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Differential gene expression toward species of *Aristolochia* impairing the performance of the Troidini butterfly *Battus polydamas*

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Abstract

The swallowtail butterfly *Battus polydamas* L. (Lepidoptera: Papilionidae) is a specialist on *Aristolochia* (Aristochiaceae). Larvae sequester secondary compounds, such as aristolochic acids (AAs), from their host plants and transfer them to adults through the pupae. In this study, we evaluated the performance of *B. polydamas* larvae fed from 1st instar through pupation on two host plants with distinct chemical compositions, *Aristolochia ringens* Vahl. (which has several diterpenes) and *Aristolochia gigantea* Mart. (which has acyclic monoterpenoids and sesquiterpenoids, but no diterpenoids or AAs). Differential gene expression in gut and fat body tissues in response to two larval host plants was evaluated in 5th-instar larvae. We found significant differences in the survival of larvae feeding on the two host plants; the survival in *A. gigantea* is significantly higher than survival in *A. ringens*. In *A. gigantea*, 46% of the larvae persisted until pupation, whereas none of the larvae feeding on *A. ringens* survived until the end of larval development. Upregulated and downregulated contigs comprise genes encoding ribosomal proteins, protein farnesyltransferase, phosphomevalonate kinase, dolichyl-phosphate-mannose-protein mannosyltransferase 4, and O-glucosyltransferase (possibly involved in AA metabolization), as well as genes encoding superoxide dismutase, P450s, UGTs, glutathione S-transferase, and many proteases. As expected, larvae of *B. polydamas* were strongly influenced by host plants containing different compounds, leading to worse larval performance on key fitness components, such as life cycle performance attributes and larval survival. We suggest that there is a threshold of toxicity in the host plant that larvae can tolerate, and above such a threshold, the impact of plant secondary chemicals is no longer beneficial for the larvae but negative, disrupting their detoxification mechanism.

KEYWORDS
Aristochiaceae, aristolochic acids, deterrence, detoxification, diterpenoids, gold rim swallowtail, herbivorous insects, Papilionidae, secondary metabolites, transcriptomic response

INTRODUCTION

The interaction between herbivore insects and their host plants has imposed adaptations and counter adaptations for both sides through evolutionary time. The more conspicuous adaptations in plants are the chemical armory, whereas herbivorous insects counterattack with elaborate mechanisms of metabolization and detoxification (Ehrlich & Raven, 1964; Futuyma & Keese, 1992). These events promoted a diversification of both plants and

†In memoriam.

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insects, allowing the colonization of new environments, in a scenario described as an "escape-and-radiate coevolution" (Thompson, 1989).

Plant allelochemicals referred to as volatile organic compounds (VOCs), which include volatile terpenes (VTs), may facilitate these interactions (Boncan et al., 2020). Terpenoids, one of the largest classes of natural products (Beran et al., 2019), mediate several exchanges between plants and insects, including pollinators, herbivores, parasitoids, and predators, usually to the benefit of the plants' interests and harm for herbivores. For instance, lepidopterans, such as swallowtail butterflies, emit terpenes that repel predators from the osmeterium, an organ unique to Papilionidae larvae (Cong et al., 2015).

On the opposite side of these interactions, herbivorous insects can use both preingestive (involve taste cells that respond selectively to unpalatable and/or toxic compounds) and postingestive mechanisms for detecting noxious secondary plant compounds (Glendinning, 2002). Genes encoding ribosomal proteins in several insect species, such as *Helicoverpa armigera* Hübner, are variably regulated in response to a range of host plants (Celorio-Mancera et al., 2012). Furthermore, of all the genes involved in insect digestion, a great deal of research has been done on those that code for proteases, possibly because obtaining nitrogen is a high-priced process necessary for insect nutrition (Celorio-Mancera et al., 2012).

Insects can store plant-derived xenobiotics to protect them from their own natural predators, or they can quickly eliminate or metabolize these compounds into nontoxic chemicals. Insects may successfully adapt to their host plants by overcoming chemical plant defenses through a number of molecular pathways (Heidel-Fischer & Vogel, 2015). Most xenobiotics undergo detoxification and metabolization via a set of enzymes, such as Phase I enzymes, such as cytochrome P450 proteins (P450s), and Phase II enzymes, such as UDP-glycosyltransferases (UGTs), responsible for glycosylation catalyzed by UGTs. Other genes involved in the detoxification of plant-obtained compounds are carboxylesterases (CEs) (Celorio-Mancera et al., 2012).

The gold rim swallowtail butterfly *Battus polydamas* L. (Lepidoptera: Papilionidae) has a Neotropical distribution and also occurs in the warmer areas of the Nearctic region (Tyler et al., 1994). This species, as well as other swallowtail butterflies in the tribe Troidini, is a specialist on plants in the genus *Aristolochia* (Aristochiaceae) (Brown et al., 1981; Tyler et al., 1994). In southeastern Brazil, larvae of *B. polydamas* have been found in at least 11 species of *Aristolochia* (*Aristolochia arcuata* Mast., *Aristolochia labiata* L., *Aristolochia cymbifera* Mart., *Aristolochia esperanzae* Kuntze, *Aristolochia ringens* Vahl. (synonymous for *A. galeata*), *Aristolochia gigantea* Mart., *Aristolochia littoralis* Parodi, *Aristolochia macroura* Ortega, *Aristolochia melastoma* Silva, *Aristolochia paulistana* Hoehne, and *Aristolochia triangularis* Cham.) (Brown et al., 1981; Brown et al., 1995; Morais & Brown, 1991; Silva-Brandão & Solferini, 2007) (Figure 1). *Aristolochia* includes over 400 species of perennial herbaceous plants, undershrubs, and shrubs that produce essential oils and have a worldwide distribution (González et al. (2014) and references therein). They are rich in natural products such as terpenoids (Shi et al., 2004; Wu et al., 2004), lignans (Conserva et al., 1990; Nogueira & Lopes, 2013), β-phenylethylamines (βPEA) (Bentley, 2006), aporphine and isoquinoline alkaloids (Bentley, 2006; Ferreira et al., 2010), aristolactams (Holzbach & Lopes, 2010), and aristolochic acids (AAs), a group of nitrophenanthrene carboxylic acids found naturally in the family Aristolochiaceae and some types of plants known as *Asarum* (wild ginger), and their derivatives (Michl et al., 2014). Some of these compounds, such as AAs, are sequestered by larvae of *B. polydamas* and transferred to adults through the pupae (Dimarco & Fordyce, 2017; Klitzke & Brown, 2000; Urzúa et al., 2013). AAs are considered defensive compounds against natural enemies in Troidini butterflies (Nishida & Fukami, 1989; Sime, 2002), although their antipredator activity may not be strong against some predators, such as birds and ants (Morais et al., 2013). AAs were also suggested to be phagostimulant for *B. polydamas archidamas* subspecies (Pinto et al., 2009), although other assays failed to associate larval preference with AA content (Dimarco & Fordyce, 2017). However, the amount of AA in the larvae's diet had an effect on their performance, with elevated larval mortality in the diet when AA levels were greater, which could imply a cost for eating plants containing AA (Dimarco & Fordyce, 2017). Larvae of *B. polydamas* are also strongly influenced by host plants and exhibit feeding preferences based on the concentration of AAs in various plant tissues, with higher concentrations leading to better larval performance on key fitness components, such as life cycle performance attributes and larval survival (Rios et al., 2016).

