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Gut bacteria facilitate leaf beetles in adapting to dietary specialization by enhancing larval fitness

Meiqi Ma[1,4], Jing Luo[1,4], Xiaotong Chen[1], Chong Li[1], Siqun Li[1], Jianghua Sun[2,3] ✉ & Letian Xu[1] ✉

Dietary specialization between insect stages can reduce intraspecific food competition. The involvement of gut bacteria and the mechanisms underlying this phenomenon received limited attention. *Plagiodera versicolora* is a pest harming Salicaceae trees. Here, we confirmed dietary specialization in *P. versicolora*, wherein adults prefer new leaves, while larvae predominantly consume mature leaves when both types are available. We demonstrated the larval preference for mature leaves confers ecological advantages by promoting growth, development and immunity and this advantage is contingent upon the presence of gut bacteria. Gut microbiota in larvae revealed a significant enrichment of *Pantoea* when feeding new leaves, with *P. anthophila* exhibiting the most pronounced inhibitory effect on larval development. Further exploration identified specific metabolites, such as Tyrosyl-valine, with higher content in new leaves, which serve as substrates for the entomopathogenic gut bacterium to facilitate its proliferation. This study provides a fresh perspective on the ecological role of gut bacteria.

Food quality and quantity are vital for the survival of insects, but these resources are often limited, prompting them to evolve various mechanisms for efficient acquisition and allocation[1,2].These strategies encompass a range of adaptations, including long-distance migration[3,4], expanding dietary breadth[5–7], regulating population density on a limited food source[8,9], and enhancing the digestive efficiency of existing food[10,11]. Furthermore, many insects employ dietary specialization to exploit different ecological resources during different life stages[12,13], thus minimizing food competition and niche overlap within their own species, and ultimately reducing intraspecific competition[14–16]. For example, most Lepidoptera larvae feed on plant leaves, while their adults utilize floral resources[17]; similar phenomenon are also observed in various wood-boring beetles and mosquitos[18,19]. Clearly, larvae and adults of these insects occupy distinct niches, allowing for the consumption of different food. Nevertheless, insects sharing the same niche may face heightened dietary competition, necessitating more delicate dietary specialization. One of the most notable such insects is leaf-eating beetles, a significant group of herbivores insects. Generally, thousands of the insects inhabit the same plants, leading to intense competition of food resource. The presence and underlying mechanisms of dietary specialization in these insects remain largely unexplored.

Previous studies have indicated that the structure of insect guts exhibits numerous modifications tailored to specialized ecological niches and feeding habits, with many of these specializations evolving to accommodate specific gut microbiota within distinct gut compartments[20]. Thereby, insect gut bacteria play an extensive role in digestion, detoxification and defense against pathogen infections[21,22]. Additionally, they can regulate insect behaviors, including mating, feeding and social interactions, through a gut-brain axis[23–27]. Meanwhile, diet stands out as one of the most prominent factor to shape insect gut microbiota[28,29], often serving as a representative indicator of the host insect's feeding habits[30,31]. Given this, gut bacteria have the potential to mediate insect dietary specialization. Previous studies have provided some insights. For instance, both adults and larvae of Scarabaeinae dung beetles thrive on the dung of herbivores, exhibiting dietary specialization where adults feed on smaller dung balls while larvae prefer larger ones. In addition to structural disparities in the guts of dung beetle adults and larvae, gut bacteria have been shown to highly adapt to the respective foods of the adult and larval stages, aiding in their digestion[12,32]. However, whether these phenomena are ubiquitous and whether gut bacteria play similar roles and mechanisms in other insects exhibiting dietary specialization remains a topic warranting further exploration.

[1]State Key Laboratory of Biocatalysis and Enzyme Engineering, School of Life Sciences, Hubei University, Wuhan, 430062, China. [2]Hebei Basic Science Center for Biotic Interactions/College of Life Sciences, Institutes of Life Science and Green Development, Hebei University, Baoding, 071002, China. [3]State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, 1 Beichen West Road, Chaoyang District, 100101 Beijing, China. [4]These authors contributed equally: Meiqi Ma, Jing Luo. ✉e-mail: sunjh@ioz.ac.cn; letian0926@163.com

The willow leaf beetle *Plagiodera versicolora* (Laicharting) (Coleoptera: Chrysomelidae) is a holometabolous and widely distributed leaf-eating insect. *P. versicolora* primarily inflicts damage on trees of the Salicaceae family, such as willow (*Salix* spp.) and poplar (*Populus* spp.), with both adults and larvae feeding on leaves. This species has resulted in significant economic losses in certain commercial forests and urban ornamental green spaces[33–35]. Previous studies have indicated that the adult *P. versicolora* exhibit a preference for new leaves while their female counterparts tend to lay eggs on mature leaves even when both types of leaves are available, larvae subsequently feed on mature leaves[34,36]. It has been proven that the preference of adults for new leaves facilitates the beetle's oviposition and induces the plant to regrow, resulting in the production of more tender leaves[36,37]. This dietary specialization in *P. versicolora* likely arises from long-term coevolution with host plants and serves a beneficial role in the adult's biology. As for *P. versicolora* larvae, this phenomenon does not appear to be contingent on the insect's synchrony with the tree's development. Given that the hatching of laid eggs typically takes 2-3 days, whereas willow or poplar leaves take over 7 days to mature[38–40], newly emerged larvae still have the opportunity to feed on new leaves or migrate to surrounding newly sprouted ones if the eggs are laid on new leaves. However, larval development also requires a substantial amount of protein. Additionally, mature leaves are more tough than new leaves, and research indicates that feeding tough leaves can lead to mandibular wear, which is detrimental to their own development[41,42]. Therefore, the aforementioned dietary specialization may not be conducive to larval development, which apparently contradicts to the 'mother knows best' principle and warrants further investigation.

In addition to the seemingly contradictory dietary specialization, previous studies have identified an abundance of gut bacteria in *P. versicolora* known for serving crucial physiological functions[43–45]. This makes *P. versicolora* an ideal experimental subject for investigating the role of gut bacteria in mediating dietary specialization across different life stages. In this study, we first assess the ecological implications of dietary specialization for *P. versicolora* larvae and examine whether and how gut bacteria influence this specialization. Our findings demonstrate that dietary specialization positively impacts larval development, with this effect contingent upon the presence of gut bacteria. Further exploration into the underlying mechanism reveals that the variation in gut microbiota, induced by metabolite difference between new and mature leaves, contributing to the observed ecological phenotype.

Results

Dietary specialization enhances *P. versicolora* larval fitness through gut bacteria

To confirm dietary specialization between *P. versicolora* developmental stages, we compared the consumption of new and mature leaves by *P. versicolora* adults and larvae. The average daily consumption of new and mature willow (*Salix*) leaves (New-Sa, Mature-Sa) for one pair *P. versicolora* adults was 0.545 cm^2 and 0.08 cm^2, respectively, indicating that adults prefer to eat new leaves (Supplementary Fig. 1a, $P < 0.05$). The average number of eggs laid on willow mature leaves by one pair adults within 48 h was 20, while that on new leaves was 4, suggesting adults prefer to lay eggs on mature leaves (Supplementary Fig. 1b, $P < 0.01$). The average feeding area of each larva on mature willow leaves within 72 h was 4 cm^2, significantly higher than that on new leaves (1.288 cm^2), indicating that larvae prefer to feed on mature leaves (Supplementary Fig. 1c, $P < 0.001$). The similar results were found when poplar trees (*Populus*) were used as experimental materials (New-Pa, Mature-Pa) (Supplementary Fig. 1). These results demonstrate that *P. versicolora* larvae and adults feed on different types of willow/poplar leaves, confirming that dietary specialization exists between developmental stages.

