Feeding preference of the Oak moth (*Phargandia californica*) and the possibility of tannin sequestration on a diet of Costal Live Oak (*Quercus agrifolia*)

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Abstract   The chemical ecology of the Oak moth's (*Phargandia californica*) (Lepidoptera: Notodontidae) relationship with its host plant the Coast Live Oak (*Quercus agrifolia*) has not been well studied. The Coast Live Oak contains the secondary plant metabolite tannins in its leaves. This study will determine if the presence of tannins in *Q.agrifolia* act as feeding stimulants to the larvae of *P.californica*. This study will also investigate if *P. californica* larvae sequester phenols and condensed from their diet of Coast Live Oak leaves. A four feeding choice experiment based on a tannin extract and two chemical analyses testing for total phenolics and condensed tannins were conducted. The results of the bioassay showed that tannins were not necessary to stimulate *P. californica* to feed. The results of that phenolic assay showed that the larval stage contained a suggestively significant amount of phenols compared to the other life stages, while the condensed tannins assay showed no significant variation between the life stages. This study confirmed the presence of phenols and condensed tannins in the tissue of *P. californica*. Further investigation is needed to fully understand the role that phenolics and condensed tannins play in the life cycle of *P. californica*.

Introduction

Chemical ecology is a field of research which investigates and explains the complex chemical interactions between plants and animals. Much of what we know about chemical interactions between species has come from plant-insect interactions (Dethier 1954 Eherlich and Raven 1964 Feeny 1976). These relationships between herbivores and plants are more then arbitrary interactions; they are often highly specialized and multifaceted. A prime example of insect plant interactions is pollination. During pollination, for example, the Hawk moth (*Hyles lineata* (Fabricius 1775)) is attracted to the Scarlet Gila (*Ipomosis aggregata* (Linneaus 1758)) which has changed its petal color, decreasing anthocyanin presence in the petal, in time to attract *H. lineata* from it southern migration *(*Paige and Whitham 1985). Another interaction that exists between plant and insects is plant defense. A defensive mechanism exercised by the Elm (*Ulmus Americana (*Linnaeus 1759)), is in releasing a volatile chemical response through its leaves that attracts the parasitoid of Elm leaf beetle (*Xantthogaleruca luteola* (Muller 1864)) after it has laid its eggs on the Elm leaf (Schoonhoven *et al*. 2005). Many plant-insect interactions involve specialized plant produced chemicals, specifically secondary plant metabolites (Rosenthal and Berenbaum 1991). These interactions are not always beneficial to the herbivore, chemical feeding deterrents such as alkaloids in the Potato (*Solanum tuberosum* (Linneaus 1753)) can reduce the herbivory of the Colorado Beetle (*Leptinotarsa decemlineata* (Say 1824)) (Harbourne 1993). Yet, a metabolite may deter some insects while others are not affected by the presence of the chemical. An example is of the Monarch butterfly (*Danaus plexippus* (Linneaus 1758)) on its host plant the Milkweed (*Asclepias syriaca* (Chamomile 1753)) (Bower 1970). The cardioglycosides present in Milkweed deter most herbivores, yet *D. plexippus* larvae are unaffected and in fact sequester the glycosides for defense against predators.

The interaction between the Coast Live Oak (*Quercus agrifolia* (Bickford, 1979)) and the Oak Moth (*Phryganidia californica* (Packard 1864)) is another example of how secondary plant metabolites, specifically tannins, play a role between insects and their host plant. There are 14 documented species of insects that are feeding specialists on *Q. agrifolia* but little research has been done on the Oak moth (Conner *et al*. 2003). Even though the leaves of *Q. agrifolia* contain tannins, which are considered a feeding deterrent, the Oak moth still selectively feeds on this species. The focus of this study will be on the role of tannins in the relationship between the Oak moth and its host the Coast Live Oak.

