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RESEARCH ARTICLE

Host plant defense produces species-specific alterations to flight muscle protein structure and flight-related fitness traits of two armyworms

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ABSTRACT

Insects manifest phenotypic plasticity in their development and behavior in response to plant defenses, via molecular mechanisms that produce tissue-specific changes. Phenotypic changes might vary between species that differ in their preferred hosts and these effects could extend beyond larval stages. To test this, we manipulated the diet of southern armyworm (SAW; *Spodoptera eridania*) and fall armyworm (FAW; *Spodoptera frugiperda*) using a tomato mutant for jasmonic acid plant defense pathway (*def1*), and wild-type plants, and then quantified gene expression of Troponin t (*Tnt*) and flight muscle metabolism of the adult insects. Differences in *Tnt* spliceform ratios in insect flight muscles correlate with changes to flight muscle metabolism and flight muscle output. We found that SAW adults reared on induced *def1* plants had a higher relative abundance (RA) of the *A* isoform of Troponin t (*Tnt A*) in their flight muscles; in contrast, FAW adults reared on induced *def1* plants had a lower RA of *Tnt A* in their flight muscles compared with adults reared on *def1* and controls. Although mass-adjusted flight metabolic rate showed no independent host plant effects in either species, higher flight metabolic rates in SAW correlated with increased RA of *Tnt A*. Flight muscle metabolism also showed an interaction of host plants with *Tnt A* in both species, suggesting that host plants might be influencing flight muscle metabolic output by altering *Tnt*. This study illustrates how insects respond to variation in host plant chemical defense by phenotypic modifications to their flight muscle proteins, with possible implications for dispersal.

KEY WORDS: *def1*, Dispersal, Jasmonic acid, Muscle metabolism, Phenotypic plasticity, Spodoptera

INTRODUCTION

Phenotypic plasticity refers to the ability of organisms to respond to environmental variation by modifying gene expression to alter their morphology, organ system development, physiological processes and/or behavior (Bradshaw, 1965; Simmons and Emlen, 2006; Marden, 2008; Whitman and Agrawal, 2009; Murren et al., 2015). Understanding how particular environmental conditions affect the expression of genes and how gene expression patterns produce modifications to organ systems has applications in the fields of ecology, conservation biology, functional genomics, population dynamics and pest management, because it provides insight into the genes and fitness traits that are being targeted by natural selection. Despite the important role phenotypic plasticity plays in species survival and evolution (Chippindale et al., 1993; Whitman and Agrawal, 2009; Murren et al., 2015), linking environmentally induced changes to the expression patterns of specific genes (or gene suites) with distinct phenotypes has been poorly documented.

Herbivorous holometabolous insects make excellent systems to study phenotypic plasticity on a mechanistic level because variation in host plant quality can be manipulated to produce tissue-specific phenotypic changes in the insects (Cloutier et al., 2000; Saha et al., 2012; Thaler et al., 2014; Portman et al., 2015a; Zinna et al., 2018). Although potential host plants are numerous, individual species of phytophagous insects feed on only a small fraction of available plant species (Strauss and Zangerl, 2002). A major factor that reduces an insect's ability to access nutrients stored in plant tissue is the plant's defense response – both constitutive and induced (Howe et al., 1996; Johnson et al., 1989; Steppuhn et al., 2004; Haviola et al., 2007; Chen et al., 2005; Kariyat et al., 2017a,b). Induced defenses are triggered by herbivory, whereupon plants upregulate the production of physical defenses (e.g. trichomes, spines; Valverde et al., 2001; Kariyat et al., 2017a,b) and secondary metabolites (e.g. alkaloids, phenolics, glycosides, volatile organic compounds). Secondary metabolites function as toxins and feeding deterrents, and interfere with nutrient absorption, thus reducing the herbivore's access to the plant's nutritional resources (Agrawal, 2001; Chen et al., 2006; Howe and Jander, 2008; Tayal et al., 2020). For a plant to be a suitable food source, it must be possible for a herbivore to efficiently acquire the nutrients contained within its tissues (Courtney, 1981; Scriber, 1981), and also metabolize, sequester, excrete or detoxify the plant's defensive secondary metabolites (Rausher, 1988; Bolter and Jongsma, 1995; Gaertner et al., 1998; Kareiva, 1999; Cloutier et al., 2000; Nishida, 2002; Ali and Agrawal, 2012).