We investigated the performance of larvae of *B. polydamas* fed from 1st instar through pupation on two host plants with dissimilar chemical composition, *Aristolochia ringens*, a plant with AAs in all its tissues but leaves, several diterpenes, such as clerodenes and derived from labdanoic acids, flavonoids, steroids, and tri- and sesquiterpenes (Klitzke & Brown, 2000; Pacheco et al., 2009; Silva-Brandão et al., 2006), and *A. gigantea*, which has acyclic monoterpenoids and sesquiterpenoids and aristolactams, but no diterpenoids or AAs (Holzbach & Lopes, 2010; Wu et al., 2004; Yamaguchi et al., 2017). Differential gene expression as a response to two larval host plants was evaluated in three biological replications of guts and fat body tissues of six 5th-instar larvae. Not many studies have focused on transcriptome response toward host plants in wild lepidopterans (Celorio-Mancera et al., 2013; Celorio-Mancera et al., 2022; Nallu et al., 2018; Tan et al., 2019b), whereas most of them focus on pest species (Breeschoten et al., 2019; Breeschoten et al., 2022; Lü et al., 2022; Silva-Brandão et al., 2017; Zhong et al., 2017). It is still unclear how Troidini butterflies process the various secondary chemicals that *Aristolochia* contains; therefore, transcriptomic

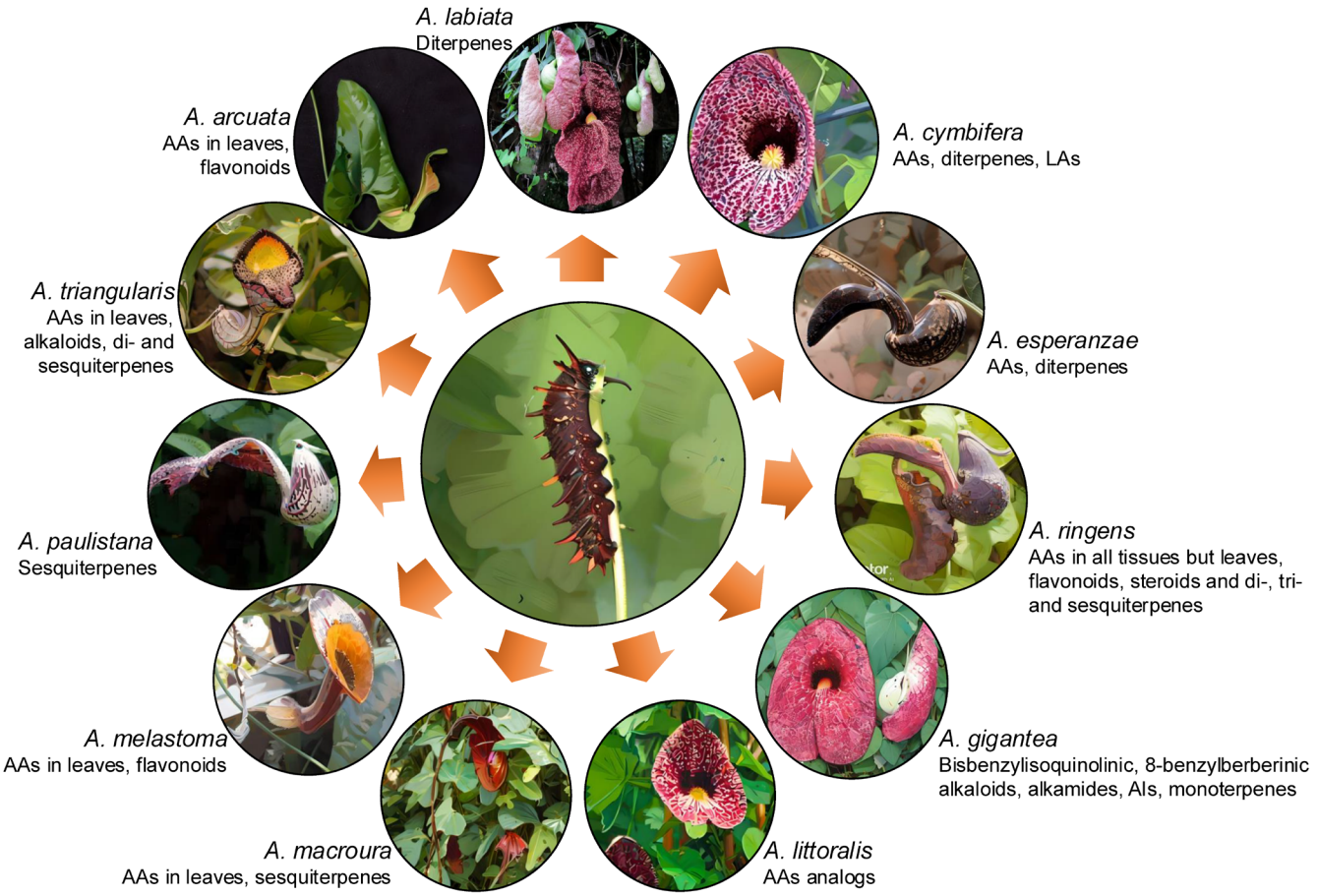


FIGURE 1 Host plants of the genus *Aristolochia* used by *Battus polydamas* larvae as food, and their main chemical composition. AAs, aristolochic acids; LAs, labdanoic acids; AL, aristolactams.
Sources: (Chen & Zhu, 1987; Han et al., 2019; Holzbach & Lopes, 2010; Klitzke, 1992; Klitzke & Brown, 2000; Kumar et al., 2003; Kuo et al., 2012; Mueller et al., 2022; Pacheco et al., 2009; Shi et al., 2004; W et al., 2005; Wu et al., 2004).

patterns in response to species of host plants with distinct chemicals can provide some insight into the genes and metabolic pathways involved in the metabolization of these compounds.

MATERIALS AND METHODS

Study system

We sampled eggs of *B. polydamas* from *A. ringens* and *A. gigantea* in Campinas, São Paulo, Brazil (22°49′15.38″ S, 47°04′8.87″ W). Eggs are abundant on both host plants from November to April during the wet-warm season.

Consumption of *Battus polydamas* on two host plants and in leaves treated with purified diterpenoids

We compared larval consumption of the two host plants by using 18-mm leaf disks offered to 1st instar larvae in a Petri dish with a moist filter paper in the bottom ($N = 12$ for each host plant). Larvae were left to feed for 3 h, and the area of any remaining leaf tissue was immediately measured. Differences in consumption were evaluated with a Wilcoxon signed-rank test (p value <0.05).

We used the same procedure to assess the effect of the acidic diterpene fraction from *A. ringens* leaves on larval consumption and growth. We applied a two-mass equivalent of the acidic diterpene fraction in each leaf disk of the otherwise acceptable larval host *A. gigantea* (which has no diterpenoids). As the acidic diterpene fraction was 0.8% of *A. ringens* fresh leaves, representing one mass equivalent, we applied 1.6% of the fresh weight of *A. gigantea* leaf disk to compensate for any loss when applied to the leaf disk surface. The acidic diterpene fraction was dissolved in 1 mL EtOH, and 250 μL was applied with a micro syringe to each leaf disk. As a control we applied the same amount of pure EtOH to each leaf disk. Leaf disk consumption was calculated by subtracting the dry weight of eaten leaf from the dry weight mean of intact leaves (in mg, mean ± standard error, n). The larval consumption (relative consumption rate [RCR]) was calculated as milligrams of dry weight of leaf eaten per milligram of initial fresh weight of larva and larval growth

(relative growth rate [RGR]) as milligrams of fresh weight gained per milligram of initial fresh weight of larva (modified from Miller & Feeny, 1983).

Performance of *Battus polydamas* on two host plants

To evaluate larval performance, we reared 1st-instar larvae hatched from eggs until pupation, individually, in young leaves (5th leave from the apical meristem) of *A. ringens* ($N = 60$) or *A. gigantea* ($N = 110$). For the trials, newly hatched larvae were fed on the same host plant from which the eggs were taken. For a representative *N*, eggs were collected from many plants in each species. We anticipated that any variations in the larval genetic backgrounds would be minimal because they were all feeding on the same host plant.

Intact expanded leaves were dipped into a 5-mL vial with water to prevent the leaf from drying out. First-instar larvae were placed on the adaxial surface of a leaf and kept in a 500-mL plastic container (8 cm high × 12 cm diameter) under controlled temperature and luminosity (27°C, photoperiod L12:D12). Containers were inspected daily for cleaning feces and providing leaves ad libitum for each larva. Survival from 1st instar to pupae, time of larval development, and pupal weight were recorded individually.

We compared the difference in frequency of survival between host plants by means of a generalized linear model (GLM) using the function GLM in R v. 3.4.0 with a binomial family (Crawley, 2005).

RNA extraction, library preparation, and sequencing

We dissected six 5th-instar larvae fed on *A. ringens* or *A. gigantea* (three biological replications from each host plant) to remove guts (crucial for both absorbing nutrients and detoxifying) and fat body tissues, totaling 12 samples, and stored them separately in RNAlater® (Life Technologies, Carlsbad, CA, USA) for subsequent RNA extraction, at a proportion of 1:5 of mass: RNAlater.