Tannins are plant polyphenols, a class of secondary metabolites which are produced by woody trees (Mauffette 1983 Harborne 1993 Bergvall 2007). Tannins are present in two forms, condensed or hydrolysable (Gonclaves, 2007). Tannins are considered a feeding deterrent, exemplified by the Winter Oak moth study done by Feeny (1970). The Winter oak moth (*Operophtera brumata* (Linnaeus, 1759)) typically fed on the English Oak (Q*uercus robur* (Bechest 1832)) during the spring and summer months but ceased feeding in late summer due to the increase in leaf tannin content. Once ingested in the gut tannins bind with the peptides of digestive enzymes prohibiting enzymatic activity (Gonclaves 2007). Tannins will bind with digestive tissue and cause lesions if the herbivore is not adapted to tannins in its diet (Harborne 1993). Oppositely, some plant chemicals act as feeding stimulants, called phagostimulants (Joop *et al*. 2002 Kim *et al.* 2007 Reifenrath *et al.* 2008). Tannins have not been shown to act as feeding stimulants but they may, due to their presence in *Q. agrifolia* leaves and *P. californica's* specialization on the Coast Live Oak.

The Oak larvae have overcome the toxic effects of tannins in their host plant and are not deterred from consuming *Q. agrifolia* leaves (Mauffette 1989). The Oak larvae may have adapted to tannins through the presence of surfactants in the gut or an alkaline gut pH (Martin *et al.* 1984). Once the Oak larva has ingested the tannin containing leaf and begun to digest it the insect must sequester, detoxify or allow the tannins to pass through the gut unmetabolized.

Other Lepidoptera insects incorporate chemicals from their host plant and use them for their own defensive purpose. For example, the Buckeye butterfly (*Junonia cocenia* (Hübner 1981)) incorporates iridoid glycosides into its tissues from a diet of Plantain (*Plantago lanceolata* (Durande 1782)) and uses them as a defensive mechanism against predators (Bowers 1992). The possibility of sequestration seems most promising due to the Oak moth's coloration. The Oak moth larvae have bright yellow bands down their back and underbelly (personal observation). This coloration may suggest the insect is either aposematic or has developed a mimicry defense. Aposematism advertises the undesirability to potential predators based on chemical, olfactory or physical defenses (Rothschild 1979). A mimicry defense is when an animal has no means of defenses against predators but imitates another animal which does, such as how the Viceroy (*Limenitis archippus* (Linneaus 1758)) butterfly imitates the Monarch butterfly (*D. plexippus*) (Ritland *et al.* 2000). When an insect sequesters a toxin as a feeding deterrent it becomes unpalatable due to the bitter taste the predator experiences upon attack

(Kobue *et al.* 2000). The Oak larvae have not been studied for any potential secondary chemical protection against predators. This study will explore which method the Oak moth uses to deal with the tannins in its diet through sequestration, detoxification or allowing them to pass through intact.

   P. californica and *Q. agrifolia* are both endemic to California and have overlapping coastal home ranges from San Diego to Oregon (Monahan 2006). The Oak moth is monophagus on *Q. agrifloia* with an average of 2-3 generations per year (Mauffette 1989). *P. californica* experiences population outbreaks every 4-7 years, lasting 2-3 years. In these years *P. californica* can defoliate a mature *Q. agrifolia* tree (Horn 1974). The larvae are often easy to spot when feeding, sitting on the tops or fringes of leaves and on open branches (personal observation). Predators of the larvae are wasps, parasitic wasps (Bernays 1988) and birds (Weston 1947). Past research on the Oak moth has focused on parasitism and feeding patterns (Feeny 1970 Bernays 1988). Research done by Bernays (1988) on predation by the Yellow paper Wasp (*Mischocyttarus flavitarsus* (Jakobson, 1978)) found mixed results of the wasps' response to *P. californica*. The researchers paired the Oak larvae with another larva and presented the two to *M. flavitarsus*. The wasp either ignored the Oak larvae, or if the wasp did attack the larvae, it left the larvae's body behind, suggesting some undesirability on the part of the Oak larvae.