Mutations, inbreeding or selective breeding within host plant populations can produce variation in a plant's defense output via genetic changes that affect the expression of defense-related genes (Howe et al., 1996; Gols et al., 2008; Kariyat et al., 2012a,b; Portman et al., 2015b). Defenseless1 (*def1*) is a loss of function mutant in the Castlemart tomato cultivar, caused by a genetic deletion in the octadecanoid pathway that affects peroxisomal synthesis of a lipid-derived phytohormone (Howe et al., 1996; Wasternack et al., 2006; Howe and Jander, 2008). *def1* mutants have a reduced titer of jasmonic acid (JA) and fail to induce defense responses through the JA signaling pathway (Schilmiller and Howe, 2005; Wasternack et al., 2006). Once synthesized, JA is mobilized to distal parts of the plant to promote the expression of defense genes such as protease

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inhibitors (Schilmiller and Howe, 2005; Howe and Jander, 2008). Compared with wild-type Castlemart plants, *def1* mutants are unable to produce large amounts of protease inhibitors (PIs) in response to herbivore feeding damage (Schilmiller and Howe, 2005). The presence of PIs decreases the overall nutritional quality of the plant by reducing the insect's ability to metabolize proteins (Chen et al., 2005; Howe and Jander, 2008). An experimentally useful characteristic of *def1* is that levels of PI production can be partially restored by treating mutant plants with a solution of commercially available methyl-jasmonate (Thaler et al., 1996).

Insect herbivores are excellent study systems to examine how variation in nutrition intake can influence phenotypic plastic traits, because their diet can be easily manipulated and controlled. Moreover, diet-induced changes to immature insects can cascade through later development stages to produce phenotypic alterations to ecologically important adult fitness traits such as flight muscle development (Portman et al., 2015a). Flight ability is crucial to the survival of most insects because flight allows insects to escape predation (Chai and Srygley, 1990), locate food and mates (Zera and Denno, 1997; Langellotto and Denno, 2001), and colonize new habitat (Haag et al., 2005; Niitepõld et al., 2009; Zheng et al., 2009). Although flight capability can be a discrete variable (flight capable versus flightless; e.g. Roff and Fairbairn, 1991; Zera and Zhou, 2006), most flight-capable insects exhibit continuous variation in flight performance (Marden and Chai, 1991). Insects with better flight performance have increased survival, reproduction and dispersal (Chai and Srygley, 1990; Roff, 1994; Langellotto and Denno, 2001; Hanski, 2011). Flight performance can also influence population dynamics in insects (Wheat et al., 2011). For example, greater flight capacity in Glanville fritillary butterflies produced a 2-fold increase in population size because the colonization rate of new habitat patches increased and local extinctions of isolated populations decreased (Zheng et al., 2009); hence, quantitative differences in insect flight ability are ecologically important at both the individual and population levels.

Insect flight capability correlates strongly with flight muscle size and power output (Hill et al., 1999; Berwaerts et al., 2002; Marden and Cobb, 2004). However, construction and maintenance of large flight muscles can be metabolically expensive (Zera et al., 1998; Zera and Denno, 1997) and internal physiological conditions, such as parasite infection or poor nutrition, that perturb metabolism can result in changes to flight muscle development and decreased muscle performance (Marden and Cobb, 2004; Schilder and Marden, 2007; Marden et al., 2008). Consequently, insects modify their flight muscles on a molecular level in response to changes to their internal biochemistry (Schilder and Marden, 2006, 2007; Marden et al., 2008). Troponin t (*Tnt*), an alternatively spliced subunit of the muscle tropomyosin complex, influences flight muscle force production and power output (Marden et al., 1999, 2001; Schilder and Marden, 2007).

Changes in the relative abundance (RA) of *Tnt* spliceforms are quantitatively related to flight muscle power output and maximum flight metabolic rate (Marden, 2008; Marden et al., 2001, 2008; Portman et al., 2015a). The higher RA of longer isoforms (i.e. *Tnt A*, *B*, *C*, *D*) correlates with increases in muscle force production and rates of muscle metabolism (Marden et al., 1999, 2001, 2008; Schilder and Marden, 2007). We recently reported that a reduction in a host plant (horsenettle; *Solanum carolinense*, Solanaceae) defense response due to inbreeding (Kariyat et al., 2012b, 2013a) altered the RA of *Tnt* spliceforms in the flight muscles of a specialist herbivore, tobacco hornworm (*Manduca sexta*, Sphingidae). Changes to the RA of *Tnt* correlated with increased flight muscle metabolic output (Portman et al., 2015a,b) and provided an insect quantitative molecular phenotype that responds to genetically derived variation in host plants.

The effects of plant defenses on a single species of insect have been tested in many plant–insect systems (Scriber, 1981; Felton et al., 1989; Johnson et al., 1989; Bolter and Jongsma, 1995; Charity et al., 1999; Cloutier et al., 2000; Haviola et al., 2007; Saha et al., 2012; Thaler et al., 2014; Portman et al., 2015b). However, plants in natural ecosystems are most often attacked by multiple insect herbivores (Iwao and Rausher, 1997) and plant defenses and herbivore counter-defenses are co-evolving and species specific. Consequently, plant defenses will not have the same effects on different insect species (Mullin et al., 1997; Stotz et al., 2000; Nishida, 2002; Harris et al., 2003; Viswanathan et al., 2005; Gols et al., 2008). Individual insect species will likely exhibit distinct phenotypic modifications to key fitness traits (e.g. survival, body size, reproduction and dispersal ability) in response to a host plant's unique suit of defenses.