We removed tissues from RNAlater, ground each sample separately in liquid nitrogen, and extracted total RNA from gut tissue using TRIzol™ (Life Technologies, Waltham, MA, USA) combined with Direct-zol™ RNA MiniPrep (Zymo Research, Irvine, CA, USA). To extract RNA from fat body tissues, we employed the RNeasy Mini Kit (Qiagen Aarhus A/S, Denmark). We eluted each sample in 32 µL final volume of Ultrapure water (Qiagen). We then evaluated RNA quality using a Nanodrop UV spectrophotometer (Techno Scientific, Wilmington, DE, USA). Libraries were prepared in the Central Laboratory of High-Performance Technologies (LaCTAD), University of Campinas, Brazil using the Denaturing and Diluting Libraries for the HiSeq® and GAIIx (using 15 pM of sample per lane), TruSeq® RNA Sample Preparation v2, and cBot (Illumina, San Diego, CA, USA). We ran each of the three biological replicates per host plant in a separate lane in an Illumina HiSeq 2500 System (Illumina).

Gene differential expression analysis

Differential expression analyses on both gut and fat tissues of larvae fed on either *A. ringens* or *A. gigantea* were developed using CLC Genomics Workbench v. 7.5.5 (Qiagen), based on de novo assembly. To account for biological variability, we normalized the number of reads by totals, and compared original expression values (filtered by $\log^2$ fold change >2 or <–2 and *p*-value <0.05) using the "Empirical Analysis of DGE" command (Robinson & Smyth, 2008).

We used R v. 4.3.2 to create a heatmap that depicts the gene expression patterns of the *B. polidamas* larvae's tissues fed on *A. gigantea* or *A. ringens*. To make it easier to compare genes, the expression data were row-scaled. Each cell in the heatmap represents a gene relative expression level toward each species, with colors ranging from red (lower expression) to blue (higher expression). Genes and host plants were ordered without using hierarchical clustering, allowing for a more direct visualization of expression differences as response to the two species. A legend on the left side of the heatmap provides a guide to interpreting the expression levels. We used the RColorBrewer (https://doi.org/10.32614/CRAN.package.RColorBrewer) package's "Spectral" color palette to ensure a clear visual distinction between expression levels.

Genes were functionally annotated as previously described (Silva-Brandão et al., 2017), and imported to the CLC Genomics Workbench using a Generic Annotation File (GAF). To gain a comprehensive understanding of how distinct host plants affect gene expression in *B. polydamas*, we performed a comparative analysis of Gene Ontology (GO) terms enriched in the fat body and gut tissues of larvae reared on *A. gigantea* or *A. ringens*. We ranked molecular function GOs based on differential expression using the Gene Set Enrichment Analysis (GSEA) option (Tian et al., 2005), for both up- and downregulated contigs, considering categories with more than five genes (i.e., features) and removing duplicate features using FeatureID. We kept the features with the highest normalized expression value and used 10 000 permutations to calculate the *p*-value. We then used REVIGO to summarize the filtered sequence annotations (Supek et al., 2011).

The enrichment analysis results were visualized using a scatter plot created with R's ggplot2 package (Wickham, 2016). The x-axis shows the negative logarithm (base 10) of the *p*-value for each GO term, which provides a visual representation of the enrichment's strength. Smaller *p*-values indicate greater significance, which corresponds to larger negative log values. As a result, GO

terms located further to the right on the x-axis are considered more enriched. We focused the plot on significant terms by including only those with a p-value ≤0.05. This filtering step focuses on the GO terms with the most compelling evidence of enrichment within the analyzed dataset.

RESULTS

Consumption and performance of *Battus polydamas* on two host plant species

Leaf disks of *A. ringens* were significantly less consumed than leaf disks of *A. gigantea* (Figure 2). Only three out of 12 disks of *A. ringens* were consumed, whereas nonconsumption was recorded for one disk of *A. gigantea*.

When leaf disks of *A. gigantea* were painted with purified diterpenoids from *A. ringens*, they were significantly less consumed than control leaf disks painted with pure EtOH (RCR_{di}) = 1103.9 mm^2/mg/day; RCR_{EtOH} = 3043.2 mm^2/mg/day, whereas larvae feeding on them showed a fourfold lower relative growth rate (RGR_{di} = 0.4 mg/mg/day; RGR_{EtOH} = 1.6 mg/mg/day) (Figure 3).

Larvae feeding on leaves of *A. gigantea* also survived significantly more than larvae reared on *A. ringens* (GLM binomial, likelihood ratio test, $\chi^2 = 76.082$, df = 1, $p < 0.001$). Of the larvae fed on *A. ringens*, none reached pupa, therefore, for larvae in this condition, no measurements of larval development up to pupation are reported, whereas 46% of the larvae in *A. gigantea* survived to that stage. When reared on *A. gigantea*, the average pupal mass weighted 1.3 ± 0.2 mg and the duration of larval development to pupation was 16.2 ± 0.4 days.

Differential gene expression analyses

We recovered 298 unique contigs upregulated in the gut of larvae of *B. polydamas* fed on *A. gigantea* (logFC >2) and 807 upregulated contigs in larvae reared on *A. ringens* (logFC <−2) (Figure 4A and Figure S1). In the larval fat body, 233 contigs were upregulated in larvae reared on *A. gigantea*, whereas 1317 were upregulated in larvae raised on *A. ringens* (Figure 4B and Figure S1).

Unigenes are described as upregulated and downregulated, in both gut and fat body tissues, in larvae reared on *A. ringens*, relative to larvae fed on *A. gigantea* (Table S1). Annotations focused on molecular functions associated with terpenoids metabolization in insects, such as farnesyltransferase and phosphomevalonate kinase, as well as functions related to the metabolism of plants' secondary compounds, such as ABC transporters, associated with excretion, by utilizing a range of physiological metabolites and xenobiotics by using the energy released by ATP hydrolysis, and the general response of insects toward plant compounds produced to avoid herbivory, such as cytochrome P450 proteins, UDP-glycosyltransferases (UGTs), glutathione S-transferase, and carboxylesterases (CEs).

Differential expression of genes associated with storage proteins and the molting process

The genes for storage proteins (SP) were found to be upregulated in the fat body of larvae fed on *A. ringens* (Table S2). SP genes are members of the superfamily of hexameric larval serum proteins and serve as an amino acid reserve for the production of adult proteins in

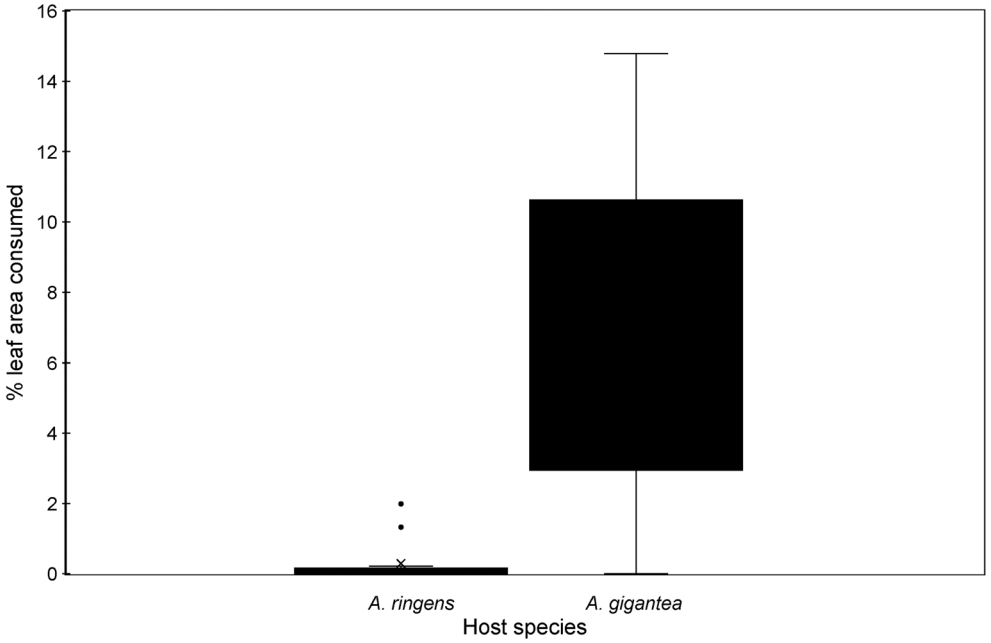


FIGURE 2 Consumed area of leaf disks of *Aristolochia ringens* and *Aristolochia gigantea* offered to 1st-instar larvae of *Battus polydamas* ($p < 0.05$).