   To uncover a possible mechanism by which the Oak moth is able to selectively forage on the tannin-rich leaves of *Q. agrifolia*, I will ask the following questions. My first question is: do the larvae prefer a diet containing tannins? A feeding experiment will be conducted to determine if the Oak larvae have a preference for a diet containing tannins. I hypothesize that the larvae will prefer a diet with tannins to a diet that contains no tannins. Since tannins are abundant in their natural diet, they may act as a feeding stimulant to the larvae. My second research question is: does *P. california* sequester tannins? If so, does the presence of tannins change during the insect's life cycle? I plan to collect *P. californica* as larvae and rear them through the adult stage. These samples will allow me to determine if *P. californica* sequester tannins during its development. I hypothesize that phenols and condensed tannins will be present in the early stages. The larval stage should establish a baseline concentration of phenols and condensed tannins, but the pupal concentration of condensed tannins and phenols will be greater than the larval stage and much greater than the adult stage. The larval stage should contain both metabolites because the larvae are actively feeding on *Q. agrifolia* leaves. The pupal stage may

have the highest concentration of phenols and condensed tannins because if the larvae do sequester tannins they would accumulate in the tissue from feeding on the Oak leaves. The phenols and condensed tannins in the pupal stage may be broken down into different compounds during metamorphosis so neither metabolite would be present in the adult stage. I expect the adult moths to have a very low tannin concentration, if any, because they no longer feed on Oak leaves. In order to test these hypotheses I will collect samples of Coastal Oak leaves from the trees which the larvae were collected to make a general extract for the feeding choice experiment. I will use a spectrometer to determine the presence of phenols and condensed tannins in *P. californica* in the larval, pupal and moth life stages.

Methods

   Data Collection In order to determine if *P. californica* sequesters tannins, live samples were collected in the field from sites in Marin and Santa Cruz County. Larvae were collected from two locations to better account for variation in the wild. The Marin location was along a residential street; the lane was shaded and lined with mature Coast Live Oak trees. The Santa Cruz site was located adjacent to the UCSC campus; the Coast Live Oaks were located in a shady thicket. Late instar larvae and Oak leaves were collected simultaneously in September and October 2007. We collected leaves that had been consumed, to feed the larvae after collection, and leaves that had not been consumed, for the feeding experiment. The leaves for the feeding experiment were frozen upon return to the lab in order to preserve the integrity of the sample (Kawakami *et al.* 2003). Although the Oak moth is widely distributed in California, it is not continually present in one location over time. Therefore, collection of larvae was opportunistic. Once the larvae were collected, they were placed in mason jars (Kerr, SandSpring, OK) with 2-3 other larvae along with fresh Coast Live Oak leaves from their collection site. The jars were fitted with cheese cloth to promote air circulation.

   Lab rearing To replicate the larvae diet prior to collection they were fed Coast Live Oak leaves from their respective collection sites. Once the collected leaves were completely consumed the larvae fed on leaves from a juvenile Coast Live Oak provided by the Berkeley Botanical Garden. Larval frass was periodically collected from the mason jars with a small brush. The frass was collected to account for one possible pathway of how *P. californica* deals with tannins. The frass was frozen in a conventional freezer (approximately -20º C) for later

analysis. Once the larvae pupated they were left to develop to the adult stage. Adult moths were given a cotton ball which was soaked in a generic sugar water mixture (5 grams of sugar/ cup of water). Any individuals that expired at any time were frozen as soon as possible in a conventional freezer (approximately -20º C) for chemical analysis. A total of 21 larvae, 23 pupae and 12 adults comprised the first generation. The larvae from the collected sample that survived to the adult stage were bred for their eggs. These offspring were reared to provide the specimens for the feeding choice experiment. The second generation of larvae was raised in Kerr mason jars fitted with cheese cloth. There were up to 3-4 larvae in a jar. The second generations' diet was controlled, they fed on an artificial Lepidoptera diet provided by Bioserve (Princetown, NJ, USA) which contained no tannins. The larvae were reared on the artificial diet until the feeding experiment began. The larvae were kept at room temperature, misted twice daily and exposed to 14 hours of light and 10 hours of darkness.