Few studies have investigated the performance of multiple insect herbivores in relation to variation in a host plant's defense response (Stotz et al., 2000; Gols et al., 2008), and even fewer studies have documented host plant effects on specific insect organ systems and/or physiological processes (Thaler et al., 2014; Portman et al., 2015a). To test this, we examined two herbivores that are similar in many ways (e.g. closely related, same feeding guild, similar body size and development time) but differ in their preferred host plants. Both fall armyworm [*Spodoptera frugiperda* (J. E. Smith)] and southern armyworm (*Spodoptera eridania* Stoll) are noctuid moths that can successfully feed and develop on tomato, but fall armyworm (FAW) larvae are mostly associated with grasses (monocots), while southern armyworm (SAW) larvae prefer leafy dicots (Capinera, 2001). To compare phenotypic changes to flight-related fitness traits of both species, we manipulated host plant quality using genetically derived variation in the tomato plant's JA-induced defense pathway to elicit changes to adult insect body development, flight muscle metabolism and *Tnt* gene expression. This is the first study to investigate how genetic variation in a host plant affects flight muscle gene expression and flight muscle physiology in two species of herbivorous insects.

MATERIALS AND METHODS

Plants and insects

Tomato (*Solanum lycopersicum*, Solanaceae), one of the host plants of FAW and SAW (Capinera, 2001), are unpalatable to many herbivores because they produce a battery of antinutritive proteins such as PIs and polyphenol oxidases (Felton, 2005), as well as secondary metabolites such as glycoalkaloids, phenolics and terpenes (Harborne, 1986; Schilmiller and Howe, 2005). Despite these defenses, both species are reported to feed and successfully complete their life cycle on tomato leaves (Capinera, 2001). For this study, tomato seeds from Castlemart (control) and *def1* (JA-deficient) genotypes were planted in 500 ml pots filled with a peat-based potting soil (Pro-Mix, Premier Horticulture Inc., Quakertown, PA, USA) and maintained in a greenhouse (16 h:8 h light:dark; 25°C:22°C day:night; 65% relative humidity, RH). After planting, pots with seeds were covered with a clear plastic Solo® cup (Dart Container, Inc., Mason, WI, USA) creating small rearing cages. To increase ventilation and prevent condensation build-up, the bottoms of the cups were cut away and covered with tulle. Seeds were watered on alternate days and allowed to sprout in the confines of the cages. When plants reached a height of approximately 15 cm (~2 weeks), a subset of *def1* seedlings were removed from the

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greenhouse and sprayed with a 0.8 mmol l^{-1} solution of methyl-jasmonate in 10% ethanol to induce the JA pathway (Farmer and Ryan, 1990). After 24 h, plants sprayed with methyl-jasmonate (MeJA induced) were returned to the greenhouse.

Eggs of both SAW and FAW were purchased from Benzon Research, Inc. (Carlisle, PA, USA). Eggs were hatched in Petri dishes (90 mm×15 mm; Fisher Scientific, Pittsburgh, PA, USA) on moist Whatman® filter paper in a growth chamber (16 h:8 h light: dark, 25°C, 65% RH). To improve survival, neonate larvae were moved to a wheatgerm–casein-based artificial diet (BioServ, Inc., Frenchtown, NJ, USA) immediately after hatching. Larvae were individually assigned to one of three host plant treatments: control, *def1* mutants or MeJA-induced plants. Larvae were placed on host plants after molting to 2nd instars, and were subsequently allowed to grow, molt and pupate.

FAW larvae suffered high mortality with only 14.1% surviving to pupation. Hence, the sample size for FAW was far smaller than that for SAW. Pupae (SAW *N*=76, FAW *N*=27) were extracted from the soil, weighed, then placed on moist paper towels inside screened insect cages (71×57×66 cm L×W×H). Pupae were separated into cages according to host plant, and cages were housed in a growth chamber (14 h:10 h light:dark, 25°C, 65% RH). After eclosing, adults (SAW *N*=76, FAW *N*=27) were placed individually into small cylindrical insect cages and assigned individual tracking numbers. Cotton wicks soaked in a mixture of lemon-lime Gatorade®, sucrose (Gatorade+added sucrose: 0.54 mol l^{-1} sucrose), fructose (0.25 mol l^{-1}) and glucose (0.26 mol l^{-1}) were placed in each cage to provide nourishment to the moths. Measures of pupal mass and adult body mass were used to determine whether differences in larval growth translated into differences in body size of the adult stages (pupae, adult).