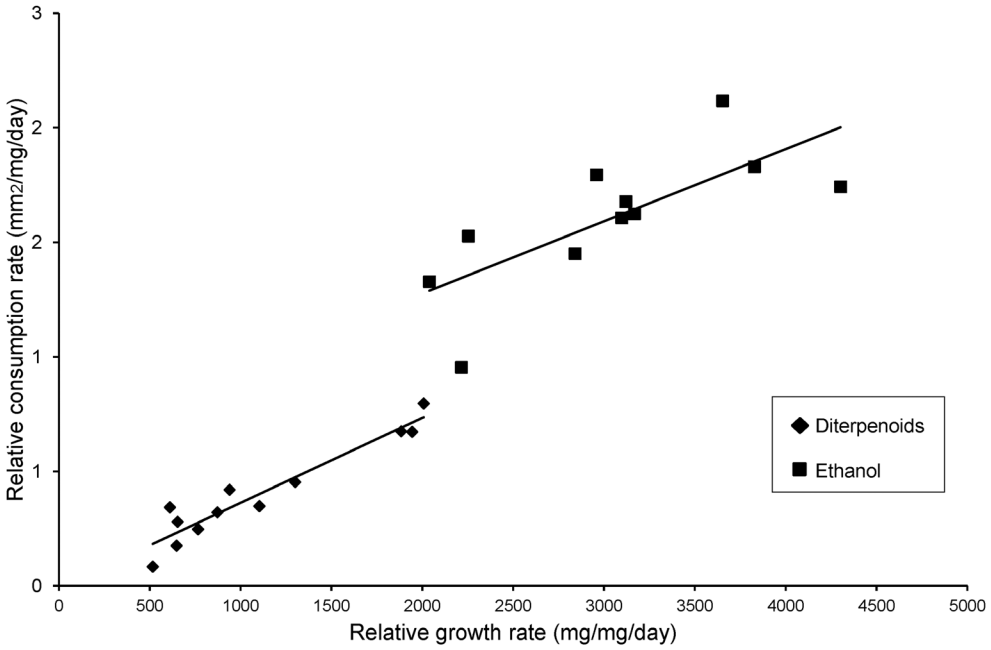


FIGURE 3 Relative consumption rate and relative growth rate of *Battus polydamas* larvae fed on *Aristolochia gigantea* leaf disks painted either with purified diterpenoids from *Aristolochia ringens* or pure ethanol (EtOH).

insects (Haunerland, 1996). Molting process (MP) genes, such as endochitinase, insulin-degrading enzyme, odorant-binding protein, and TRAP1 (tumor necrosis factor receptor-associated protein-1), and akirin genes, responsible for controlling development in both vertebrates and invertebrates (Si et al., 2020), were also found to be upregulated in the gut and fat body of larvae raised on *A. ringens*.

Enrichment analyses for gene ontology

The results of the enrichment analyses revealed significant differences in the enrichment of GO terms between larval fat body and gut tissues, regardless of the host plant where the larvae were reared (Figure S2 summarizes the top 50 enriched GO terms and their corresponding statistic tests). The fat bodies of larvae reared on *A. gigantea* exhibited a broader range of molecular functions and biological processes, implying a more diverse set of molecular activities. In contrast, larvae fed on *A. ringens* had a higher prevalence of terms related to DNA binding, protein kinase activity, and signal transduction. For instance, terms related to metabolic pathways, such as "oxidoreductase activity" and "transferase activity," were more common in this condition (Figure S3).

The analysis of gut revealed a higher enrichment of terms related to energy metabolism and detoxification, such as cytochrome-c oxidase activity and proton-transporting ATP synthase activity, in larvae fed on *A. gigantea*. The gut of larvae reared on *A. ringens*, on the other hand, contained a greater number of terms related to DNA-related processes, protein kinase activity, and nucleic acid binding.

DISCUSSION

Larvae reared on *A. gigantea* ingested substantially more and performed better than larvae fed on *A. ringens*, the plant that contains AAs (although not in the leaves) and diterpenoids. Furthermore, after being painted with the diterpenoid fraction of *A. ringens*, the otherwise acceptable leaf disks of *A. gigantea* were less eaten than control leaf disks, suggesting an antifeeding activity of these chemicals against *B. polydamas* larvae. A similar result was found when larvae of *B. polydamas* were reared on five species of *Aristolochia* (Klitzke, 1992): They survived less when fed on *A. ringens* (named as *A. galeata* in the referred reference) and showed the lowest life cycle on this plant. Likewise, none of the larvae reared on *A. ringens* pupated, even after 50 days as larvae.

As expected, the majority of the genes that are differentially expressed in the gut and fat body of *B. polydamas* larvae fed on *A. gigantea*, a species that lacks aristolochic acids (AAs) but has acyclic monoterpenoids and sesquiterpenoids, and on *A. ringens*, are related to the metabolism of secondary compounds and the general response of insects toward plant compounds produced to avoid herbivory. Genes related to larval growth and amino acid reserve were upregulated only in larvae fed on *A. ringens*.

When compared to the number of upregulated contigs in larvae reared on *A. gigantea*, the number of upregulated contigs in larvae fed on *A. ringens* was 5.6× higher in the fat body and 2.7× higher in the gut. As none of the larvae reared on *A. ringens* survived to reach the pupal phase, the high expression of genes associated with the metabolism of secondary chemicals in plants was insufficient to protect the larvae from developmental harm. In