    Four Choice Feeding Assay We tested third instar larvae in a feeding experiment. The larvae were given a choice between four artificial diets. The base of the artificial diets was made according to the procedures in Harbourne (1988) with minor modifications (Table 1). A general tannin extract was added to three of the diets. To make the extract a standard method was used (Hagerman 1988). The extract was based on uneaten the leaves collected in September 2007. We choose leaves that had not been eaten because leaf chemistry changes once the tissue as been attacked (Bladwin and Schulz 1983) and this chemical change may have affected larval feeding preference. The leaves were cut with scissors and soaked in 100% methanol for 4-5days. The leaf fragments were removed and the remaining methanol was desiccated, leaving the general tannin extract. This extract was termed general because it is very likely that more than just tannins were extracted from the leaves. The concentration of extract in each diet (Table 2) started at .5% but did not exceed 2% of the total amount of diet. A concentration of 2% may have exceeded the presence of tannin presence in an Oak leaf (Harbourne 1988). These concentrations were on a weight basis. The fourth diet was the control and contained no extract. The prepared diets were cut into small cubes, their weight was recorded on a Wes 163 scale (Mettler-Toledo, Columbus, OH) and placed in to 4oz soufflé cups (Smart and Final, Los Angeles, CA). The diets were arranged as in Figure 1. Cups and their lids were punctured with a needle to allow for air circulation. Two larvae were placed into the center of the cup (N= 15) with the lid closed. Two larvae were placed in a single cup to ensure that at least some diet would be consumed. The

larvae were left for four days to feed and at the end of that period the final weight of each diet was recorded. A control jar with no larvae was under identical conditions, its final weight was recorded at the end of the experiment.

Table 1. Artificial Diet Recipe

Base Diet Ingredients	Amount
Wessons Salt Mix	100g
Wheat germ	200g
Sucrose	350g
Vitamin free casin	350g
Methyl p-hydroxybenzoate	15g
Vitamin Mix enriched with Choline	40mg/1L H20

Table 2. Extract concentration

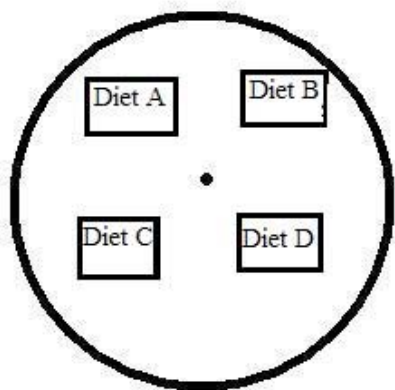| Diet	% Concentration
A	1.7
B	1.4
C	.5
D	0 , Control

Figure 1. Soufflé cup with four artificial diets.

Assay analyses A spectrometer, Spectra Mac, (Molecular Devices, Sunnyvale, CA) was used to determine the absorbance values of total phenols and condensed tannins from the collected samples. The first samples prepared were the frozen larvae. The larvae were sliced

down the ventral axis in order to remove the internal digestive system. Additionally, frozen pupae, adults and the larval frass collected from the first generations larvae were analyzed. The specimens of the three life stages and frass' weight were then recorded. The specimens were then cut into pieces, ground with a mortar and pestle and then soaked in methanol for 3-4 days. A single individual from each life stage was placed in its own methanol extract. A sample of the methanol extract was then used for analysis in the chemical assays. An acid butanol assay was run to determine the presence of condensed tannins. We followed the procedures described by Hartzfield *et al.* 2000 by combining an acidic methanol mixture with an iron reagent and 1ml of sample extract. This assay was standardized with unpurified Quebracho in methanol (.01g/1ml) provided by Ann Hagerman. Quebracho extract is a powdered form of tannic acid extract from the bark of the Quebracho tree. The samples were run at 550 nm. A modified Prussian blue assay by Gram (1992) was used to determine the presence of total phenols through a series of chemical reactions. We combined the 1ml of sample, reagents and a stabilizer. We standardized the assay with gallic acid (.019g/1ml methanol). The samples were run at 700nm. From these assays we determined the absorbance values of total phenols and condensed tannin presence in the tissue of the Oak moth tissue. From these absorbance values we calculated the concentration of phenols and condensed tannins.