To explore the ecological advantages conferred by dietary specialization, we fed conventionally-reared (CR) larvae (with gut microbiota) new or mature leaves, and investigated the body mass of their 2nd instar larvae, pupae, and adults, as well as their developmental time. The average body mass of 2nd instar larvae feeding mature willow leaves (CR-Mature-Sa) was significantly higher than larvae feeding new willow leaves (CR-New-Sa) (Fig. 1a, $P < 0.001$). Meanwhile, the average body mass of pupae and adults in CR-Mature-Sa were both significantly higher than that in CR-New-Sa (Fig. 1b, c, $P < 0.05$). Additionally, for CR-Mature-Sa group, the developmental time

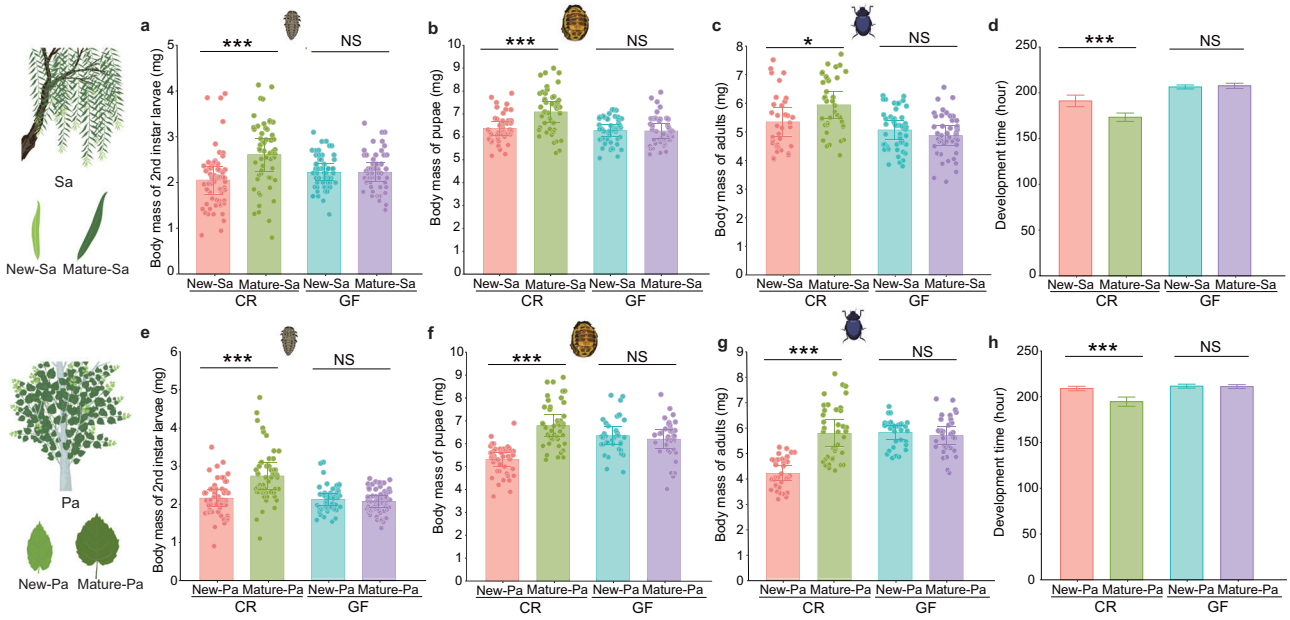

Fig. 1 | The growth and development of conventionally-reared (with gut microbiota, CR) and germ-free (without gut microbiota, GF) *P. versicolora* larvae feeding on new and mature leaves. a–c The body mass of 2nd instar larvae, pupae and adults, as well as the developmental time (**d**), of CR and GF larvae feeding on different willow leaves ($n = 70$). **e–g** The body mass of 2nd instar larvae, pupae, and adults, and (**h**) the developmental time of CR and GF larvae feeding on different poplar leaves ($n = 70$). New-Sa, Mature-Sa, New-Pa and Mature-Pa denote the groups of larvae feeding on new and mature *S. babylonica* willow or 'Shanxin' poplar leaves, respectively. The data are presented as means ± SD. *P* values were calculated using the independent-samples *t* test. * indicates the significance between two groups, $^*P < 0.05$, $^{***}P < 0.001$, NS no significance.

required for larvae to reach adulthood was significantly shorter than that in CR-New-Sa (Fig. 1d, $P < 0.001$). We obtained similar results when feeding larvae with new or mature poplar leaves. The average body mass of 2nd instar larvae, pupae and adults of CR-Mature-Pa were all significantly higher than that of CR-New-Pa (Fig. 1e–g, $P < 0.001$). The larval developmental time of CR-Mature-Pa was also significantly shorter than that of CR-New-Pa (Fig. 1h, $P < 0.001$).

To evaluate whether gut microbiota is involved in *P. versicolora* larval dietary specialization, germ-free (GF) larvae (without gut microbiota) were fed new and mature willow/poplar leaves, and their weight and developmental time were assessed. The efficacy of gut bacteria removal was validated through LB medium culture and qPCR techniques, and the results indicated no gut bacteria were detected in the GF larvae (Supplementary Fig. 2). The body mass of 2nd instar larvae, pupae and adults of GF-Mature-Sa showed no significant differences compared to GF-New-Sa (Fig. 1a–c, $P > 0.05$). The developmental time also showed no significant difference between them (Fig. 1d, $P > 0.05$). Similarly, there was no significant difference in the four indices when comparing GF-Mature-Pa with GF-New-Pa (Fig. 1e–h, $P > 0.05$).

Additionally, we evaluated the effect of dietary specialization on *P. versicolora* immunity by challenging CR larvae fed with new and mature leaves using the entomopathogenic fungi *Beauveria bassiana* (Bb) and *Aspergillus nomius* (An). The infection of *B. bassiana* resulted in a 90% mortality rate among larvae feeding on new leaves (New-Sa-Bb), which was significantly higher compared to Mature-Sa-Bb (20%) (Supplementary Fig. 3a, log-rank test, $P < 0.001$). The infection of *A. nomius* resulted in a 95% mortality rate for larvae feeding on new leaves (New-Sa-An), which was significantly higher than that of Mature-Sa-An (90%) (Supplementary Fig. 3b, log-rank test, $P < 0.05$).

To eliminate the effect of plant-associated microbiota, we fed CR larvae with non-axenic/axenic new and mature leaves. The results indicated that larvae fed with mature leaves still exhibited higher body weight and shorter developmental time compared to those fed with new leaves regardless of whether the leaves were non-axenic or axenic (Supplementary Fig. 4).

These results revealed that feeding mature leaves can improve the larval growth, development and resistance to fungal infection, indicating that the dietary specialization can improve *P. versicolora* larval fitness. Additionally, the advantages conferred to *P. versicolora* larvae by dietary specialization were unaffected by plant-associated microbiota, but were eliminated when gut bacteria were removed, raising the interesting possibility that the gut microbiota mediate *P. versicolora* larval dietary specialization.

The gut microbiota exhibited significant differences between the larvae feeding on new and mature leaves

We investigated the composition of the gut bacterial community in *P. versicolora* larvae (CR) feeding new and mature leaves by full-length 16S rRNA amplicon sequencing. High-throughput sequencing of 40 samples from four groups (New-Sa, Mature-Sa, New-Pa and Mature-Pa) yielded a total of 1,529,149 quality-filtered sequences with an average length of 1465 bp (Supplementary Table 1). The sequences were clustered to generate a total of 1065 ASVs (actual sequence variants) at 100% sequence similarity, among them, 972 ASVs were identified and classified into 22 bacterial phyla, 45 classes, 105 orders, 145 families, 253 genera, and 356 species. We calculated ACE, Chao1, Sobs, Shannon and Simpson as alpha diversity estimators based on the genus level and the significance difference was tested using the Kruskal–Wallis *H* test and Turkey-Kramer post hoc test (Supplementary Table 1). Generally, for the alpha-diversity, the larvae feeding willow leaves exhibited higher diversity than that feeding poplar leaves, with no significant differences found between the new and mature leaf groups (Supplementary Table 2).

Principal coordinates analysis (PCoA) showed that bacterial communities of New-Sa and Mature-Sa or New-Pa and Mature-Pa clustered independently (Fig. 2a, b, ANOSIM, $P < 0.05$). NMDS analysis showed a similar pattern (Fig. 2c, d, ANOSIM, $P < 0.05$). The type of trees, willow or poplar also had a slight impact on bacterial communities (Supplementary Fig. 5). These results indicated that the gut bacterial community in larvae feeding new and mature leaves showed significant differences. The taxonomic and relative abundance analysis at the genus level showed that the gut bacterial community was dominated by five bacterial genera: *Ralstonia*, *Pantoea*, *Pluralibacter*, *Enterobacter* and *Pelomonas*, which accounted for over 80% of the total sequences in all samples (Fig. 3a). Furthermore, comparing with larvae feeding mature willow or poplar leaves, the relative abundance of *Pantoea* significantly increased in larvae feeding the correspondent new leaves, meanwhile with a significantly lower relative abundance of *Ralstonia* (Fig. 3a, b, Kruskal–Wallis *H* test and Turkey-Kramer post hoc test, $P < 0.05$). RT-qPCR results confirmed a significant increase in the abundance of *Pantoea* in 1st and 2nd instar larvae of New-Sa and New-Pa groups (larvae fed on new leaves) (Fig. 3c, *t*-test, $P < 0.01$), and showed the total load of gut bacteria was also significantly higher in larvae of New-Sa and New-Pa groups (2nd instar) comparing with Mature-Sa and Mature-Pa groups (Fig. 3c, $P < 0.01$).