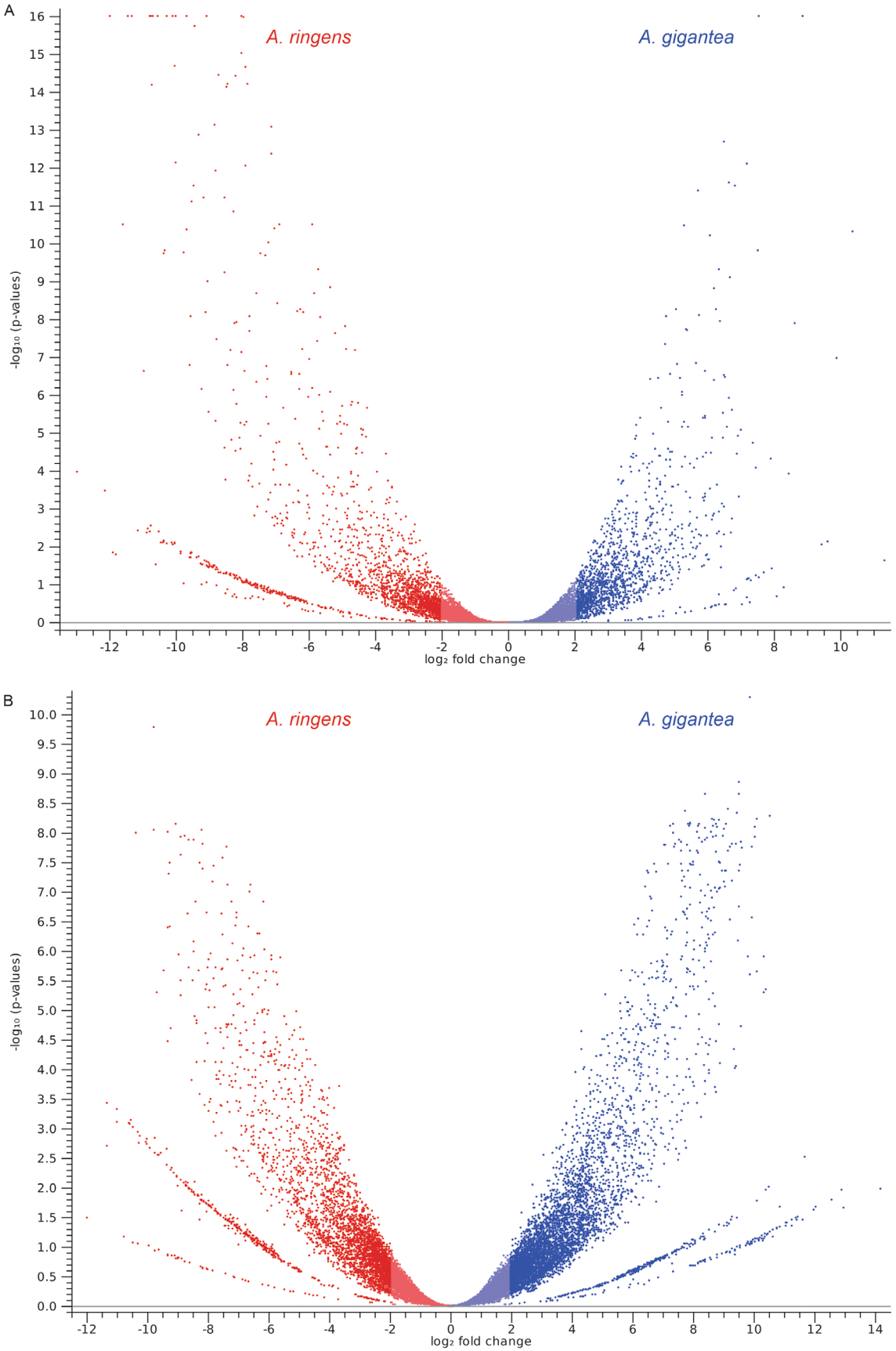


FIGURE 4 Volcano plot of differential gene expression on fat body (A) and gut (B) tissues from *Battus polydamas* reared on *Aristolochia ringens* (solid red dots) or *Aristolochia gigantea* (solid blue dots). The light blue area represents transcripts of *B. polydamas* reared on *A. gigantea* with logFC >2; the light red area corresponds to transcripts of larvae reared on *A. ringens* with logFC <–2.

addition, larvae fed this plant demonstrated an amazing upregulation of genes linked instead to storage proteins, the molting process, and invertebrate development, in addition to the gene expression increasing response of the larvae treated with the secondary compounds of *A. ringens*. The caterpillars certainly devoted all of their molecular mechanisms to survival, either by rapidly developing into the pupal stage or by expressing genes in reaction to the hazardous substances they are exposed to, causing a gene expression overloading that was insufficient to avoid their death.

Despite the improved performance of larvae reared on *A. gigantea*, the pupation rate was also very low. One may wonder why the larvae would consume such a subpar host plant in light of this poor response. Female *B. polydamas* indeed oviposits on substandard hosts, possibly due to the lack of an "optimal" host plant within their life range. Regarding this issue, a number of theories have been put forth to explain why herbivorous insects choose less-than-ideal host plants (Fox & Lalonde, 1993; Larsson & Ekbom, 1995; Thompson & Pellmyr, 1991). One of these theories is that a growing number of potential host plants, with possible preference hierarchies among them (Thompson & Pellmyr, 1991), may result from "mistakes" made by females during oviposition, eventually even leading to different host races. Two plants may be thought of as being the same (as in the instance of *A. gigantea* and *A. ringens*) even if they may differ fundamentally in ways that impact insect performance. Because selecting against a poor plant variety would also result in the loss of a high-quality plant with a similar signature, this "confusion" may cause the insect to continue feeding on the poor plant species (Fox & Lalonde, 1993). If the overall traits of the hosts are similar, we can assume that females *B. polydamas* are not overly discriminatory about where they oviposit their eggs, and larvae opportunistically feed on almost all *Aristolochia* species available in their life range, without any preference (Brown et al., 1995; Silva-Brandão & Solferini, 2007). However, *B. polydamas* larvae do not do well on plants that contain diterpenoids. Although they may be attracted to plants with high concentrations of diterpenoids at first, there is a threshold of diterpenoids that they can withstand, and beyond that, the effects of these chemicals are detrimental rather than helpful, interfering with their detoxification system.

At the evolutionary level of Troidini butterflies, this opportunistic trend is also evident: Troidines' use of host plants was unrestricted by the phylogeny of their food plants, their secondary chemicals, or their geographic similarities. Except for entirely monophagous species, all Troidini feed on *Aristolochia* species with distinct geographic ranges, and those with a wider geographic range use more host plant species than those with a more restricted distribution. This behavior might just be a reflection of the diversity and richness of host plants in various locations across a butterfly's geographic range (Silva-Brandão & Solferini, 2007).

Gene expression differences of specific genes

Even though the larvae of *B. polydamas* may not have responded as well to both host plants, feeding troidine butterflies on both *A. gigantea* and *A. ringens* surely activated their detoxification mechanism, which has evolved to deal with various species of *Aristolochia* during the group's evolution. It is not unexpected that the amount and type of genes that were differently regulated changed in the gene expression response to each of the investigated hosts due to their distinct chemical compositions. The larvae's performance was impacted by this variation; when fed the diterpenoid-containing plant *A. ringens*, they grew less and perished more. In any event, the genes that are differentially expressed in the larvae that are fed this plant can provide us with some insight into how the insects react to diterpenoids, whereas the larvae also struggle to grow as quickly as possible, failing in both challenges.

Our findings suggest that the host plant affects the gut tissue's gene expression profile in *B. polydamas* larvae, which may have an effect on the larvae's physiological adaptations and interactions with their surroundings. Ribosomal proteins, for instance, were mostly upregulated in larvae reared on *A. ringens*. Ribosomal protein genes were tightly linked to the translation processing of proteins, molecular binding activities, and ribosome biosynthesis and are involved in the oogenesis of the moth *Spodoptera litura* Fabricius (Sun et al., 2022). The differential expression of ribosomal genes in insects may target the inactivation of plant-produced ribosome-inactivating proteins (RIPs) (Celorio-Mancera et al., 2012). RIPs inhibit protein synthesis in eukaryotes (Puri et al., 2012), and they have insecticidal activity in aphids, beetles, mosquitoes, and moths in the Noctuidae family (Dowd et al., 2003; Shahidi-Noghabi et al., 2008; Zhu et al., 2018). Ribosomal proteins may indeed be considered targets for pest management strategies (Sun et al., 2022).

With regard to the protein farnesyltransferase subunit beta, it is part of the biosynthetic pathway, catalyzing the reductive dimerization of farnesyl pyrophosphate. The enzymes that make terpenes and steroids are members of the isoprenoid biosynthesis family, which includes the farnesyl pyrophosphate synthase (FPPS) homologs. It was suggested that they might work in a pathway to produce terpenes that deter predators and are secreted by the osmeterium, an organ unique to the family Papilionidae in butterflies (Cong et al., 2015). It is conceivable that the farnesyltransferase subunit beta may be crucial for *B. polydamas* to metabolize diterpenes because it was upregulated in larvae raised on the diterpene-producing *A. ringens*.

Likewise, phosphomevalonate kinase was upregulated on larvae fed on *A. ringens*. The enzyme is part of the biochemical pathway of mevalonate enzymes, involved in terpene synthases in insects (Beran et al., 2019). The biosynthesis of terpenes differs between plants and insects.

Insect genomes lack homologs of plant or microbial-type TPS (terpene synthases) genes, despite the presence of typical IDSs (isoprenyl diphosphate synthases) and mevalonate pathway enzymes (Beran et al., 2019). Recent research on Hemiptera and Coleoptera indicates that a wider evolutionary transition from IDS to TPS proteins has taken place in insects. Gene structure and phylogenetic analysis supported the TPS enzymes' evolutionary ancestry from IDS progenitors (Beran et al., 2016).

ATP-binding cassette (ABC) transporters, a broad class of transmembrane proteins, are present in many organisms and are crucial to the movement of foreign substances (Wu et al., 2019). They have attracted particular attention recently due to the association between pesticide resistance and multidrug resistance-associated ABC genes, specifically those of subfamilies B and C. Furthermore, it has been demonstrated that a few ABC transporters directly mediate the harmful effects of pesticides and biopesticides, and a number of inhibitors may be helpful in enhancing the toxicity of pesticides (Merzendorfer, 2014). Its role in the transport network needed for cardenolide sequestration was also demonstrated for the cardenolide-sequestering leaf beetle *Chrysochus auratus* Fabricius (Kowalski et al., 2020). Additionally, when *Danaus plexippus* L. caterpillars were fed low-cardenolide milkweed as opposed to high-cardenolide milkweed, there was an upregulation of ABC transporters, which was linked to a higher rate of cardenolide sequestration (Tan et al., 2019a).