Calculating Tannin concentration To calculate the concentrations in different life stages from the absorbance values attained we used the Beers Laws equation $A = \varepsilon bc$, the molar absorbtivity (ε), path length (b), concentrations (c) and absorbtivity (A). The molar absorptivity constant (ε) was different for the two assays; 43600 L mol^{-1}cm^{-1} and for the condensed tannin assay (Takos *et al.* 2005) and 10639 L mol^{-1}cm^{-1} for the phenols assay (Pellio *et al.* 2003). We rearranged the equation to solve for concentration (moles/gram), this value was then multiplied by the amount of methanol sample that was extracted for the assay, then this value was divided by the weight of the original sample yielding the (mol tannin/ gram tissue) concentration in original specimen.

Statistical Analyses

Feeding Experiment To determine the larvae feeding preference in the four choice experiment an ANOVA test was run, then followed by a Tukey test both run in JMP (SAS Institute, Cary, NC). To control for the water loss of the diets a dehydration factor was calculated by taking the difference of the control weights and dividing that by the original control weight.

This factor was then multiplied by the final weight of each of the artificial diets. These final values were used for the statistical analyses of the four choice feeding experiment.

Chemical Assays We compared the results within a chemical assay, it would have been inappropriate to compare any results between the two chemical analyses because of the inherent natural difference between condensed tannins and phenols. Two plots were made of condensed tannins and phenolic concentration to show the progression of chemical presence in the Oak moth system. An ANOVA was run for each assay to determine statistical significance between the three life stage and the larval frass.

Results

Four Choice Feeding Experiment: An ANOVA test was run between the four diets and no statistical difference was detected between the four diets $p=.6007$ (Figure 2). A post hoc Tukey test confirmed these results. There was a general trend, though, of increasing consumption as the amount of general extract increased in the diets.
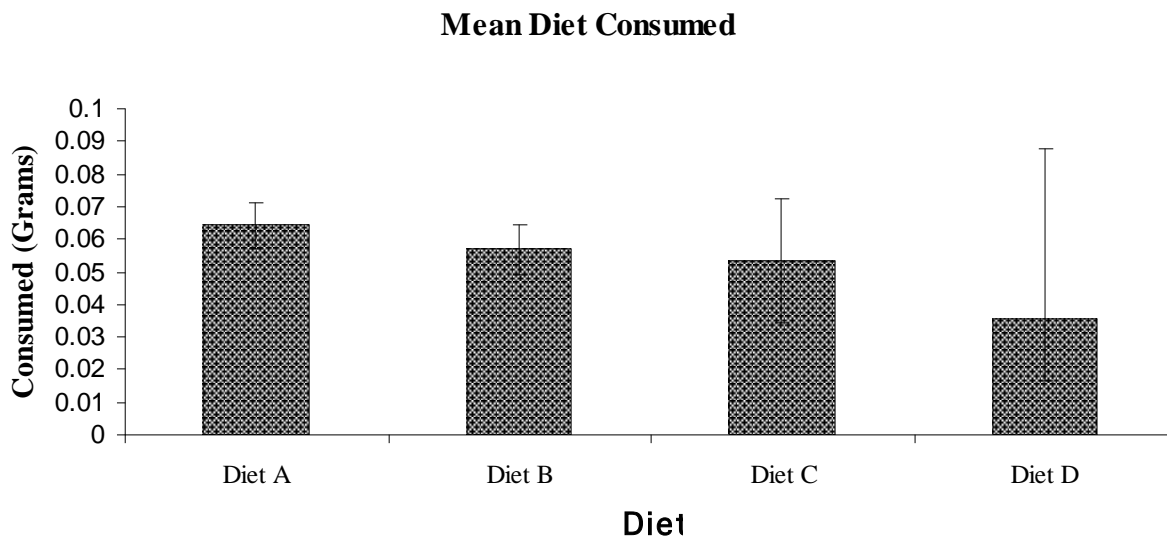
Mean Diet Consumed

Figure 2. Mean diet consumed for A (1.7% tannins), B (1.4% extract), C (.7% extract), D (0% extract). ($p=.6007$, df=3, F ratio=.6238)

Chemical Analyses

Phenolics An ANOVA was run on the calculated phenolic concentrations and yielded a possibly significant difference between the larva and pupa stages at p= .0627. A Post hoc Tukey test confirmed these results (Figure 3).