Adult flight metabolism

Adult moths (SAW *N*=76, FAW *N*=27) were flown in a 1 liter plastic jar, attached to a flow-through respirometry system, for 10 min. After a moth was introduced into the jar, 5–10 min of inactivity were required for the outflow CO_2 concentration to attain baseline metabolic output (for details, see Portman et al., 2015a). The plastic jar was attached to a Vortex Genie® (Fisher Scientific) mixing machine using elastic bands. Vibration created by the Vortex Genie stimulated the moths to fly continuously. Dry CO_2-free air was passed through the jar at an average rate of 4.7 l min^{-1}. During flight trials, the vortex–jar setup was kept in an incubator that ranged from 24 to 29°C in air temperature. Outlet air was subsampled and dried by passing through a magnesium perchlorate filter before flowing into a LI-COR 6252 gas analyzer (Lincoln, NE, USA), which measured CO_2 concentration. Custom Igor Pro® (WaveMetrics Inc. 2007) macros were used to convert LI-COR voltage data to fractional increases in CO_2 imparted by the insect. From these data, metabolic rate (ml CO_2 s^{-1}) and total metabolic output (ml CO_2) were calculated. Baseline metabolic rate was subtracted prior to calculating peak metabolic rate. We used a *Z*-transformation (Bartholomew et al., 1981) to estimate instantaneous metabolic rate, including the maximal rate. The area under the curve of CO_2 output was used to determine the total CO_2 emitted during 10 min flights.

Tnt isoform profiling

Immediately after their flight test, moths were weighed, flash-frozen in liquid nitrogen, and stored at −80°C to preserve their flight muscle tissue. Whole thoraxes were removed on dry ice and pulverized in Trizol® (Invitrogen Inc., Carlsbad, CA, USA) using a tissue homogenizer (25 Hz for 5 min; Qiagen Inc., Germantown, MD, USA). Insoluble material was removed by centrifugation and RNA was purified according to methods described in Marden et al. (2008); 0.5 µg of RNA was used for cDNA synthesis with Superscript II® (Invitrogen, Inc.) and oligo(dT) primers.

Fluorescently labeled primers for FAW *Tnt* (*Tnt*AltF 5-56FAM-CACCCGTGCGACATTAAATAAAC-3, *Tnt*AltR 5-GCGCCATT-CGTTGATGTATTC-3) corresponding to constitutively spliced regions on both sides of the 5′ alternatively spliced region (see Marden et al., 2008), successfully amplified this region from SAW (*N*=43) and FAW (*N*=19) cDNA. Capillary electrophoresis of the labeled *Tnt* fragments was performed on an ABI Hitachi 3730XL DNA Analyzer (Foster City, CA, USA) at the Pennsylvania State University Genomics Core facility. PCR products were diluted 1:50 in water before electrophoresis so that all isoform peak heights fell within the linear range (below 30,000 units) of the instrument detector.

Data analysis

Data reported for *Tnt* isoforms are based on RA calculated by dividing the height of individual isoform peaks (Fig. 1) by the sum of the heights for all isoform peaks detected for a particular insect (Marden et al., 2008). The RA of all isoforms was arcsine transformed to achieve normality. ANOVA was used to compare SAW and FAW *Tnt* isoform RA differences in adult moths; all *Tnt A* ANOVA models included host plant and sex as predictor variables. *Post hoc* comparisons (Hsu–Dunnett's test, α=0.05) of *Tnt A* from moths that developed on JA-deficient and MeJA-induced host plants versus control plants were carried out for significant host plant predictor variables. Student's *t*-tests (α=0.05) were used to compare *Tnt A*-related sex differences. An increase in the relative abundance of larger Tnt isoforms has been shown to correlate with FAW peak flight metabolic rate (Marden et al., 2008); therefore, ANOVA was used to compare SAW and FAW *Tnt A*/*F* ratios for moths that developed on JA-deficient and MeJA-induced host plants versus control plants. ANOVA models included pupal mass and host plant as predictor variables; *post hoc* comparisons (Hsu–Dunnett's test, α=0.05) tested differences in *Tnt A*/*F* ratios for significant host plant predictor variables.

To account for variation in flight metabolism due to differences in body size, we analyzed the residuals from regressions on body mass (mass-adjusted metabolic output). Many additional factors can also

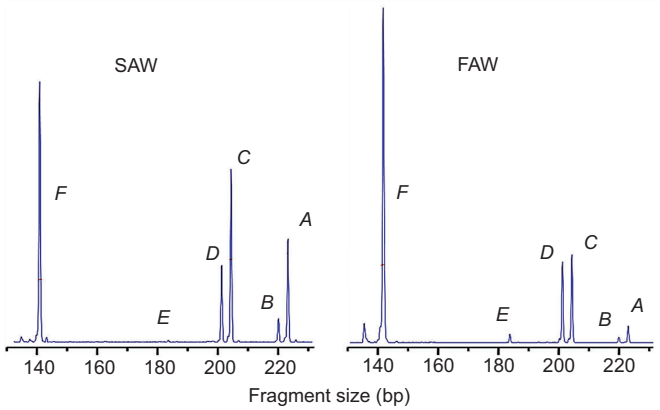

Fig. 1. Relative abundance of *troponin t* (*Tnt*) gene amplicon fragments isolated from flight muscles of southern armyworm (SAW) and fall armyworm (FAW). Amplicon fragment sizes were detected by capillary electrophoresis separation of PCR products produced by primers that flank the *Tnt* alternatively spliced region. Individual spliceforms are labeled *A*–*F*.