Reintroduction of *Pantoea* bacteria into germ-free *P. versicolora* larvae impedes their growth and development

In a bid to confirm the adverse impact of gut bacteria *Pantoea* on *P. versicolora* larvae, we fed germ-free larvae with axenic willow leaves painted with four kinds of gut bacterial suspension (*Pantoea anthophila*, *Pantoea dispersa*, *Pantoea ananatis* and *Staphylococcus xylosus*) and equivalent PBS solution respectively. Comparing with larvae fed with PBS solution (Control), larvae inoculated by *P. anthophila* or *P. dispersa* exhibited significantly lower body mass in 2nd instar larvae, pupae and adults, and had longer developmental time (Fig. 4, one-way ANOVA, $P < 0.05$). However, the larvae inoculated with *P. ananatis* or *S. xylosus* showed no significant differences in the four indices compared to the Control (Fig. 4 one-way ANOVA, $P > 0.05$). These results indicated that the gut bacteria *P. anthophila* and *P. dispersa* can impede the growth and development of *P. versicolora* larvae.

The nutrients and specialized metabolites in the new leaves of the two plants displayed distinct variations compared to the mature leaves

We measured the content of representative metabolites in willow and poplar leaves. The average total protein content in New-Sa and New-Pa were 163.9 mg/g and 371.2 mg/g, respectively, significantly higher than those of Mature-Sa and Mature-Pa (117.2 mg/g and 308.4 mg/g) (Supplementary Fig. 6a, $P < 0.05$). The soluble sugar content in New-Sa (68.8 mg/g) was similar with Mature-Sa (67.0 mg/g) ($P > 0.05$), but the soluble sugar content in New-Pa (54.16 mg/g) was significantly lower than that in Mature-Pa (89.13 mg/g) (Supplementary Fig. 6b, $P < 0.001$). The content of tannins in new willow leaves (9.470 mg/g) was significantly higher than that in mature leaves (7.575 mg/g), but there was no significant difference in tannin content between new and mature poplar leaves (Supplementary Fig. 6c, $P < 0.05$ and $P > 0.05$). The salicin content in New-Sa and New-Pa were 3.549 mg/g and 9.926 mg/g, respectively, significantly higher than that of Mature-Sa and Mature-Pa (0.4145 mg/g and 7.493 mg/g) (Supplementary Fig. 6d, $P < 0.01$). The content of chlorogenic acid between new and mature leaves showed no significant difference (Supplementary Fig. 6e, $P > 0.05$).

To elucidate the metabolite differences between new and mature leaves in detail, we also performed metabolome analysis. We chose the VIP value (Variable Importance in Projection) to assess the difference of metabolite accumulation between two groups. Comparison between New_Sa and Mature_Sa groups revealed 523 differential metabolites (metabolite collection 1) in both positive and negative ion modes (the VIP > 1, Fig. 5a). While the comparison between New_Pa and Mature_Pa groups identified 468 differential metabolites (metabolite collection 2) in both positive and negative ion modes (the VIP > 1, Fig. 5a). Considering that both new willow and poplar leaves can promote the growth of gut bacteria, we identified 191 metabolites (metabolite collection 3) with similar levels between New-Sa and New-Pa groups (the VIP < 0.5, Fig. 5a). Taken together, 29 shared metabolites among the three metabolite collections were picked out and

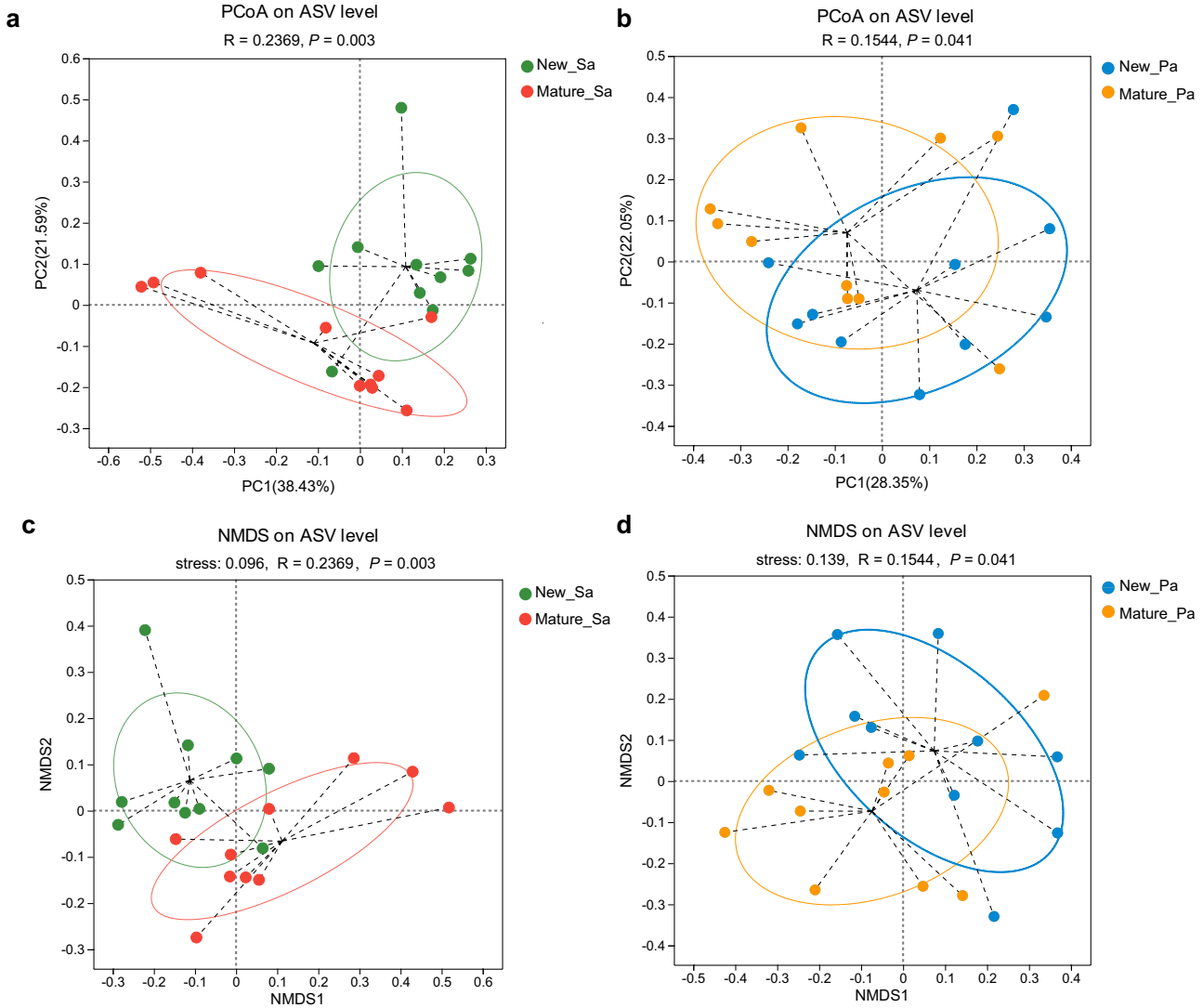

Fig. 2 | The gut bacterial community structure of conventionally-reared *P. versicolora* larvae feeding on new and mature willow or poplar leaves. a, **b** Principal coordinates analysis (PCoA) plot visualizing the data based on Bray–Curtis dissimilarity of the bacterial community ($n = 10$). **c**, **d** Non-metric multidimensional scaling (NMDS) of the bacterial communities based on Jaccard matrix ($n = 10$). Newly-hatched conventionally-reared larvae (with gut microbiota) were fed with new and mature willow or poplar leaves. After 2 days of feeding (2nd instar), their gut microbiota were investigated and compared. New-Sa, Mature-Sa, New-Pa and Mature-Pa denote the groups of larvae feeding on new and mature *S. babylonica* willow or 'Shanxin' poplar leaves, respectively.

regarded as the candidate differential metabolites between new and mature leaves that may affect the growth of gut bacteria (Fig. 5b, c, Supplementary Table 3). The 29 metabolites can be classified into more than 19 categories at HMDB (Human Metabolome Database) Subclass level, and were manually clustered into three groups based on their expression profiles in different leaves (Fig. 5b, c, Supplementary Table 3). The group 1 contained differential metabolites that were both higher in New-Sa and New-Pa compared to Mature-Sa and Mature-Pa, the group 2 contained differential metabolites that were both lower in New-Sa and New-Pa than Mature-Sa and Mature-Pa and the group 3 included metabolites that showed irregular variations in two types of leaves (Fig. 5b, c).