Condensed Tannins An ANOVA test was run on the concentrations of the different *P. californica* sources but yielded no statistically significant results p=.4893 (Figure 4).
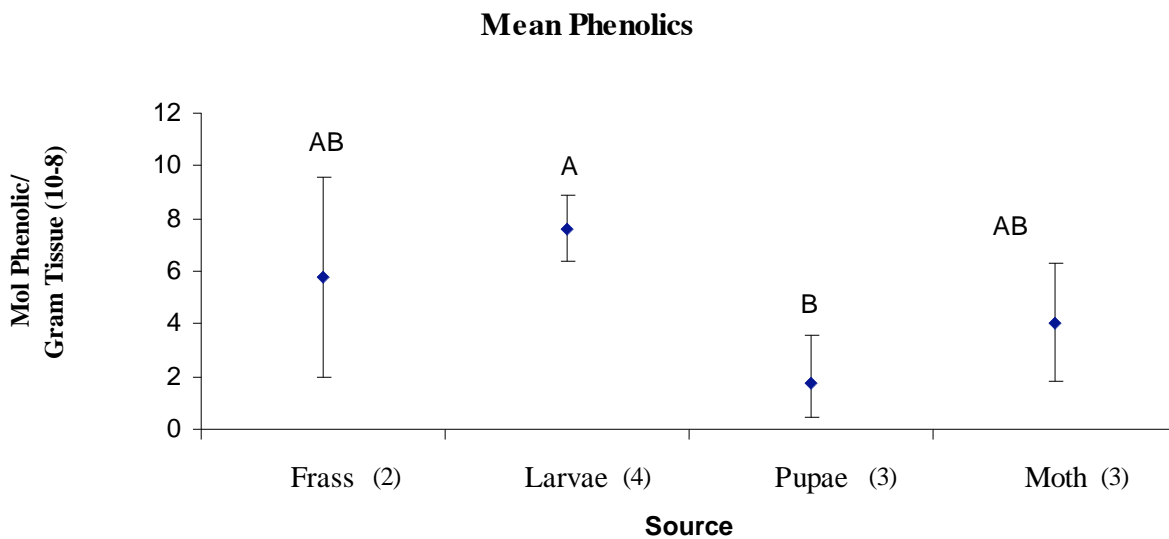
Mean Phenolics

Figure 3. Mean Phenolic Presence with standard error bars (p= .0627,df=3, F=3.67). Letters indicate suggestive statistical significance at p=.06 level. Values in Parenthesis indicate sample size.
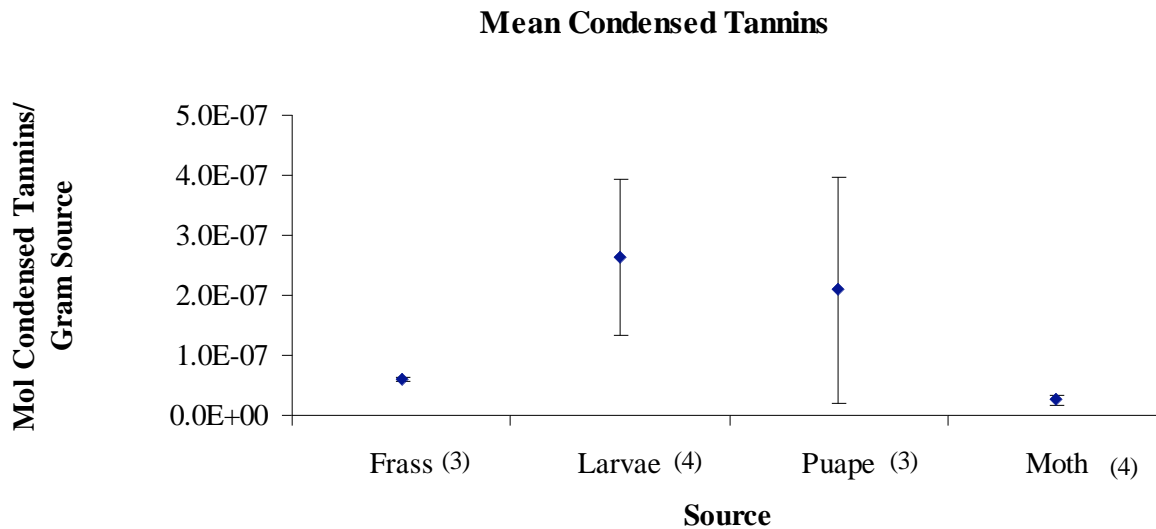
Mean Condensed Tannins

