, H., Kim, B., Kim, A.C., Lee, K., Yoon, J., Ryu, J., Lee, W., 2011. Drosophila microbiome modulates host developmental and metabolic homeostasis via insulin signaling.

Storelli, G., Defaye, A., Erkosar, B., Hols, P., Royet, J., Leulier, F., 2011. Lactobacillus plantarum promotes drosophila systemic growth by modulating hormonal signals through TOR-dependent nutrient sensing. Cell Metab. 14, 403–414. https://doi.org/10.1016/J.CMET.2011.07.012.

Tailford, L.E., Crost, E.H., Kavanaugh, D., Juge, N., 2015. Mucin glycan foraging in the human gut microbiome. Front. Genet. 5 https://doi.org/10.3389/fgene.2015.00081.

Takashima, S., Hartenstein, V., 2012. Genetic control of intestinal stem cell specification and development: a comparative view. Stem Cell Rev. Reports 8 (2), 597–608. https://doi.org/10.1007/s12015-012-9351-1.

Takashima, S., Mkrtchyan, M., Younossi-Hartenstein, A., Merriam, J.R., Hartenstein, V., 2008. The behaviour of *Drosophila* adult hindgut stem cells is controlled by Wnt and Hh signalling. Nature 454 (7204), 651–655. https://doi.org/10.1038/nature07156.

Wong, A.C., Dobson, A.J., Douglas, A.E., 2014. Gut microbiota dictates the metabolic response of *Drosophila* to diet. J. Exp. Biol. 217, 1894–1901. https://doi.org/10.1242/jeb.101725.

Xiao, X., Yang, L., Pang, X., Zhang, R., Zhu, Y., Wang, P., Gao, G., Cheng, G., 2017. A Mesh-Duox pathway regulates homeostasis in the insect gut. Nat. Microbiol. 2. https://doi.org/10.1038/nmicrobiol.2017.20.

Yamada, R., Deshpande, S.A., Bruce, K.D., Mak, E.M., Ja, W.W., 2015. Microbes promote amino acid harvest to rescue undernutrition in Drosophila. Cell Rep. 10, 865–872. https://doi.org/10.1016/j.celrep.2015.01.018.

Zheng, H., Powell, J.E., Steele, M.I., Dietrich, C., Moran, N.A., 2017. Honeybee gut microbiota promotes host weight gain via bacterial metabolism and hormonal signaling. Proc. Natl. Acad. Sci. 114 (18), 4775–4780. https://doi.org/10.1073/pnas.1701819114.

Zurek, L., Keddie, B.A., 1996. Contribution of the colon and colonic bacterial flora to metabolism and development of the American cockroach *Periplaneta americana* L. J. Insect Physiol. 42, 743–748. https://doi.org/10.1016/0022-1910(96)00028-5.