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affect an insect's flight metabolism, thus stepwise ANCOVA models were used to examine variation in SAW and FAW mass-adjusted flight metabolism (stopping rule: *P*-value threshold; direction: mixed). Variables originally included in the stepwise procedures were: block, host plant, sex, percentage thorax mass, RA of *Tnt* isoforms *A–F*, *Tnt A*/*F* ratio, and host plant×RA of *Tnt A–F* interactions. The stepwise procedures for SAW mass-adjusted flight metabolic rate and mass-adjusted total flight metabolic output resulted in ANCOVA models that included only adult host plant, sex, *Tnt A* and host plant×*Tnt A* interaction as significant predictors (α=0.05); FAW mass-adjusted flight metabolic rate and mass-adjusted flight metabolic total output ANCOVA models included host plant, *Tnt A* and host plant×*Tnt A* interaction as predictors, but only the interaction term (host plant×*Tnt A*) was significant at α=0.05. All analyses were carried out in JMP v.12 (SAS Institute, Cary, NC, USA).

RESULTS

Adult body mass and flight metabolism

SAW larvae survived best on MeJA-induced host plants (81.6%), but development time was shortest on JA-deficient plants (*P*<0.0001). In FAW, the opposite pattern was observed: larvae survived best on JA-deficient host plants (33.3%) but developed fastest on MeJA-induced plants (*P*<0.0001). The effect of host plant variation on adult body size was also different for each insect. FAW larvae that developed on MeJA-induced plants were 27.7% smaller (*P*=0.011) than adults that developed on JA-deficient or control plants (Fig. 2), while there were no statistically significant differences in SAW adult body mass for insects reared on the three host plant varieties. Adult body mass correlated with both SAW and FAW peak metabolic rate (R^2=0.42, *P*<0.0001; R^2=0.84, *P*<0.0001) and total flight metabolic output (R^2=0.40, *P*<0.0001; R^2=0.69, *P*<0.0001) during 10 min of forced flight. SAW mass-adjusted peak flight metabolic rate (ANCOVA R^2=0.35, *P*=0.001) and mass-adjusted total flight metabolic output (ANCOVA R^2=0.20, *P*=0.019) showed significant effects of sex, *Tnt A* RA and host plant×*Tnt A* RA interaction (Table 1). Male moths had a 28.5% higher peak metabolic rate (*P*<0.0001) and a 24.2% higher total metabolic output than females (*P*=0.002). FAW mass-adjusted peak flight metabolic rate (ANCOVA R^2=0.57, *P*=0.021) and mass-adjusted total flight metabolic output (ANCOVA R^2=0.76, *P*=0.002) showed only significant effects from the host plant×*Tnt A* RA interaction term (Table 1). Although host plant did not affect adult flight metabolism directly, the significant interaction suggests that the host plant may have indirect effects on flight muscle metabolism by altering the relationship between protein structure (e.g. *Tnt A*) and flight muscle metabolic output.

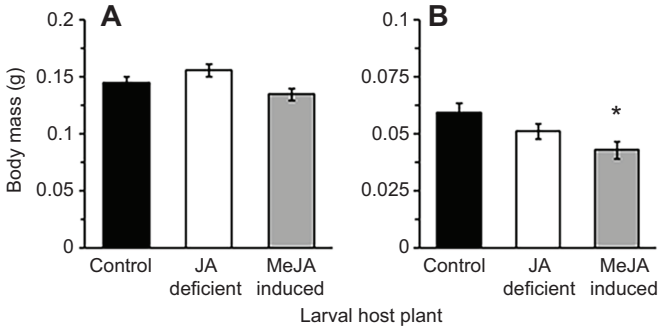

Fig. 2. Effect of larval host plant on adult body mass. Bars represent mean±s.e.m. adult body mass of SAW (A) and FAW (B) obtained from larvae reared on wild-type Castlemart tomato plants (control; *N*=20 SAW, *N*=7 FAW), jasmonic acid (JA)-deficient plants (*N*=27, 11) and methyl-jasmonate (MeJA)-induced plants (*N*=28, 9). The asterisk represents a significant difference in treatment means compared with control plants (Hsu–Dunnett test, α=0.05).

Table 1. ANCOVA for southern armyworm (SAW) and fall armyworm (FAW) body mass-adjusted peak metabolic rate

Source	d.f.	SS	MS	*F*-ratio	Prob >*F*	R^2
SAW						
Model	6	9.80	1.63	5.09	**0.001**	0.35
Error	39	12.51	0.32			
Total	45	22.31				
Host plant	2	0.41		0.64	0.531	
Sex	1	6.14		19.14	**<0.0001**	
Arcsin *Tnt A* RA	1	1.64		5.12	**0.029**	
Host plant×arcsin *Tnt A* RA	2	3.28		5.11	**0.011**	
FAW						
Model	5	1.63	0.33	4.80	**0.017**	0.56
Error	10	0.68	0.07			
Total	15	2.31				
Host plant	2	0.07		0.48	0.631	
Arcsin *Tnt A* RA	1	0.06		0.88	0.370	
Host plant×arcsin *Tnt A* RA	2	0.96		7.09	**0.012**	

Residuals are from regression on body mass. Effects of host plant, sex (for SAW), relative abundance (RA) of troponin t isoform A (*Tnt A*) and the interaction host plant×*Tnt A* RA are shown; significant factors are in bold. SS, sum of squares; MS, mean squares.