As most transcriptomics studies have demonstrated that one or more P450s genes were differently regulated (Celorio-Mancera et al., 2012; Koenig et al., 2015; Perera et al., 2015; Roy et al., 2016), it is not unexpected that P450 enzymes upregulated and downregulated unigenes in our analyses. But this might be a cascading effect as well as a direct relationship (Feyereisen, 2012). P450s play a significant role in the detoxification of xenobiotics in insects, and they are implicated in numerous instances of pesticide resistance. For instance, *Spodoptera littoralis* Boisduval responds to the terpenoids of *Origanum vulgare* L. by upregulating the activities of its antioxidant enzymes and the expression of its genes; nevertheless, this was insufficient to maintain the terpenoids' toxicity (Agliassa & Maffei, 2018). P450 gene expression is controlled by substances that enable insects to react to changing environments in two ways: directly by developing a detoxification defense and indirectly by modifying their basal metabolism, which includes hormone balance, development rate, and reproduction rate, as evidence for pheromone catabolism is mounting.

Various UDP-glycosyltransferases (UGTs) were upregulated in larvae fed both on *A. gigantea* and *A. ringens*. UGTs are a diverse superfamily of enzymes, and insects use the uridine diphosphate-glucose (UDP-glucose) as a glycosyl donor for glycosylation in vivo, involved in the glycosylation of lipophilic endosymbionts and xenobiotics, including phytotoxins. This process of O-glucosylation of sequestered phenolic AAs Ia, IIIa, IVa, and IVb was also observed in *B. polydamas*, and the analysis of its osmeterial secretion and larval tissues confirmed that the phenolic AAs are significantly O-glucosylated in this species (Priestap et al., 2012).

As *B. polydamas* is a monophagous species that only feeds on one genus of host plant, it should be well-suited to the secondary chemicals present on its larval host. It may even be able to retain and utilize these compounds to protect itself from predators. Nonetheless, this butterfly can have a hierarchical preference for some plant species (Klitzke, 1992; Thompson & Pellmyr, 1991), although no strong preference has been reported (Brown et al., 1995). In any case, *Aristolochia* plants are chemically diverse; thus, some plant chemical combinations should be simpler to handle than others, but not all of them at once. In fact, several specialized herbivores have been reported to be adversely affected by plant defence compounds (Ali & Agrawal, 2012). Specialists also appear to gain from intermediate levels of plant toxins (via protection from predators), even though toxins eventually come at a cost. The insect's goal is to maximize its own growth through induction, and induction should be minimal for all herbivore tactics (Ali & Agrawal, 2012). That means that tolerance of specialist insects to low levels of chemicals is to be expected; however, at higher levels of defence, few insects are immune to the deleterious effects of plant toxins, as we described for *B. polydamas* fed on both *A. gigantea* and *A. ringens*, but with a stronger negative effect from the first.

In conclusion, *B. polydamas* larvae raised on two distinct species of *Aristolochia* exhibit differences in both performance and gene expression levels. Although none of the reactions to the host plant can be regarded as ideal, the larvae developed better when diterpenoids were absent, and we were nevertheless able to offer some hints on how these substances are metabolized in the larval intestine at the level of gene expression.

The differential gene regulatory response of *B. polydamas* larvae toward two host plant species indeed provided cues on the metabolization and detoxification processes of plant secondary compounds by herbivorous insects. It is not always possible to investigate these topics on Neotropical wild species of lepidopterans, although the kind of data gathered with such organisms certainly provides basic knowledge potentially easily applied, for example, for pest control.

AUTHOR CONTRIBUTIONS

Karina L. Silva-Brandão: Formal analysis; funding acquisition; methodology; project administration; resources; software; writing – original draft; writing – review and editing. **Clécio Fernando Klitzke:** Validation; writing – review and editing. **Marcelo M. Brandão:** Formal analysis; methodology; resources; software; visualization; writing – review and editing. **José Roberto Trigo:** Conceptualization; formal analysis; funding acquisition; methodology.

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CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Supporting information is deposited at https://doi.org/10.25824/redu/A3GVHV.

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REFERENCES

Agliassa, C. & Maffei, M.E. (2018) Origanum vulgare terpenoids induce oxidative stress and reduce the feeding activity of *Spodoptera littoralis*. *International Journal of Molecular Sciences*, *19*, 2805.

Ali, J.G. & Agrawal, A.A. (2012) Specialist versus generalist insect herbivores and plant defense. *Trends in Plant Science*, *17*, 293–302.

Bentley, K.W. (2006) Beta-phenylethylamines and the isoquinoline alkaloids. *Natural Product Reports*, *23*(3), 444–463. Available from: https://doi.org/10.1039/b509523a

Beran, F., Köllner, T.G., Gershenzon, J. & Tholl, D. (2019) Chemical convergence between plants and insects: biosynthetic origins and functions of common secondary metabolites. *New Phytologist*, *223*, 52–67.

Beran, F., Rahfeld, P., Luck, K., Nagel, R., Vogel, H., Wielsch, N. et al. (2016) Novel family of terpene synthases evolved from trans-isoprenyl diphosphate synthases in a flea beetle. *Proceedings of the National Academy of Sciences*, *113*, 2922–2927.

Boncan, D.A.T., Tsang, S.S.K., Li, C., Lee, I.H.T., Lam, H.M., Chan, T.F. et al. (2020) Terpenes and terpenoids in plants: interactions with environment and insects. *International Journal of Molecular Sciences*, *21*(19), 7382. Available from: https://doi.org/10.3390/ijms21197382

Breeschoten, T., Ros, V.I.D., Schranz, M.E. & Simon, S. (2019) An influential meal: host plant dependent transcriptional variation in the beet armyworm, *Spodoptera exigua* (lepidoptera: Noctuidae). *BMC Genomics*, *20*, 845.

Breeschoten, T., Schranz, M.E., Poelman, E.H. & Simon, S. (2022) Family dinner: transcriptional plasticity of five Noctuidae (lepidoptera) feeding on three host plant species. *Ecology and Evolution*, *12*, e9258.

Brown, K.S., Damman, A.J. & Feeny, P. (1981) Troidine swallowtails (lepidoptera: Papilionidae) in southeastern Brazil: natural history and foodplant relationships. *Journal of Research on the Lepidoptera*, *19*, 199–226.

Brown, K.S., Klitzke, C.F., Berlingeri, C. & Santos, P.E.R. (1995) Neotropical swallowtails: chemistry of food plant relationships, population ecology, and biosystematics. In: Scriber, J.M., Tsubaki, Y. & Lederhouse, R.C. (Eds.) *Swallowtail butterflies: their ecology and evolutionary biology*. Gainesville, FL: Scientific Publishers, pp. 405–444.

Celorio-Mancera, M.D., Heckel, D.G. & Vogel, H. (2012) Transcriptional analysis of physiological pathways in a generalist herbivore: responses to different host plants and plant structures by the cotton bollworm, *Helicoverpa armigera*. *Entomologia Experimentalis et Applicata*, *144*, 123–133.

Celorio-Mancera, M.D., Steward, R.A., Pruisscher, P., Smialowska, A., Pires Braga, M., Janz, N. et al. (2022) Larval transcriptomes reflect the evolutionary history of plant–insect associations. *Evolution*, *77*, 519–533.

Celorio-Mancera, M.D., Wheat, C.W., Vogel, H., Söderlind, L., Janz, N. & Nylin, S. (2013) Mechanisms of macroevolution: polyphagous plasticity in butterfly larvae revealed by RNA-seq. *Molecular Ecology*, *22*, 4884–4895.

Chen, Z.-L. & Zhu, D.-Y. (1987) Aristolochia Alkaloids. In: Brossi, A. (Ed.) *The alkaloids: chemistry and pharmacology*, Vol. *31*. New York: Academic Press, pp. 29–65.

Cong, Q., Borek, D., Otwinowski, Z. & Grishin, N.V. (2015) Tiger swallowtail genome reveals mechanisms for speciation and caterpillar chemical defense. *Cell Reports*, *10*, 910–919.

Conserva, L.M., da Silva, M.S. & Filho, R.B. (1990) Lignans from *Aristolochia* birostris. *Phytochemistry*, *29*, 257–260.