The enriched metabolites in new leaves promote certain gut bacterial growth

To investigate whether these differential metabolites can promote the growth of pathogenic *Pantoea* bacteria, we selected LysoPC (Class: Glycerophosphocholines), Tyrosyl-Valine (Class: Amino acids, peptides and analogs) and Palmitoylcarnitine from group 1; Toxin-T2 (Class: Sesquiterpenoids) from group 2; and Citrulline (Class: Amino acids, peptides, and analogs) from group 3 (Fig. 5c); These metabolites were individually added to 1/2 LB medium to cultivate four gut bacteria: *P. anthophila*, *P. dispersa*, *P. ananatis*, and *S. xylosus* (Fig. 5d). Results showed that the OD_{600} values of *P. anthophila* in the Tyrosyl-Valine and Toxin-T2 groups were significantly higher than those in the control group, while the values in the LysoPC and Palmitoylcarnitine groups were significantly lower than those in the control group (Fig. 5d, $P < 0.05$). For *P. dispersa* and *P. ananatis*, the OD_{600} values in the Toxin-T2 group were significantly higher compared to the control group, while in the LysoPC and Palmitoylcarnitine groups, they were significantly lower than those in the control group (Fig. 5d, $P < 0.001$). As for *S. xylosus*, the OD_{600} values in the Toxin-T2 and LysoPC groups were significantly higher than those in the control group, whereas in Palmitoylcarnitine group, they were significantly lower than those in the control group (Fig. 5d, $P < 0.001$). These results indicate that Tyrosyl-Valine exclusively promotes the growth of *P. anthophila*, LysoPC inhibits the growth of *P. anthophila, P. dispersa* and *P. ananatis*, but promotes the growth of *S. xylosus*. Palmitoylcarnitine impedes the growth of all bacteria species, while Toxin-T2 promotes the growth of all bacterial

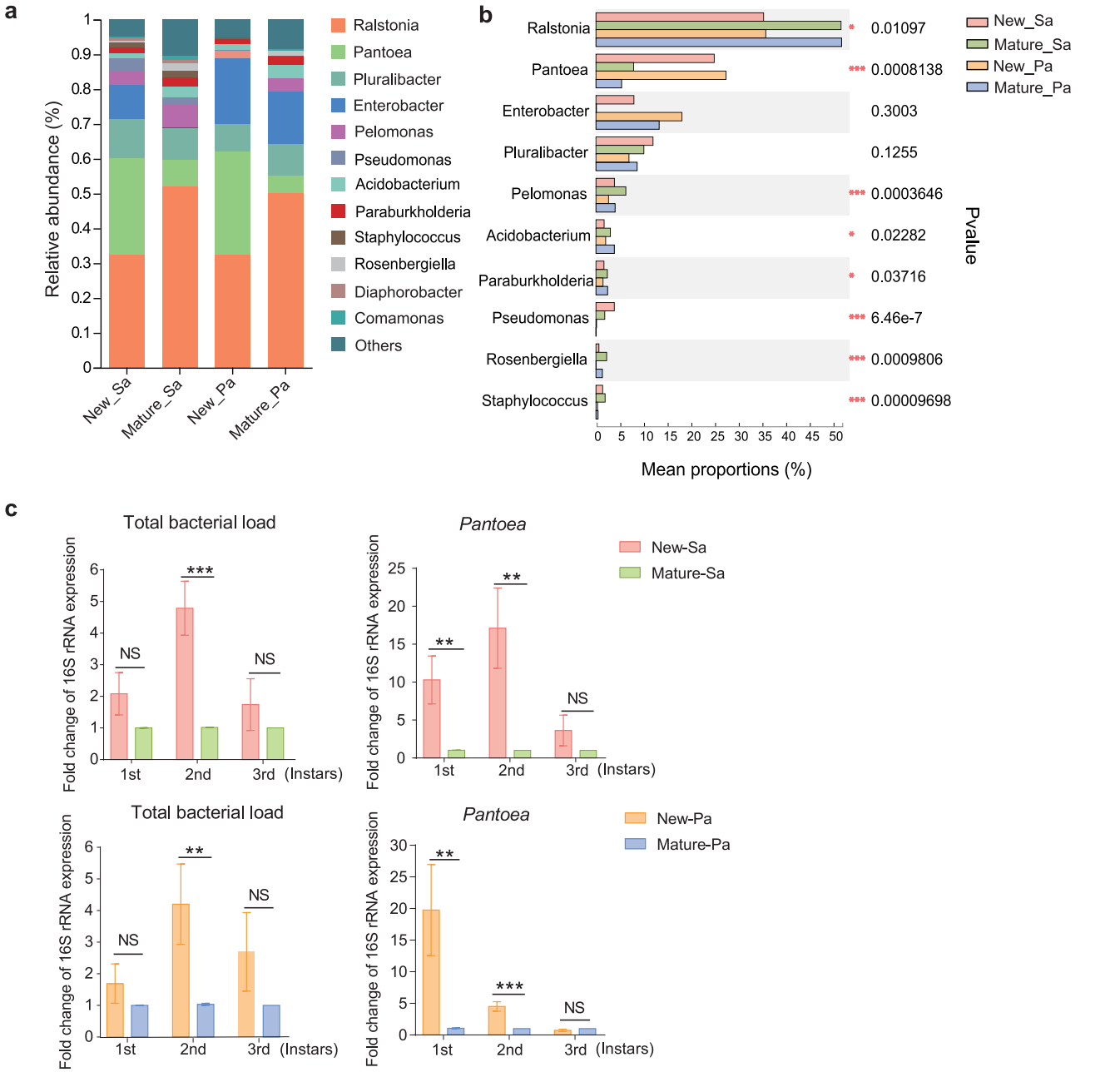

Fig. 3 | The gut bacterial composition and quantity of conventionally-reared *P. versicolora* larvae feeding on new and mature willow or poplar leaves. a The relative abundance of bacterial communities at genus level across different samples. **b** Significant differences of the relative proportions of major genera of gut bacteria across different samples (relative abundances of the top 10 genera, Kruskal–Wallis *H* test). **c** RT-qPCR analysis of the relative abundance of the total gut bacteria and *Pantoea* genus of three instars in different samples ($n = 12$). Total RNA from different instars' larvae was extracted and reverse-transcribed to obtain cDNA for subsequent qPCR analysis. The 16S rRNA gene was targeted gene and the *P. versicolora RPS18* gene served as the reference gene. The $2^{-\Delta\Delta Ct}$ method was employed to calculate fold change values of gene expression levels. The data are presented as means ± SD (Independent *t* test). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, NS no significance.

species. Citrulline, on the other hand, does not significantly influence the growth of the four bacteria (Fig. 5d).

Discussion

Dietary specialization enables insects to exploit diverse ecological resources at different life stages, thereby averting food competition and niche overlap, ultimately reducing intraspecific competition[14–16]. Previous studies have shown that dietary specialization benefits *P. versicolora* adults by enhancing their egg production[36,37]. In this study, we have observed a preference among adult *P. versicolora* for new leaves, while a tendency to oviposit on mature leaves has also been identified (Supplementary Fig. 1a, b). Newly hatched larvae predominantly consume the mature leaves surrounding the oviposition site, and the mature leaf feeding larvae exhibit enhanced growth, development and resistance to entomopathogenic fungi (Supplementary Fig. 1c, Fig. 1 and Supplementary Fig. 2). These observations highlight the advantageous nature of dietary specialization for both *P. versicolora* adults and larvae, providing a detailed and novel perspective on the ecological significance of dietary specialization within insects. In contrast to the conspicuous dietary specialization observed in many Lepidopteran insects, flies, and mosquitoes, where larvae and adults feed on entirely different foods[17–19], *P. versicolora* exhibits a less pronounced dietary specialization, with both larvae and adults consuming leaves. This behavior is akin to the dietary

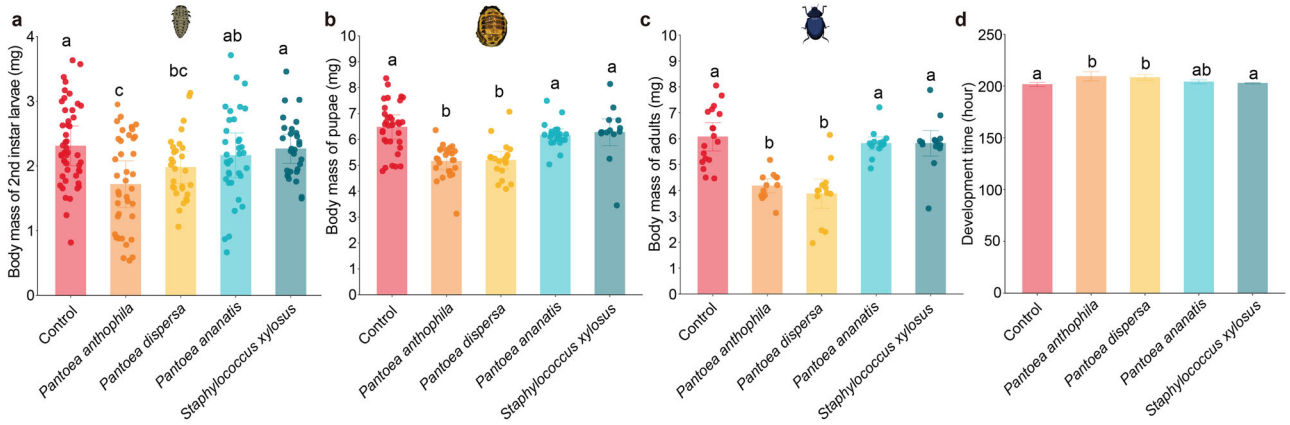

Fig. 4 | The growth and development of germ free (GF) *P. versicolora* larvae after the reintroduction of different gut bacterial strains. a–c The body mass of 2nd instar larvae, pupae and adults, and **d** the developmental time of GF larvae inoculated with PBS buffer (Control), *Pantoea anthophila*, *Pantoea dispersa*, *Pantoea ananatis*, and *Staphylococcus xylosus* (One-way ANOVA, $n = 50$, $P < 0.05$). The four gut bacteria were isolated from guts of *P. versicolora* larvae, cultured in LB medium, suspended in 0.01 M PBS solution, diluted to approximately 1×10^6 cells/mL, and applied to axenic willow leaves. These leaves, containing gut bacteria, were then fed to GF larvae. The data are presented as means ± SD.