Tnt expression in adult flight muscles

Flight metabolism in armyworm moths has been shown to positively correlate with higher RA of longer *Tnt* spliceforms (i.e. *Tnt A*, *Tnt B* and *Tnt C*) and negatively correlate with the RA of the shortest isoform, *Tnt F* (Marden et al., 2008). To further examine the effects of host plant on *Tnt* isoform expression and flight and muscle physiology, we quantified the RA of alternatively spliced transcripts of *Tnt* for both species. Consistent with previous work on this gene in insects (Fitzhugh and Marden, 1997; Marden et al., 2008; Portman et al., 2015a), we found six *Tnt* fragments corresponding to the known spliceforms *A–F*. SAW expressed isoforms *A*, *C* and *F* in the highest RA; FAW expressed isoforms *C*, *D* and *F* in the highest RA (Fig. 1).

A greater RA of *Tnt A* in SAW flight muscles correlated with an increase in mass-adjusted peak flight metabolic rate (*P*=0.029) and total flight metabolic output (*P*=0.014). RA of *Tnt A* in SAW (ANOVA R^2=0.35, *P*<0.0001) also showed significant effects from host plant (*P*=0.006) and sex (*P*<0.0001; Table 2). On average, SAW adults reared on MeJA-induced host plants had 19.4% higher RA of *Tnt A* in their flight muscles than moths reared on control plants (*P*=0.003; Fig. 3); females had 23.7% higher RA of *Tnt A* in their flight muscles than males (*P*<0.0001), which could be the result of females requiring greater flight muscle force output to compensate for changes in body mass due to egg load. Regression analysis showed no relationship between *Tnt A* and mass-adjusted flight metabolics in SAW (Fig. S1).

RA of *Tnt A* from FAW flight muscle (ANOVA R^2=0.62, *P*=0.002) showed significant effects of host plant (*P*=0.001) and pupae mass (*P*=0.002; Table 2). The flight muscles of FAW adults reared on MeJA-induced plants had a 31.3% decline in *Tnt A* RA compared with moths reared on control plants (*P*=0.003; Fig. 1B), which is the opposite effect to that occurring in SAW adults. Overall, RA of *Tnt A* in FAW decreased as pupae mass increased (Fig. 4), which is contrary to what occurs in this species when reared on artificial diet and when body weight is manipulated by attaching

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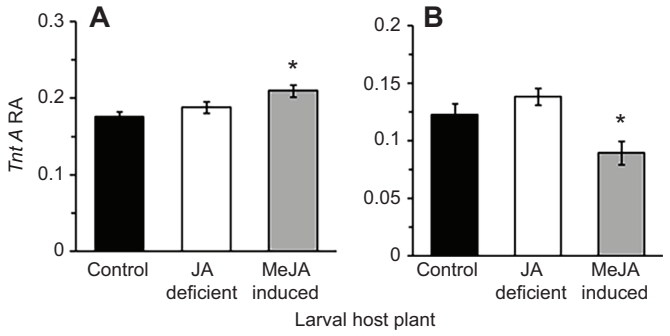

Fig. 3. Effect of larval host plant on the relative abundance (RA) of *Tnt A* in adult flight muscles. Bars represent mean±s.e.m. RA of *Tnt* A isolated from SAW (A) and FAW (B) adult moths that were reared on control (*N*=15 SAW, *N*=5 FAW), JA-deficient (*N*=13, 9) and MeJA-induced plants (*N*=15, 5). Asterisks above bars indicate significant differences in treatment means compared with control plants (Hsu–Dunnett test, α=0.05).

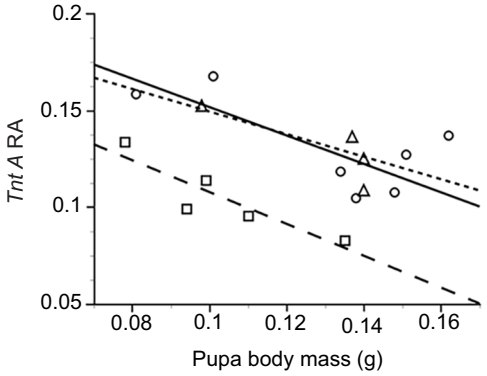

Fig. 4. Scatter plot with regression lines showing *Tnt A* RA in adult flight muscles in relation to pupa body mass. Data are shown for moths reared on control (open triangles and solid line), JA-deficient (open circles and dotted line) and MeJvnA-induced tomato plants (open squares and dashed line).

a weight load (Marden et al., 2008). Regression lines also highlight the effects of the host plant (MeJA induced: R^2=0.73, P=0.041; control: R^2=0.51, P=0.181; JA deficient: R^2=0.39, P=0.08). Flight muscle peak metabolic rate decreased linearly with higher RA of *Tnt A* (R^2=0.70, P=0.012) for FAW reared on JA-deficient host plants. Although not significant (R^2=0.44, P=0.13), the opposite trend was observed for FAW reared on MeJA-induced host plants (Fig. S1), which indicates that the host plants altered the effect of *Tnt A* on flight muscle metabolic output. These results show that gene expression patterns of a flight muscle protein in FAW responded to differences in body mass and host plants – possibly as a result of variation in the plants' nutritional quality.