Crawley, M.J. (2005) *Statistics: an introduction using R*. England: John Wiley & Sons Ltd.

Dimarco, R.D. & Fordyce, J.A. (2017) Not all toxic butterflies are toxic: high intra- and interspecific variation in sequestration in subtropical swallowtails. *Ecosphere*, *8*, e02025.

Dowd, P.F., Zuo, W.-N., Gillikin, J.W., Johnson, E.T. & Boston, R.S. (2003) Enhanced resistance to *Helicoverpa zea* in tobacco expressing an activated form of maize ribosome-inactivating protein. *Journal of Agricultural and Food Chemistry*, *51*, 3568–3574.

Ehrlich, P.R. & Raven, P.H. (1964) Butterflies and plants: a study in coevolution. *Evolution*, *18*(4), 586–608. Available from: https://doi.org/10.2307/2406212

Ferreira, M.L., de Pascoli, I.C., Nascimento, I.R., Zukerman-Schpector, J. & Lopes, L.M. (2010) Aporphine and bisaporphine alkaloids from *Aristolochia lagesiana* var. *intermedia*. *Phytochemistry*, *71*(4), 469–478. Available from: https://doi.org/10.1016/j.phytochem.2009.11.010

Feyereisen, R. (2012) In: Gilbert, L.I. (Ed.) *Insect CYP genes and P450 enzymes: insect molecular biology and biochemistry*. San Diego: Academic Press, pp. 236–316.

Fox, C.W. & Lalonde, R.G. (1993) Host confusion and the evolution of insect diet breadths. *Oikos*, *67*, 577–581.

Futuyma, D.J. & Keese, M.C. (1992) Evolution and coevolution of plants and phytophagus arthropods. In: Rosenthal, G.R. & Berenbaum, M.R. (Eds.) *Herbivores: their interactions with secondary plant metabolites*. San Diego, CA: Academic Press, Inc., pp. 439–475.

Glendinning, J.I. (2002) How do herbivorous insects cope with noxious secondary plant compounds in their diet? *Entomologia Experimentalis et Applicata*, *104*, 15–25.

González, F., Wagner, S.T., Salomo, K., Symmank, L., Samain, M.-S., Isnard, S. et al. (2014) Present trans-Pacific disjunct distribution of *Aristolochia* subgenus *Isotrema* (Aristolochiaceae) was shaped by dispersal, vicariance and extinction. *Journal of Biogeography*, *41*, 380–391.

Han, J., Xian, Z., Zhang, Y., Liu, J. & Liang, A. (2019) Systematic overview of aristolochic acids: nephrotoxicity, carcinogenicity, and underlying mechanisms. *Frontiers in Pharmacology*, *10*, 648.

Haunerland, N.H. (1996) Insect storage proteins: gene families and receptors. *Insect Biochemistry and Molecular Biology*, *26*, 755–765.

Heidel-Fischer, H.M. & Vogel, H. (2015) Molecular mechanisms of insect adaptation to plant secondary compounds. *Current Opinion in Insect Science*, *8*, 8–14.

Holzbach, J.C. & Lopes, L.M.X. (2010) Aristolactams and alkamides of *Aristolochia gigantea*. *Molecules*, *15*, 9462–9472.

Klitzke, C.F. (1992) Ecologia química e coevolução na interface Troidini (Papilionidae)/*Aristolochia* (Aristolochiaceae): Instituto de Biologia (ed. Universidade Estadual de Campinas, Campinas, p. 73).

Klitzke, C.F. & Brown, K.S. (2000) The occurrence of aristolochic acids in neotropical troidine swallowtails (lepidoptera: Papilionidae). *Chemoecology*, *10*, 99–102.

Koenig, C., Bretschneider, A., Heckel, D.G., Grosse-Wilde, E., Hansson, B.S. & Vogel, H. (2015) The plastic response of *Manduca sexta* to host and non-host plants. *Insect Biochemistry and Molecular Biology*, *63*, 72–85.

Kowalski, P., Baum, M., Körten, M., Donath, A. & Dobler, S. (2020) ABCB transporters in a leaf beetle respond to sequestered plant toxins. *Proceedings of the Royal Society B*, *287*, 20201311.

Kumar, V., Kumari, P., Prasad, A.K. & Parmar, V.S. (2003) Naturally occurring aristolactams, aristolochic acids and dioxoaporphines and their biological activities. *Natural Product Reports*, *20*, 565–583.

Kuo, P.-C., Li, Y.-C. & Wu, T.-S. (2012) Chemical constituents and pharmacology of the *Aristolochia* (馬兜鈴 mădōu ling) species. *Journal of Traditional and Complementary Medicine*, *2*, 249–266.

Larsson, S. & Ekbom, B. (1995) Oviposition mistakes in herbivorous insects: confusion or a step towards a new host plant? *Oikos*, *72*, 155–160.

Lü, D., Yan, Z., Hu, D., Zhao, A., Wei, S., Wang, P. et al. (2022) RNA sequencing reveals the potential adaptation mechanism to different hosts of *Grapholita molesta*. *Insects*, *13*, 893.

Merzendorfer, H. (2014) Chapter one—ABC transporters and their role in protecting insects from pesticides and their metabolites. In: Cohen, E. (Ed.) *Advances in insect physiology*, Vol. *46*. New York: Academic Press, pp. 1–72.

Michl, J., Ingrouille, M.J., Simmonds, M.S. & Heinrich, M. (2014) Naturally occurring aristolochic acid analogues and their toxicities. *Natural Product Reports*, *31*, 676–693.

Miller, J.S. & Feeny, P. (1983) Effects of benzylisoquinoline alkaloids on the larvae of polyphagous lepidoptera. *Oecologia*, *58*, 332–339.

Morais, A.B.B. & Brown, K.S. (1991) Larval foodplant and other effects on Troidine guild composition (Papilionidae) in southeastern Brazil. *Journal of Research on the Lepidoptera*, *30*, 19–37.

Morais, A.B.B., Brown, K.S., Stanton, M.A., Massuda, K.F. & Trigo, J.R. (2013) Are aristolochic acids responsible for the chemical Defence of aposematic larvae of *Battus polydamas* (L.) (lepidoptera: Papilionidae)? *Neotropical Entomology*, *42*, 558–564.

Mueller, L., Souza, V.B., de Aquino, C.D., Garcia, L., Tanaka, L., Bachega, T. et al. (2022) The *Aristolochia* (Aristolochiaceae) genus: therapeutic properties, biological effects and toxicity. *Research, Society and Development*, *11*, e293111133504.

Nallu, S., Hill, J.A., Don, K., Sahagun, C., Zhang, W., Meslin, C. et al. (2018) The molecular genetic basis of herbivory between butterflies and their host plants. *Nature Ecology & Evolution*, *2*(9), 1418–1427.

Nishida, R. & Fukami, H. (1989) Ecological adaptation of an Aristolochiaceae-feeding swallowtail butterfly, *Atrophaneura alcinous*, to aristolochic acids. *Journal of Chemical Ecology*, *15*, 2549–2563.

Nogueira, C.R. & Lopes, L.M. (2013) Sequestration and biotransformation of lignans from *Aristolochia giberti* by *Battus polydamas* larvae (Papilonidae: Troidini). *Planta Medica*, *79*, PI67.

Pacheco, A.G., de Machado Oliveira, P., Piló-Veloso, D. & de Flávio Carvalho Alcântara, A. (2009) 13C-NMR data of diterpenes isolated from *Aristolochia* species. *Molecules*, *14*(3), 1245–1262. Available from: https://doi.org/10.3390/molecules14031245

Perera, O.P., Shelby, K.S., Popham, H.J.R., Gould, F., Adang, M.J. & Jurat-Fuentes, J.L. (2015) Generation of a transcriptome in a model lepidopteran pest, *Heliothis virescens*, using multiple sequencing strategies for profiling midgut gene expression. *PLoS One*, *10*, e0128563.

Pinto, F.C., Troncoso, J.A., Urzúa, A. & Niemeyer, M.H. (2009) Aristolochic acids affect the feeding behaviour and development of *Battus polydamas archidamas* larvae (lepidoptera: Papilionidae: Troidini). *European Journal of Entomology*, *106*, 357–361.