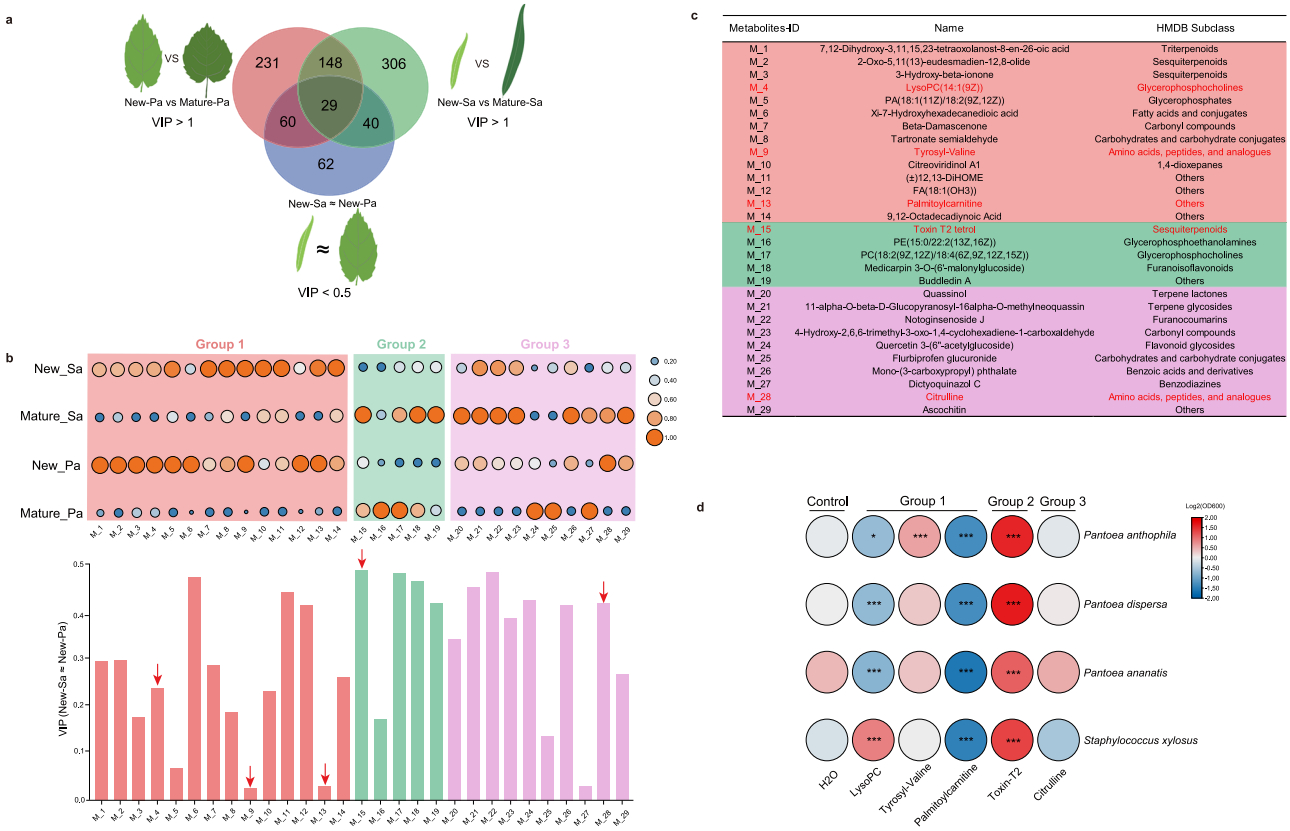

Fig. 5 | Differential metabolite screening between new and mature leaves, and metabolites enriched in new leaves can promote gut bacterial growth in *P. versicolora* larvae. a Venn diagram illustrates the overlap among the three metabolite collections. **b** Heatmap displays 29 metabolites, manually grouped into three groups, along with a diagram showing VIP values of the 29 metabolites in the New_Sa vs New_Pa metabolite collection. Metabolites marked with red arrows were selected for further experiment. **c** Table displays the names and HMDB Subclass classification of the 29 metabolites. **d** Heatmap shows that metabolites enriched in new leaves can promote gut bacterial growth ($n = 10$). Four bacteria species were cultivated for 24 h in 1/2 LB liquid medium supplemented with 1000 nmol/mL of the five metabolites from three groups (LysoPC, Tyrosyl-Valine, Palmitoylcarnitine, Toxin-T2 and Citrulline) or H_2O. *P* values were calculated using independent samples *t* test. * indicates the significance between H_2O and treatment groups (*$P < 0.05$, ***$P < 0.001$).

specialization observed in dung beetles[12]. Further investigation into the specific regulatory mechanisms governing this phenomenon among different insect stages provides valuable insights into understanding insect population dynamics.

Drawing from established hypotheses such as "Mother Knows Best" and the "preference performance hypothesis" tested in various insect species, it is known that the oviposition preference of females is intricately tied to the performance of their offspring[46–48]. While *P. versicolora* adults