Figure 4. Mean Condensed Tannins Presence with standard error bars (p=.4893, df=3, F=.8681). Values in the parenthesizes are the sample size.

Discussion

Feeding experiment The feeding responses of the individual larvae varied among the trials. During the four days which the larvae were allowed to feed, some did not begin feeding right away, some starting on the second or third day. It is shown (Figure 2) that there was a general trend of the larvae consuming more of the diets as the amount of extract increased. Yet, there was no statistically significant difference between the four diets. This result does not support the hypothesis that *P. california* larvae would prefer a diet which contained the highest amount of tannins available and means that tannins are not necessarily needed to stimulate the *P. california* to feed.

Past studies that have conducted artificial diet experiments have reported varied feeding preference results. There is no general trend that can be applied from a single species to another, but comparisons can be made among closely related species. A study done by Walter and Gardiner (1965) found that larvae of the Large White butterfly (*Pieris brassica (*Linnaeus 1758)) preferred an artificial diet which had higher concentrations of glycosides, compounds found naturally in cabbage related plants upon which they feed, to those that had lesser amounts. Larval preference may also stem from a feeding stimulant found in its native diet (Thorsteinson 1960 Nishida 1995 Haung *et al.* 2003). This seems unlikely to be the case in this feeding experiment since there was no significant difference between the mean consumption of the three extract diets

and control diet. Since the larvae were raised on a diet that contained no tannins this may have influenced their feeding preference and contributed to the statistically insignificant results. A study done by Jermey *et al.* (1968) found that Tobacco hornworm (*Manduca sexta* (Linneaus 1763)) larvae had a strong preference for the diet on which they were raised. Once the larvae fed on a particular host plant they would prefer the same diet through the rest of their larval molts. Yet, the study was conducted on actual plant tissue, unlike this study which was based on artificial diets. Additional research is needed to be able to conclude if there are any possible feeding stimulants present in *Q. agrifolia* that act on *P. californica*.

Chemical Assays

Phenolics   The results of the chemical assay showed that *P. californica* contains phenols in the larval, pupal, adult stages and larval frass. There was a suggestive statistical difference between the larval and the pupal stages. The results confirm the hypothesis that *P. californica* contains phenols in its larval tissue but the phenolic variation was different than hypothesized. We hypothesized that the pupal concentration would be greater than the larval concentration because of the accumulation of phenols in the pupal tissue. The difference between the larval and pupal phenol concentrations may be because the larvae metabolize the phenols during digestion and excreted them in a different form that would not be detected by the phenolic assay. The larvae may also metabolize the phenolics for nutrients (Calacagno *et al*. 2004) or another unknown pathway. Since there was no significant variation between the larval tissue and larval frass it would seem that excreting the phenols would be an unlikely pathway. There was no significant variation between the pupal stage and the adult moth. The adults had no access to phenols in their sugar water diet, the phenols in the puapl stage seems to be the source of phenolic presence in the adults. Since phenolics are present in each life stage further research is needed to be able to understand what role phenolics play and if they contribute to a defensive mechanism in *P. californica.*

Condensed Tannins The results of the chemical assay show that *P. californica* does contain condensed tannins in its larval, pupal, adult stages and larval frass. The hypothesis that *P. californica* contains tannins in its larval tissue was confirmed but the hypothesis that condensed tannin presence would vary throughout the life stages was not confirmed. The statistical insignificance between the three stages suggests that once the Oak larvae have reached a certain concentration this concentration remains relatively constant into the later stages of development.