Priestap, H.A., Velandia, A.E., Johnson, J.V. & Barbieri, M.A. (2012) Secondary metabolite uptake by the *Aristolochia*-feeding papilionoid butterfly *Battus polydamas*. *Biochemical Systematics and Ecology*, *40*, 126–137.

Puri, M., Kaur, I., Perugini, M.A. & Gupta, R.C. (2012) Ribosome-inactivating proteins: current status and biomedical applications. *Drug Discovery Today*, *17*, 774–783.

Rios, S.R., Salgado-Luarte, C., Stotz, C.G. & Gianoli, E. (2016) Co-occurrence of host plants associated with plant quality determines performance patterns of the specialist butterfly, *Battus polydamas archidamas* (lepidoptera: Papilionidae: Troidini). *European Journal of Entomology*, *113*, 150–157.

Robinson, M.D. & Smyth, G.K. (2008) Small-sample estimation of negative binomial dispersion, with applications to SAGE data. *Biostatistics*, *9*, 321–332.

Roy, A., Walker, W.B., Vogel, H., Chattington, S., Larsson, M.C., Anderson, P. et al. (2016) Diet dependent metabolic responses in three generalist insect herbivores *Spodoptera* spp. *Insect Biochemistry and Molecular Biology*, *71*, 91–105.

Shahidi-Noghabi, S., Van Damme, E.J. & Smagghe, G. (2008) Carbohydrate-binding activity of the type-2 ribosome-inactivating protein SNA-I from elderberry (*Sambucus nigra*) is a determining factor for its insecticidal activity. *Phytochemistry*, *69*, 2972–2978.

Shi, L.-S., Kuo, P.-C., Tsai, Y.-L., Damu, A.G. & Wu, T.-S. (2004) The alkaloids and other constituents from the root and stem of *Aristolochia elegans*. *Bioorganic & Medicinal Chemistry*, *12*, 439–446.

Si, W., Wang, Q., Li, Y. & Dong, D. (2020) Label-free quantitative proteomic analysis of insect larval and metamorphic molts. *BMC Developmental Biology*, *20*, 24.

Silva-Brandão, K.L., Horikoshi, R.J., Bernardi, D., Omoto, C., Figueira, A. & Brandão, M.M. (2017) Transcript expression plasticity as a response to alternative larval host plants in the speciation process of corn and rice strains of *Spodoptera frugiperda*. *BMC Genomics*, *18*, 792.

Silva-Brandão, K.L. & Solferini, V.N. (2007) Use of host plants by Troidini butterflies (Papilionidae, Papilioninae): constraints on host shift. *Biological Journal of the Linnean Society*, *90*, 247–261.

Silva-Brandão, K.L., Solferini, V.N. & Trigo, J.R. (2006) Chemical and phylogenetic relationships among *Aristolochia* L. (Aristolochiaceae) from southeastern Brazil. *Biochemical Systematics and Ecology*, *34*, 291–302.

Sime, K. (2002) Chemical defence of *Battus philenor* larvae against attack by the parasitoid *Trogus pennator*. *Ecological Entomology*, *27*, 337–345.

Sun, R., Liu, J., Xu, Y., Jiang, L., Li, Y., Zhong, G. et al. (2022) Genome-wide identification and stage-specific expression profile analysis reveal the function of ribosomal proteins for oogenesis of Spodoptera litura. *Frontiers in Physiology*, *13*, 943205.

Supek, F., Bosnjak, M., Skunca, N. & Smuc, T. (2011) REVIGO summarizes and visualizes long lists of gene ontology terms. *PLoS One*, *6*, e21800.

Tan, W.-H., Acevedo, T., Harris, E.V., Alcaide, T.Y., Walters, J.R., Hunter, M.D. et al. (2019) Transcriptomics of monarch butterflies (*Danaus plexippus*) reveals that toxic host plants alter expression of detoxification genes and down-regulate a small number of immune genes. *Molecular Ecology*, *28*, 4845–4863.

Thompson, J.N. (1989) Concepts of coevolution. *Trends in Ecology & Evolution*, *4*, 179–183.

Thompson, J.N. & Pellmyr, O. (1991) Evolution of oviposition behavior and host preference in lepidoptera. *Annual Review of Entomology*, *36*, 65–89.

Tian, L., Greenberg, S.A., Kong, S.W., Altschuler, J., Kohane, I.S. & Park, P.J. (2005) Discovering statistically significant pathways in expression profiling studies. *Proceedings of the Natural Academy of Science USA*, *102*(38), 13544–13549. Available from: https://doi.org/10.1073/pnas.0506577102

Tyler, H., Brown, K.S. & Wilson, K. (1994) *Swallowtail butterflies of the Americas—a study in biological dynamics, ecological diversity, biosystematics, and conservation*. Gainesville, FL: Scientific Publishers, Inc.

Urzúa, A., Olguín, A. & Santander, R. (2013) Fate of ingested Aristolactams from *Aristolochia chilensis* in *Battus polydamas archidamas* (lepidoptera: Papilionidae). *Insects*, *4*, 533–541.

W, T.-S., Amooru, G.D., S, C.-R. & K, P.-C. (2005) Chemical constituents and pharmacology of *Aristolochia* species. In: Atta ur, R. (Ed.) *Studies in natural products chemistry*, Vol. *32*. Amsterdam: Elsevier, pp. 855–1018.

Wickham, H. (2016) *ggplot2—elegant graphics for data analysis*, 2nd edition. USA: SpringerCham.

Wu, C., Chakrabarty, S., Jin, M., Liu, K. & Xiao, Y. (2019) Insect ATP-binding cassette (ABC) transporters: roles in xenobiotic detoxification and Bt insecticidal activity. *International Journal of Molecular Sciences*, *20*(11), 2829. Available from: https://doi.org/10.3390/ijms20112829

Wu, T.-S., Damu, A.G., Su, C.-R. & Kuo, P.-C. (2004) Terpenoids of *Aristolochia* and their biological activities. *Natural Product Reports*, *21*(5), 594–624. Available from: https://doi.org/10.1039/b401950d

Yamaguchi, L.F., Torres, H. & Kato, M. (2017) Sequestration of compounds from *Aristolochia gigantea* by caterpillars and adults of *Battus polydamas archidamas* (Lepidoptera: Papilionidae: Troidini): Proceedings of Brazilian Conference on Natural Products and Annual Meeting on Micromolecular Evolution, Systematics and Ecology. (ed., Campinas).

Zhong, H., Li, F., Chen, J., Zhang, J. & Li, F. (2017) Comparative transcriptome analysis reveals host-associated differentiation in *Chilo suppressalis* (lepidoptera: Crambidae). *Scientific Reports*, *7*, 13778.

Zhu, F., Zhou, Y.K., Ji, Z.L. & Chen, X.R. (2018) The plant ribosome-inactivating proteins play important roles in defense against pathogens and insect Pest attacks. *Frontiers in Plant Science*, *9*, 146.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Heatmap visualization of differentially expressed genes in (A) the gut and (B) fat body tissues of larvae reared on *Aristolochia ringens* or *Aristolochia gigantea*. The color-coded expression patterns suggest potential differences in biological process, for example, metabolic pathways, developmental processes in larvae fed associated with each one of the two different host plants. Each cell in the heatmap represents a gene relative expression level toward each species, with colors ranging from red (lower expression) to blue (higher expression).

Figure S2. Summary of gene set enrichment analysis (GSEA) on *Battus polydamas* tissues fed on different host plants. Each data point on the scatterplot corresponds to a gene ontology (GO) term and represents the log-transformed absolute test statistics (log10(Teststatistic)). Four distinct comparisons are displayed: (A) fat body of larvae reared on *Aristolochia ringens*; (B) fat body of larvae reared on *Aristolochia gigantea*; (C) gut of larvae reared on *A. ringens*; (D) gut of larvae reared on *A. gigantea*.

Figure S3. TreeMap of enriched gene ontology terms resulting from gene set enrichment analysis. Each rectangle corresponds to a specific term, clustered into most significant molecular function. A: Fat body of larvae reared on *Aristolochia ringens*; B: Fat body of larvae reared on *Aristolochia gigantea*; C: Midgut of larvae reared on *A. ringens*; D: Midgut of larvae reared on *A. gigantea*.

Data S1. Unigenes are indicated as up-and downregulated in larvae reared on Aristolochia ringens, relative to larvae fed on Aristolochia gigantea. Annotation is associated to their molecular function.

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