DISCUSSION

In natural environments plant–insect associations are generally more complex than a single herbivore species feeding on one host plant; host plants are often attacked by multiple herbivores (Iwao and Rausher, 1997; Burdon and Thrall, 1999). Plants respond to these challenges by employing various defenses against herbivores (e.g. increased production of PIs and toxic secondary metabolites), which reduces the nutritional quality of the plants' tissues (Howe et al., 1996; Gols et al., 2008; Ali and Agrawal, 2012; Portman et al., 2015b). Insects, in turn, respond by altering gene expression of nutritionally dependent biochemical pathways, resulting in phenotypic changes to body structures, organ systems and/or physiological processes (Leclaire and Brandl, 1994; Agrawal, 2001; Awmack and Leather, 2002; Portman et al., 2015a; Mullin et al., 1997; Stotz et al., 2000; Nishida, 2002; Harris et al., 2003; Viswanathan et al., 2005; Gols et al., 2008). Each herbivore species has likely evolved unique mechanisms to cope with their host plants' defenses and therefore will exhibit species-specific phenotypic responses.

Table 2. ANOVA for RA of *Tnt A* isolated from SAW and FAW flight muscle

Source	d.f.	SS	MS	*F*-ratio	Prob>*F*	R^2
SAW						
Model	3	0.021	0.007	9.18	**<0.0001**	0.35
Error	42	0.032	0.001			
Total	45	0.053				
Host plant	2	0.009		5.71	**0.006**	
Sex	1	0.016		21.0	**<0.0001**	
FAW						
Model	3	0.006	0.002	9.30	**0.002**	0.62
Error	12	0.003	0.000			
Total	15	0.008				
Host plant	2	0.005		11.97	**0.001**	
Pupal mass	1	0.004		16.86	**0.002**	

Effects of host plant and sex (for SAW) and pupal mass (for FAW) are shown; significant factors are in bold.

We found that variation in the tomato plant defense response (defenseless versus control or induced versus defenseless) produced species-specific differences in the expression of a gene that affects flight muscle performance. SAW adults reared on MeJA-induced host plants had a higher RA of *Tnt A* in their flight muscles compared with adults reared on JA-deficient and control plants. In contrast, FAW adults reared on MeJA-induced host plants were smaller in size and had a lower RA of *Tnt A* in their flight muscles compared with adults reared on JA-deficient and control plants. Interestingly, FAW raised on MeJA-induced plants also showed an inverse relationship between body size and the RA of *Tnt A* (Fig. 4), a surprising response given that this species was previously reported to have a positive correlation with total body mass (mass+added weight) and the RA of *Tnt A* (Marden et al., 2008), but may indicate that this species responds differently to host plant-related metabolic stress versus starvation on artificial diet.

Previous studies have shown that environmental factors can bring about phenotypic changes to insect flight muscles (Zera et al., 1998; Schilder and Marden, 2007; Marden et al., 2008; Portman et al., 2015a). While both SAW and FAW can feed and successfully develop on tomato, FAW is a common turf pest, and its host range includes mostly grasses (monocots), whereas SAW typically feeds on leafy dicots (Sparks, 1979; Capinera, 2001), indicating some degree of specialization between these two species. The survival of SAW larvae on all three plant types was significantly higher than that of FAW; SAW had an average survival of 70.9%, compared with only 16.8% for FAW. SAW adults that were reared on artificial diet were only 17.1% larger on average than adults reared on tomato plants, while diet-reared FAW adults were 113.6% larger than their plant-reared counterparts (data not shown). The higher percentage survival of SAW compared with FAW, and >2× increase in FAW body mass when reared on artificial diet, implies that FAW was less adapted than SAW to feed on tomato and cope with its defensive secondary metabolites. The lack of nutrition and/or toxic effects from the secondary metabolites, resulting in lower protein and energy available may not have allowed FAW to build a large body and powerful flight muscles. This is consistent with other studies that link nutrition deficits to changes in flight muscle protein

composition. Marden et al. (2008) reported that FAW adults that developed from starved larvae were smaller and had higher RA of the shortest *Tnt* isoform (*Tnt F*); and, gregarine parasite infection in a libellulid dragonfly was associated with a 10-fold decrease in the abundance of a ∼155 kDa fragment of myosin heavy chain in their flight muscles (Schilder and Marden, 2007).