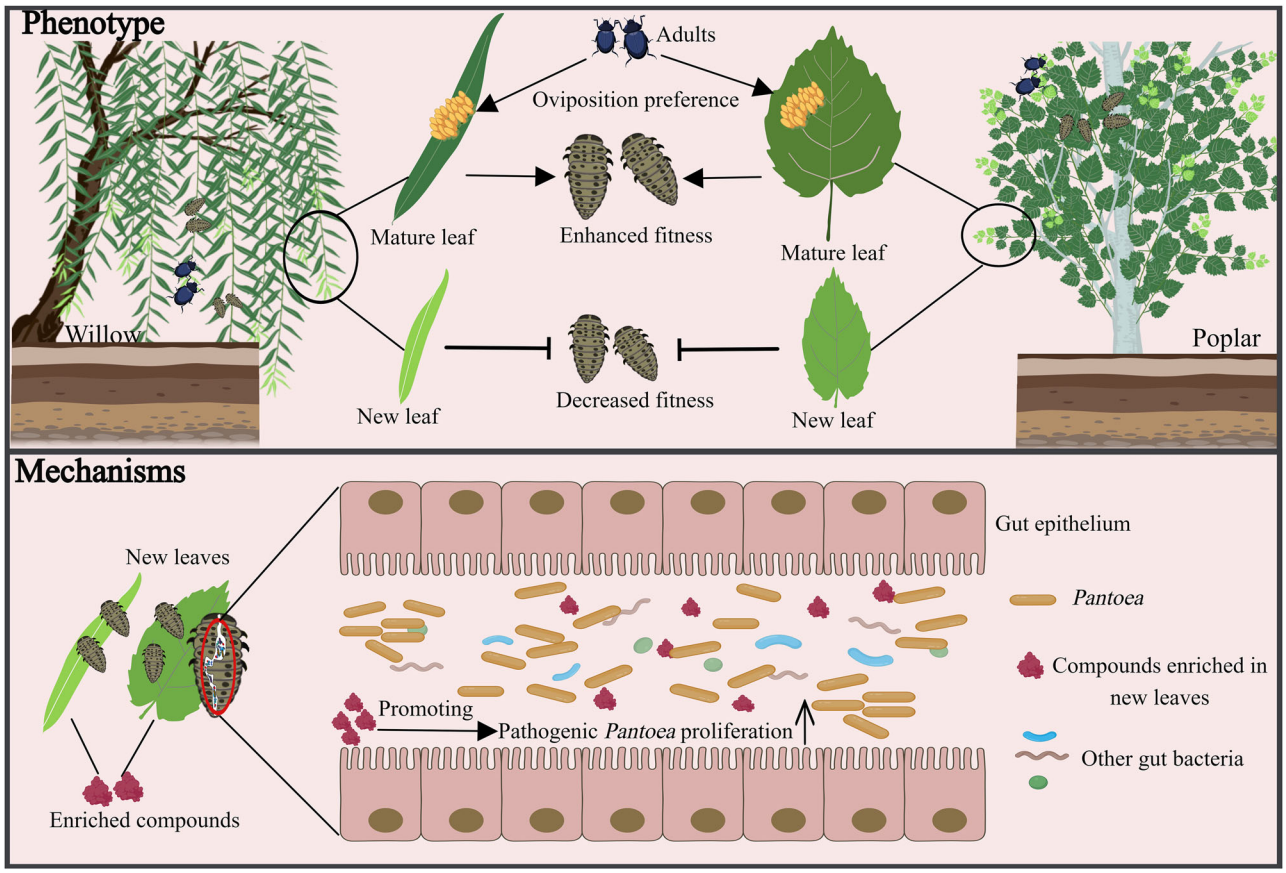

Fig. 6 | Schematic diagram illustrating the role of gut bacteria in mediating dietary specialization between *P. versicolora* adults and larvae. Dietary specialization exists between adults and larvae, contributing to improved *P. versicolora* larval fitness. The gut microbiota mediate *P. versicolora* larval dietary specialization: Pathogenic *Pantoea* bacteria utilize specific compounds enriched in new leaves for their proliferation, which further inhibits the larval growth and development.

predominantly feed on new leaves, they choose to lay their eggs on mature leaves, a behavior that proves beneficial for larval fitness (Figs. 1 and 6), despite the potential for larval mouthpart wear caused by mature leaves. This affirms the validity of both aforementioned hypothesizes. Furthermore, our findings suggest that the adult females' capacity to discern the ecological repercussions of their oviposition preferences is mediated by gut microbiota. This is substantiated by the observed loss of the fitness advantage in subsequently hatched larvae on mature leaves following the removal of their gut bacteria (Figs. 1 and 6). We initially considered whether the underlying mechanism mirrors that observed in dung beetles, where gut bacteria play a pivotal role in enhancing digestion abilities. Specifically, larvae feeding on dung balls with higher fiber content harbor a greater abundance of gut bacteria that facilitate cellulolytic and nitrogen fixing processes[12]. It is worth noting that we fed CR larvae with both non-axenic and axenic new and mature leaves to eliminate the effect of plant-associated microbiota (Supplementary Fig. 4). According to existing research, while gut bacteria in Coleoptera insects are transmitted through non-strict vertical transmission, these gut bacteria can adhere to the surface of the eggs during oviposition. This makes the eggs a route for vertical transmission and a source of gut bacteria for the next generation[49]. In the case of *P. versicolora*, larvae consume the eggshells after hatching. Our unpublished data also indicate substantial overlap between the bacteria on the egg surface and the gut microbiota of *P. versicolora* larvae and adults. Consequently, we consider that the bacteria on the eggs of *P. versicolora* are an important source of functional gut bacteria for the larvae. Additionally, the dietary specialization in *P. versicolora* occurs across two species of the Salicaceae, which contain abundant phenolic glycosides as anti-herbivore defenses[50]. *P. versicolora* is considered an oligophagous pest, and we hypothesize that dietary specialization may also be driven by the substantial differences in defense compound composition between new and mature leaves of the Salicaceae. Furthermore, another possibility is that larvae growing on mature leaves may be protected from predators or high solar temperatures because new leaves are brighter and smaller[48]. These hypotheses warrant further investigation.

To ascertain whether the improved fitness of larvae consuming mature leaves can be attributed to the involvement of gut bacteria in nutrition provision, it is imperative to delineate the specific bacterial dynamics and their temporal shifts. The RT-qPCR results demonstrated a significant increase in gut bacteria counts of 2nd instar larvae, whereas no obvious differences were observed in the 3rd instar larvae (Fig. 3c). Additionally, we noted differences in larval body weight as early as the onset of the 2nd instar stage when feeding on different leaves (Fig. 1a). Taken together, dietary specialization plays an important role in the early larval stage with limited mobility. Therefore, we sampled the 2nd instar larvae for 16S rRNA sequencing to better illustrate the distinctions in gut bacteria composition (Fig. 3a, b). The sequencing results revealed significant disparities in the gut bacterial structures of larvae feeding on new and mature leaves (Figs. 2 and 3). Notably, *Ralstonia* and *Pantoea* emerged as the dominant bacterial genera in *P. versicolora* larval guts, collectively constituting approximately 60% of the total microbiota, furthermore, the two genera exhibited the most pronounced changes (Fig. 3a, b). Subsequent RT-qPCR results confirmed a significantly higher abundance of *Pantoea* in larvae feeding on new leaves (Fig. 3). Our focus on the increased abundance of bacteria in larvae consuming new leaves stems from the substantial developmental disadvantages observed in this group, as compared to larvae feeding on mature leaves. Besides, germ-free larvae exhibited no such

differences, indicating that the observed phenotypic variation may be attributed to the significant enrichment of bacteria in new leaves. As for *Pantoea*, a typically yellow rod-shaped gram-negative bacterium within the Enterobacteriaceae family, it has been shown to closely associate with insects[51,52]. For instance, *P. stewartidine*, which colonizes in *Chaetocnema pulicaria*, has the potential to infect plants[53]. This bacterial species can also colonize the guts of *Acyrthosiphon pisum*, albeit with pathogenic effects[54]. These studies suggest that *Pantoea* may engage in opportunistic interactions with insect hosts in response to environmental changes. Our reintroduction of *Pantoea* bacteria into germ-free *P. versicolora* larvae confirmed its toxic effects (Fig. 4). However, further research is needed to determine whether the reduction in *Ralstonia* and related bacteria is responsible for the delayed development observed in larvae consuming new leaves, as there is a possibility that *P. versicolora* larvae may struggle to adapt to mature leaves after the removal of gut bacteria.

To elucidate the enrichment of entomopathogenic *Pantoea* while feeding on new leaves, we performed a metabolite analysis on both new and mature leaves from both willow and poplar plants. However, there are numerous chemicals with similar or distinct compositions in the four groups; thus, a focused strategy was necessary for the identification of key chemicals. Since the bacterial shifts in the gut microbiota of larvae feeding on new leaves from both plant species were largely consistent, including a significant increase in *Pantoea*, we hypothesized that shared compounds in the new leaves of the two plants significantly influenced the varied gut microbiota and facilitated the growth of *Pantoea*. Consequently, we employed the following strategy for the metabolic profiling of the four types of leaves: focusing on substances exhibiting differences between mature and new leaves, along with compounds common in new leaves of willow and poplar. Through this approach, we identified a total of 29 metabolites. Among these, Tyrosyl-Valine exclusively promoted the growth of *P. anthophila*, which was highly toxic to *P. versicolora* larvae (Figs. 4, 5d). As a degraded product of proteins, the enriched Tyrosyl-Valine in new leaves aligns with the higher protein content observed in new leaves compared to their mature counterparts (Supplementary Fig. 6a). Prior research has also demonstrated that the utilization of peptide-bound amino acids contributes to the predominance of specific gut bacteria[55]. Some previous studies have reported that bacteria from soils and waters or animal guts can utilize Toxin T2 as carbon source and energy for growth, and *Pantoea* bacteria can even reduce the production of Toxin T2 of *Fusarium*[56,57]. Thus, we speculate that the four species of bacteria may be able to metabolize Toxin T2 as carbon source for growth. However, further testing is required to explore other potential metabolites that may also facilitate the growth of *Pantoea* bacteria or inhibit the growth of other bacteria.

Taken together, this study initiates by confirming the occurrence of dietary specialization among different stages of *P. versicolora* and demonstrates its significant enhancement of larval fitness. Subsequently, we establish the pivotal role of gut bacteria in mediating this dietary specialization by utilizing specific metabolites present in new leaves to promote the proliferation of certain gut bacteria. This, consequently, impedes the growth and development of *P. versicolora* larvae (Fig. 6). This study provides a fresh perspective on the ecological significance of gut bacteria and introduces a novel idea for the gut bacteria-mediated control of *P. versicolora*. For instance, by artificially manipulating gut bacteria to disrupt dietary specialization and increase intra-species competition, we can effectively regulate the population. Additionally, this study provides a valuable reference for investigating intraspecific and interspecific dietary specialization in other insect species.

Methods

P. versicolora rearing and preparation of germ-free larvae

The wild *P. versicolora* population was captured from Sha Lake Park in Wuhan, China. The rearing conditions were 26 ± 1 °C, with 70% ± 5% relative humidity and a photoperiod of 14 h light/10 h dark. Larvae and adults were fed with detached fresh willow (*Salix babylonica* L.) leaves.