Since the condensed tannin presence in the larval frass did not vary from the larval tissue, the mechanism of passing the condensed tannins through the gut unmetabolized seems less likely. The concentrations between the larval stage and the pupal stage were statistically insignificant, so the mechanism of detoxifying the condensed tannins during metamorphosis seems less promising. There was no statistical difference between the pupal and adult stage. The adult Oak moth does not consume Coast Oak leaves so sources for tannin consumption have become limited except from nectar sources which may contain tannins (Harborne 1988). It is possible that the condensed tannins are sequestered through to the adult stage. The moths in this study were reared in an artificial setting and only fed a generic sugar water mixture. Thus, the pupal stage seems to be the main source for the condensed tannin presence. We originally speculated a possible defensive mechanism stemming from the sequestration of condensed tannins. If *P. californica* does sequester condensed tannins as chemical defense each life stage is equally defended. Further research is needed to be able to uncover a potential defense mechanism in *P. californica* based on the presence of condensed tannins in its tissue.

General Discussion There has been little research in tannin presence in insects, so the results of the condensed tannins and phenols analyses can be compared to past studies which have investigated other insects and other chemicals derived from their diet. A study done by Bowers (1992) reported that the larvae of *J. cocenia* sequestered acubin and catapol, which were derived from its host plant. Another study done by Nishida & Fukami (1989) found the larvae of the Chinese Windmill (*Atrophaneura alcinous* (Klug 1836)) and pupae contain each of the seven aristolochic acids found in its native food plant. The presence of aristolochic acids was shown to deter A. *alcinous* natural vertebrate predators.

The implications of the presence of condensed tannins and phenolics in the tissue of *P. californica* to its natural predators have not been researched but studies on other relationships have been done. Muller *et al.* (2006) reared gypsy moth (*Lymantria dispar* (Lineaus 1766)) larvae on diets with varying amounts of phenols and condensed tannins. The larvae were presented to Chickadees (*Poecile atricapilla* (Linneaus 1758)) and the bird consistently preferred caterpillars that had fed on foliage with lower concentrations of condensed tannins and phenolic glycosides, or caterpillars that fed on foliage with more condensed tannins but low levels of phenolic glycosides. Additionally, *P. atrcapilla* would sample the larvae and choose the above mentioned combinations against the additional choice of larvae that fed on foliage with high

levels of condensed tannins and high levels of phenols. If these results could be replicated in a feeding trial with Californian chaparral birds, the role of secondary plant metabolites as defensive chemical for *P. californica* could be better understood.

The limits of this study are very important when considering the outcome of this project. The results of the feeding experiment were determined in an artificial setting much different from the coastal shrub ecosystem. To better control for unidentified chemicals in *Q. agrifolia* leaves future researchers could run a more rigorous evaluation to screen the leaves for any known Lepidoptera feeding stimulants or other secondary plant metabolites. Additionally, the results of the chemical assays must be weighted carefully because the assays employed were not intended for insect tissue. Since the chemical analyses were designed for plant tissue is also possible that contamination from unidentifiable compounds in the insect tissue altered the results of the chemical assays. Sample sizes could also have been larger.

Future research that could stem from the results of this study could investigate a potential defensive mechanism based on the presence of phenols and condensed tannins in the tissue of *P. californica*. Furthermore, researcher could investigate tritropic interactions rooted in the presence of condensed tannins and phenols. Future research could focus on hydrolysable tannins, the other known form. Additionally, feeding experiments could be continued with different diets over longer periods of time. One of the problems of this study was keeping a population of *P. californica* alive and large enough to have a large sample size. Since *P. californica* hatches biannually it is important to collect as many samples as possible when they are available.

Based on the results of the feeding choice experiment, tannins do not necessarily have to be present to stimulate *P. californica* to feed. The chemical assays confirmed the presence of phenols and condensed tannins in each developmental stage of *P. californica*. The results of the phenolic assay showed that phenols were present in each stage but with a notable difference between the larval and pupal stage. The condensed tannins assay showed that the tannins were equally present in each developmental stage. Overall, this study may inform the understanding of how *P. californica* deals with the tannins in its diet and broaden the knowledge of insect tannin interactions in chemical ecology.

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