In this study, SAW adults that were reared on MeJA-induced host plants expressed a higher RA of *Tnt* A in their flight muscles, which was opposite to the reaction of FAW. This suggests that SAW that fed on MeJA-induced plants developed more powerful flight muscles even though they ingested higher levels of secondary metabolites compared with cohorts that fed on JA-deficient host plants. Indeed, SAW adults that developed on the wild-type Castlemart and MeJA-induced host plants had higher body mass-adjusted peak flight metabolic rates than adults that developed on JA-deficient host plants (data not shown). Other studies also found that some insect species can shift their development strategy to become better dispersers under conditions of nutritional stress. For example, the plant hopper *Prokelisia marginata* (Delphacidae) was shown to invest more resources into the development of wings and flight muscles (i.e. dispersal capability) in response to low-nitrogen host plants, while another plant hopper species, *Prokelisia dolus*, invested in larger esophageal muscles (i.e. compensatory feeding; Huberty and Denno, 2006). Female tropical butterflies (*Bicyclus anynana*: Nyphalidae) that were starved as larvae or adults had higher thorax/total body mass ratios (i.e. increased flight muscle mass), and longer bouts of forced flight, compared with well-fed females (Saastamoinen et al., 2010), indicating that starvation led to greater flight muscle development and better dispersal capability.

Flight muscles are metabolically costly to build and maintain (Zera et al., 1998) and neuromotor systems are affected by plant toxins (Huang et al., 2011); thus, it is not surprising that plant-produced secondary metabolites can impact flight muscle development and performance. We recently reported (Portman et al., 2015a) that *Manduca sexta* (Sphingidae) adults reared on inbred horsenettle (*Solanum carolinense*: Solanaceae) grew larger, developed faster, and had higher peak flight metabolic output when reared on inbred plants with compromised defenses (Kariyat et al., 2012a,b, 2013a), compared with moths reared on outbred (wild-type) host plants. Changes in the insects' flight metabolism were also associated with changes in the RA of a particular *Tnt* isoform in their flight muscles. Here, we show that variation in a host plant's induced defense response also produced changes in *Tnt* isoform expression in two armyworm species, and changes to *Tnt* correlated with differences in their flight metabolic output (Table 1). This is consistent with previous studies showing that better larval nutrition produced a lower RA of the short *Tnt* isoforms and higher flight metabolic rates in FAW (Marden et al., 2008), and a higher RA of short *Tnt* isoforms is associated with mechanically weaker flight muscles in dragonflies (Marden et al., 2001). Changes in the alternative splicing of *Tnt* reported here and in other studies (Marden et al., 2001, 2008; Portman et al., 2015a) infers a general but species-specific homeostatic mechanism, where *Tnt* splicing responds to variation in body weight and/or nutritional quality. Although we did not find direct host plant effects on mass-adjusted flight metabolism, host plants had indirect effects on flight metabolic performance in both species, via changes to the RA of *Tnt A*. Our results for both SAW and FAW support a broader pattern of diet affecting alternative splicing, protein structure and performance of insect flight muscle.

Flight-related traits, such as flight muscle metabolism, can impact the dispersal ability and spatial dynamics of particular species, especially insects that are not well adapted to feeding on specific host plants. Recent studies of the Glanville fritillary butterfly (*Melitaea cinxia*) show that relatively small changes in the flight muscle metabolic performance (15–20%) strongly correlated with dispersal distance in the field (Niitepõld et al., 2009) and colonization rate of new habitat patches (Haag et al., 2005). Our results also suggest that phenotypic changes in species that are resistant to a host plant's defenses may increase their ability to disperse longer distances to find new patches of host plants. Greater dispersal capability might increase herbivore-associated selection pressure on host plant populations, which is likely to also negatively impact other herbivore species that rely on the host plant (Kokko and López-Sepulcre, 2006).

To our knowledge, this is the first study to compare how two insect herbivores respond to changes in a host plant's induced defense, and document distinct phenotypic effects to their flight muscle gene expression and flight muscle physiology. Our results show that different insect species cope with variation in plant secondary metabolite production by employing unique adaptive strategies, via phenotypic modification to important fitness traits, such as flight muscle development. Identifying specific insect genes, biochemical pathways and/or fitness traits that are actively responding to host plant selection pressure provides tools and insights that are useful to better understand the mechanisms of plant–insect co-evolution.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: S.L.P.; Methodology: S.L.P.; Software: J.H.M.; Validation: R.R.K., J.H.M.; Formal analysis: S.L.P.; Investigation: S.L.P.; Resources: G.W.F., R.R.K., J.H.M.; Data curation: S.L.P.; Writing - original draft: S.L.P.; Writing - review & editing: S.L.P., G.W.F., R.R.K., J.H.M.; Supervision: J.H.M.; Project administration: J.H.M.; Funding acquisition: J.H.M.

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Data availability
Data are available from the Dryad digital repository (Portman 2020): dryad.wh70rxwkc

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