After adult oviposition, fresh eggs were collected and placed on moist absorbent paper. Eggs were surface-sterilized with 75% ethanol for 8 min and washed with sterilized water to obtain germ free (GF) larvae[33]. To better simulate the situation in field, leaves collected from field grown plants were used for testing. Thus, we applied antibiotic mixtures containing 0.2 mg/mL erythromycin, gentamicin, kanamycin and tetracycline to coat the surfaces of willow (*S. babylonica*) and hybrid poplar 'Shanxin' (*Populus davidiana* × *Populus bolleana*) leaves[58,59]. Then GF larvae were fed with the antibiotics coated wild willow or poplar leaves to suppress gut bacteria. The efficiency of microbial elimination was confirmed by culture-dependent (Luria-Bertani medium) and culture-independent (transcription-quantitative PCR, RT-qPCR analysis) methods. Briefly, three 3rd instar treated larvae (GF) or conventionally-reared (CR) larvae were dissected under the dissecting microscope (Mshot, Guangzhou, China) in a clean bench to collect guts. For culture-dependent method, three guts were pooled and pulverized in 300 μL of sterile water. Then 100 μL suspensions were dropped on LB agar medium and grown for 48 h at 30 °C. For culture-independent method, guts of 3rd instar GF or CR larvae were collected and homogenized using a bead-beating homogenizer (Jingxin, Shanghai, China), and three guts were pooled together as a replicate and each group had five replicates. Total RNA of the gut tissues was extracted using RNAiso Plus (Takara, Shiga, Japan). A total 2 μg RNA was reverse-transcribed to obtain cDNA using PrimeScript^{TM} RT Master Mix (Takara). RT-qPCR was performed using SYBR mix (Takara) and universal primers targeting 16S rRNA genes of all gut bacteria (Supplementary Table 2). The *P. versicolora RPS18* gene was used as the reference gene[60]. Gut bacterial 16S rRNA gene expression levels were calculated as fold change values using the $2^{-\Delta\Delta Ct}$ method[61].

Preference of *P. versicolora* to new and mature leaves

We selected willows (*S. babylonica*) and poplar 'Shanxin' leaves from the wild as plant materials. New leaves were defined as the first and second pieces of branches with the top buds removed, while mature leaves were considered as the fourth, fifth and sixth pieces. Ten willow or poplar trees were randomly selected at intervals of approximately 300 m for leaf collection. New and mature leaves were randomly collected. Ten pairs of *P. versicolora* adults (3 days after emergence) were randomly selected and placed in pairs within plastic boxes (14 × 8.3 × 7 cm). Every box contained enough fresh new willow/poplar leaves (New_Sa, New_Pa) and mature willow/poplar leaves (Mature_Sa, Mature_Pa). Before insect feeding, we photographed the leaves with a scale. After 24 h of feeding, we photographed all leaves consumed by the adults and used Image J software (https://imagej.net/ij/index.html) to calculate the consumption area of both new and mature leaves by comparing the pre-feeding photos.

Ten pairs of *P. versicolora* adults (five days post emergence) were randomly selected and placed in plastic boxes. Similarly, enough new and mature willow/poplar leaves were placed inside. The number of eggs laid on different types of leaves were counted 48 h later. Another piece of leaf with ten eggs was placed in each of ten axenic plastic boxs ($n = 10$). After the larvae hatched, enough new leaves and mature leaves were placed into the box, and the average feeding area of each larva on new leaves and mature leaves was calculated after 72 h.

The measurement of the body mass and developmental time of *P. versicolora* larvae feeding on new and mature leaves

A total of 140 newly-hatched CR larvae were divided into two groups: CR-New-Sa group (feeding new willow leaves) and CR-Mature-Sa group (feeding mature willow leaves). Due to the fragility of first instar larvae, which makes weighing them potentially lethal, we measured four indicators: the body mass of 2nd instar larvae (3 days old), pupae and adults, as well as the developmental time required for larvae to grow to adulthood. Body mass was measured using an electric microbalance (±0.01 mg, Mettler Toledo, Zurich, Switzerland). In addition to *S. babylonica* willow trees, we conducted the same experiments using leaves from the wild hybrid poplar 'Shanxin' leaves. The larvae were randomly divided into the CR-New-Pa group and CR-Mature-Pa group. For GF larvae feeding on antibiotics coated new and

mature willow/poplar leaves in the wild, the groups named GF-New-Sa, GF-Mature-Sa, GF-New-Pa and GF-Mature-Pa, respectively. To ensure a continuous supply of new or mature leaves during the experiment, we replaced the consumed leaves in the petri dishes with fresh, antibiotic-coated leaves daily. This procedure minimizes bacterial colonization in the GF larvae, and all experimental procedures were conducted on a sterile clean bench.

To eliminate the effect of plant-associated microbiota, we fed newly-hatched CR larvae with non-axenic/axenic new or mature leaves (Non-axenic-New-Sa/Pa, Non-axenic-Mature-Sa/Pa, Axenic-New-Sa/Pa, Axenic-Mature-Sa/Pa, $n = 60$). Axenic leaves were from tissue-cultured plantlets. Axenic willow (*S. babylonica*) plantlets were raised aseptically on a solid medium containing 2.2 g/L Murashige & Skoog (MS) basic salts plus vitamins, 0.1 mg/L NAA and 1% (w/v) sucrose, and were cultured at 28 °C, 60% ± 5% relative humidity under a photoperiod of 16 h light and 8 h dark[62]. Axenic 'Shanxin' poplar were cultured on MS medium containing 0.1 mg/L NAA and 3% (w/v) sucrose at the same condition as the willow. After growing for a month in MS medium, these plantlets were transferred into axenic soils in sterile glass jars (height 80 cm) to continue to grow for two months.

Infection of entomopathogenic fungi to larvae feeding on different leaves

We used *Beauveria bassiana* (isolate 476) and *Aspergillus nomius* to infect larvae feeding new and mature willow leaves. Fungal mycelia were collected into a 50 mL centrifuge tube with 20 mL sterile water, resuspended fully using a vortex mixer and the obtained conidial suspension were filtered with sterile cotton and injection syringes. Microscopes and blood cell counting plates were used to count the number of conidia, and the concentrations of *B. bassiana* and *A. nomius* conidial suspension were both 10^8 conidia/mL. Two-hundred larvae were divided into two parts and fed new and mature willow leaves for one day, respectively. Then 50 larvae feeding new or mature leaves for 1 day were soaked in conidial suspension for 5 s, the other larvae were soaked with water and regarded as the control groups, and later these larvae continued to feed new or mature willow leaves in petri dishes. The number of larval deaths was recorded daily.

Analysis of gut microbiota by 16S rRNA gene Illumina sequencing

Newly-hatched larvae were fed with new and mature willow or poplar leaves. After 2 days of feeding (2nd instar), these larvae were surface-sterilized in 75% ethanol for 15 s, washed three times with sterile water and were dissected under sterile conditions to collect guts. Three guts were pooled together as a replicate. Each group has ten biological replicates. Bacterial DNA was extracted from gut samples with the FastDNA Spin Kit for Soil (MP Biomedicals, California, USA) and a negative control (NC) without any tissue was included.

The DNA quality was checked by gel electrophoresis using 1% agarose gel and visualized using UV transillumination. The DNA concentration was determined with a Nanodrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, USA). The full-length 16S rRNA gene was amplified using 27 F and 1492 R primers (Supplementary Table 2). Each PCR reaction contained a 20 μL PCR mix with 5 × FastPfu Buffer (4 μL), 2.5 mM of dNTPs (2 μL), 0.5 mM of each primer (0.8 μL), 0.4 μL FastPfu polymerase (Transgene, China), 10 ng of template DNA and nuclease-free water, and was conducted on an ABI GeneAmp 9700 (ABI, CA, USA). The amplification program included an initial 3-min denaturation step at 95 °C, followed by 29 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 1 min, and a final elongation of 10 min at 72 °C. The PCR products were then purified with AxyPrep DNA gel extraction kit (Axygen, USA) and were used for subsequent secondary short PCR with different barcoded primers and Illumina adapter sequences[63]. The amplicon library was then sequenced on the Illumina MiSeq PE300 platform in Majorbio (Shanghai, China). The negative control was also sequenced. The raw data was submitted to the NCBI Sequence Read Archive (SRA) (accession number: PRJNA1160435).

Raw reads were quality filtered by fastp (v0.19.6) and FLASH (v1.2.7) was used to assemble paired-end reads to obtain clean reads[64,65]. Then, the DADA2 pipeline (https://github.com/benjjneb/dada2) was employed to identify ASVs (actual sequence variants) according to 100% similarity through the Quantitative Insights Into Microbial Ecology version 2 (QIIME2) platform (https://qiime2.org/)[66–68]. Taxonomic classification of ASVs was performed using QIIME 2 and reference data sets from the SILVA 16S rRNA and Greengenes databases[69,70]. Only four ASVs were detected in the negative control (NC) sample, indicating the absence of contaminants in the experiment (Supplementary Table 1). Thus, the NC was excluded in the following analysis. Microbial diversity and community composition were analyzed using vegan packages in R (version 4.0.3)[71]. Principal coordinate analysis (PCoA) based on the Bray–Curtis distance and non-metric multidimensional scaling (NMDS) using the Jaccard similarity matrix were performed at the ASV level to evaluate the differences between microbial communities and significant differences were tested using ANOSIM with 1000 permutations[72,73]. The R package ggplots were used to plot bar diagrams based on the genus level to display bacterial composition and their relative abundance among different samples[74]. The R package stats was used to assess the significance differences in alpha diversity estimators and species abundance using Kruskal–Wallis *H* test and Turkey-Kramer post hoc test.

The quantification of the gut and plant-associated bacteria by RT-qPCR analysis

Newly-hatched larvae were fed with new and mature willow/poplar leaves. Different instar larvae or leaves were subjected to a snap freeze with liquid nitrogen. Three larvae were pooled together as a replicate and each group contained 12 replicates. Four cm^2 leaves were regarded as a replicate and each group contained 4 replicates. Total RNA of the samples was extracted and reverse-transcribed to obtain cDNA as described above. The RT-qPCR primers used for the quantification of the total bacterial load and the *Pantoea* genus are based on sequences used in previous studies[75,76] (Supplementary Table 2). The *RPS18*, *Actin* and Tubulin genes were used as the reference genes of *P. versicolora* larvae, willow and poplar leaves, respectively. The $2^{-\Delta\Delta Ct}$ method was used to calculate fold change values of gene expression levels.

Preparation of gut bacterial strains and reintroduction of bacteria into insect guts

For isolation of gut bacteria, individual surface-sterilized 2nd instar *P. versicolora* larvae were dissected to collect the contact gut under the dissecting microscope in a clean bench ($n = 10$). The guts were crushed in 100 μL of 0.01 M PBS solution, the suspension was vortexed for 30 s, and the diluted suspension (10^2 to 10^4) was plated on LB agar medium, followed by incubation at 30 °C for 24–72 h. Genomic DNA of the different morphological bacterial isolates cultured on LB agar medium for 24 h was extracted with bacterial genomic DNA extraction kit (TIANGEN, China). Then the DNA was used to amplify 16S rRNA gene by performing PCR using 27F and 1492R primers (Supplementary Table 2). The PCR products were then sequenced by Sanger sequencing (Sangon Biotech, Shanghai, China), and the sequences were matched with the known 16S rRNA gene sequences to identify the bacterial species by NCBI Blast tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Strains of the gut bacteria *Pantoea anthophila*, *Pantoea dispersa*, *Pantoea ananatis* and *Staphylococcus xylosus* were isolated from *P. versicolora*. After cultured in LB liquid medium for 24 h, the four bacteria were collected and centrifuged at 4000 rpm for 5 min to remove the supernatant. The bacterial cells were then washed three times with sterile 0.01 M PBS solution, resuspended in PBS solution, and diluted to a final concentration of approximately 1×10^6 cells/mL[77]. The axenic willow leaves were coated with bacterial suspension, then these leaves were fed to 1st instar axenic *P. versicolora* larvae and the control group were fed with leaves inoculated with PBS solution ($n = 50$). The body mass of 2nd instar larvae, pupae and adults, and the developmental time were also recorded.

Metabolite analysis of new and mature leaves

We collected wild new and mature willow or poplar leaves, each sample contained 1 g leaves and each group had six replicates. Samples were frozen in liquid nitrogen and sent for non-target LC-MS-based metabolomic analysis (Majorbio, Shanghai, China). The LC-MS/MS analysis was conducted on a Thermo UHPLC-Q Exactive HF-X system equipped with an ACQUITY HSS T3 column (100 mm × 2.1 mm i.d., 1.8 μm; Waters, USA). The mobile phases were 0.1% formic acid in water (95:5, v/v) (solvent A) and 0.1% formic acid in acetonitrile: isopropanol (47.5:47.5, v/v) (solvent B). The flow rate was 0.40 mL/min, and the column temperature was 40 °C. Mass spectrometric data were collected in positive and negative modes under the following conditions: source temperature at 42 °C; sheath gas flow rate at 50 arb (arbitrary unit); Aux gas flow rate at 13 arb; ion-spray voltage at − 3500 V (negative mode) and 3500 V (positive mode); normalized collision energy at 20-40-60 V rolling for MS/MS. Full MS resolution was 60,000, and MS/MS resolution was 7500. Data acquisition was performed in Data Dependent Acquisition mode over a mass range of 70–1050 m/z. Mass-calibrated raw data files were converted to .mzML format using CompassXport software. All data preprocessing and analysis were performed in R (v4.0.3). The metabolites were identified by matching mass spectrum data with the HMDB (http://www.hmdb.ca/), Metlin (https://metlin.scripps.edu/), and Majorbio Database. The raw data was submitted to the National Genomics Data Center (NGDC) OMIX database (accession number: PRJCA030209). In order to find the metabolites that may promote the growth of gut bacteria, we first listed the differential metabolites of New_Sa vs Mature_Sa and New_Pa vs Mature_Pa with VIP > 1 (Variable Importance in Projection) as the criterion. Then we screened the similar metabolites between New_Sa and New_Pa with VIP < 0.5 as the criterion[78]. Finally, we found out 29 common metabolites among the three metabolite collections, and then these metabolites were classified at HMDB Subclass level and made into a heatmap based on the metabolite expression levels (Supplementary Table 3).

Total protein content in different leaves were measured using the Plant Protein Extraction Kit (Solarbio, Beijing, China) and the BCA protein assay kit (TransGen Biotech, Beijing, China). Soluble sugar content was measured using the Plant Soluble Sugar Content Assay Kit (Solarbio). Tannin content was measured by Tannin Assay Kit (Abbkine, Wuhan, China). All assays were performed according to the manufacturer's instructions. The content of salicin and chlorogenic acid were quantified with HPLC (High Performance Liquid Chromatography)[79]. Twenty mg standard salicin or chlorogenic acid (MACKLIN, Shanghai, China) were accurately weighed and revolved into 1 mL 70% methanol. The 0.1 g of different leaves were dissolved in 1 mL 70% methanol and the solution was passed through a 0.45 μm filter. The injection volume was 5 μL, the flow rate was 0.35 mL/min and triplicate injections were used for each sample. The equation of the regression line was determined as Y = 36301 X + (3E + 06) for salicin and as Y = 37072 X − (2E + 06) for chlorogenic acid.

Bacterial growth promotion and inhibition assays

Tyrosyl-Valine, Citrulline, Toxin-T2 (all from MACKLIN), Palmitoylcarnitine and LysoPC (both from MedChemexpress, New Jersey, USA) were prepared into 0.01 mol/L solution, and 0.22 μm filter membrane was used for filtration sterilization. The five substances were added into 1 mL 1/2 LB medium with a final concentration of 1000 nmol/mL. The four bacteria (*P. anthophila*, *P. dispersa*, *P. ananatis* and *S. xylosus*) were cultured with LB medium at a frequency of 220 rpm at 37 °C for 24 h and the OD_{600} values were determined by spectrophotometer. Then the bacteria culture was collected into 2 mL centrifuge tubes and centrifuged at 4000 g for 5 min. The supernatant LB medium was removed to obtain bacteria cells, and then 2 mL 0.01 M PBS solution was added to resuspend the bacteria cells. The 20 μL bacteria suspension (approximately 10^7 cells/mL) were inoculated into 1 mL 1/2 LB medium containing the five substances or H_2O (Control), and cultured at 30 °C with a shaker speed of 220 rpm. After being cultured for 24 h, the OD_{600} values were measured using a spectrophotometer, with each group comprising 10 replicates. Subsequently, a heatmap of the Log2 (OD_{600}) was generated.

Statistical analysis

The experimental data containing two groups were analyzed using unpaired independent-samples t test, and data comprising more than two groups were analyzed using One-way ANOVA coupled with Bonferroni (equal variances) or Dunnett' s T3 (unequal variances) test. Survival curves were analyzed using the Kaplan–Meier method, and the log-rank test was used to evaluate the significance of differences between two groups. When the value of $P < 0.05$, there is a significant difference. * means $P < 0.05$, ** means $P < 0.01$, and *** means $P < 0.001$. Data were statistically analyzed using SPSS version 19.0, figures were plotted using R (v4.3.1) and GraphPad Prism 8.0, and assembled using Adobe Illustrator CS6 and Photoshop CS6.

Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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Author contributions

L.X. and J.S. designed research; M.M., J.L., X.C., C.L. and S.L. did experiments; M.M. and J.L analyzed data. M.M. and J.L. wrote the paper and contributed equally to this work.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to Jianghua Sun or Letian Xu.