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Nutritional Ecology of a Generalist Herbivore *Vanessa cardui* Linnaeus (Lepidoptera: Nymphalidae) on Variable Larval and Adult Diets

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Nutritional Ecology of a Generalist Herbivore *Vanessa cardui* Linnaeus (Lepidoptera: Nymphalidae) on Variable Larval and Adult Diets.

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

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in

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by

Dustin Robert VanOverbeke

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Dissertation Committee:
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DEDICATION

To my lovely wife Jessica C. VanOverbeke for your unwavering love and support.

To my beautiful twins Ansel and Emerson.

You bring more joy to me than you will ever know.

ABSTRACT OF THE DISSERTATION

Nutritional Ecology of a Generalist Herbivore *Vanessa cardui* Linnaeus (Lepidoptera: Nymphalidae) on Variable Larval and Adult Diets.

by

Dustin Robert VanOverbeke

Doctor of Philosophy, Graduate Program in Entomology
University of California, Riverside, December 2011
Dr. Richard A. Redak, Chairperson

This dissertation research examined whether generalist feeding *Vanessa cardui* larvae display dietary self-selection, what rules of compromise are followed when larvae are constrained to suboptimal diet, how larval and adult nutrition affect butterfly fitness, and whether adults are able to compensate for variations in larval nutrition with differential adult foraging. First, I examined dietary self-selection and rules of compromise for protein and digestible carbohydrate intake by 5th instar *V. cardui* larvae. Larvae regulated nutrient intake to a near equal-ratio protein:carbohydrate in choice trials, consistent with results found for other Lepidoptera. Larvae on no-choice diets had reduced fitness on protein-biased or carbohydrate-biased larval diets relative to equal-ratio, and the rule of compromise exhibited by *V. cardui* is consistent with that expected for a generalist herbivore.

Second, I determined whether larval nutrition affects adult foraging behaviors. Females reared on carbohydrate-biased diet were more likely to probe towards nectar mimic containing sugars and amino acids than towards mimic containing sugars alone. Butterflies reared on protein-biased diet consumed more nectar than butterflies reared on carbohydrate-biased diet. Butterflies, however, did not exhibit preference between sugar and sugar plus amino acids solutions, regardless of larval nutrition.

Third, I examined the relative importance of larval and adult dietary nutrient intake on *V. cardui* fitness. Adult nutrition played a greater role in realized fitness than larval nutrition, but was mediated by larval dietary history. The most important factor influencing female fitness was adult carbohydrate consumption. Nectar-derived amino acids did not appear to increase fitness, but appeared to negatively affect total egg production when butterflies were reared on equal-ratio larval diet.

Lastly, I studied whether *V. cardui* exhibit mate choice based on the levels of proteins/amino acids and carbohydrates on which potential mates fed. Mate choice was not affected by larval diet, but was affected by the adult diet of potential mates. Overall, selecting butterflies were more likely to pair with animals provided sugar and amino acids than those given sugar alone. Those fed protein-biased diet as juveniles and sugars plus amino acids as adults were most preferred, while animals provided carbohydrate-biased diet and sugars were least preferred.

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CHAPTER I

INTRODUCTION

Many generalist herbivores mix food items, thereby improving performance over that of feeding on any single food item (Bernays et al., 1994). Generalist herbivores may acquire fitness benefits through feeding on several plant species by improved regulation of nutrient intake (Bernays and Lee, 1988; Pulliam, 1975) or reduced intake of defensive plant chemicals (Freeland and Janzen, 1974; Singer et al., 2002). Herbivorous insects can regulate feeding such that a specified nutrient intake target optimal for growth and development is achieved (Simpson and Raubenheimer, 1993). Plants vary widely in their nutrient composition and generalist larvae feeding within and between plants may encounter differing nutritional resources. Insect herbivores growing under sub-optimal nutrient conditions may compensate through increased, or "compensatory" feeding within the immature stages (Berner et al., 2005; Lavoie and Oberhauser, 2004; Lee et al., 2004a; Simpson et al., 1989; Simpson et al., 1990; Slansky and Wheeler, 1992; Yang and Joern, 1994). Compensatory feeding, however, may still have fitness costs in the field due to increased exposure to natural enemies or increased energy costs of consuming and processing food (Lavoie and Oberhauser, 2004).

Dietary Self-Selection

Insects can regulate nutrient intake to optimize performance and growth (Raubenheimer and Simpson, 1997; Waldbauer and Friedman, 1991), while a suboptimal diet can adversely affect growth, development, survivorship, and fecundity (Awmack and Leather, 2002). Most research concerning nutritional quality and self-selection behaviors has focused on the ratio of protein to digestible carbohydrate (P:C) available to insects. This ratio is a chief aspect of dietary quality that significantly affects performance. Caterpillars allowed to self-select a dietary ratio of protein to carbohydrate generally have better growth, development, and fecundity than those fed dilute, or either protein- or carbohydrate-biased diets (Lee et al., 2004a; Lee et al., 2002; Lee et al., 2003; Lee et al., 2004b; Raubenheimer et al., 2005; Telang et al., 2001; Thompson and Redak, 2005; Waldbauer et al., 1984).

The geometric framework (Raubenheimer and Simpson, 1997, 1999; Simpson and Raubenheimer, 1993, 1995) is a powerful method used to study the regulation of multiple nutritional components in insect feeding and their subsequent effects on insect performance. In the terms of the geometric framework, regulation ensures that an organism consumes a balance of nutrients, called the "nutrient intake target" (Simpson and Raubenheimer, 1993), which is optimal for growth and development. Intake targets are dynamic and may change during development, which reflects the nutrient requirements for different stages of growth (Raubenheimer and Simpson, 1999). Intake targets can change due to shifting physiological demands such as increased physical activity (Raubenheimer and Simpson, 1999), or costs associated with fighting off pathogens (Lee et al., 2006). Regulating nutrient intake to an optimal point may benefit an herbivore for reasons beyond purely nutritional. The deterrent properties of a defensive compound may be mediated by its interaction with the nutritional content of a food, and regulating intake to an optimal point can lessen the effects of plant defensive compounds on an herbivore (Simpson and Raubenheimer, 2001). Nutritional regulation may be disabled to the detriment of the insect. In cases of parasitism, nutritional regulation behaviors may be abandoned (Thompson and Redak, 2005), thereby benefitting the parasitoid (Thompson and Redak, 2008).

Rules of Compromise

When animals are constrained to a single food having the appropriate ratio of nutrients, they need only to regulate consumption to reach the intake target. However, when constrained to a nutritionally unbalanced food, animals will be unable to reach the intake target and will have to compromise to assure their minimum nutritional requirements are met. While following "rules of compromise" insects will suffer deficiencies and/or excesses of nutrients while attempting to optimize nutrient intake. The rule of compromise exhibited reflects the costs and benefits of over and under ingesting specific nutrients (Raubenheimer and Simpson 1997, 1999). Which rule the insects follow when faced with nutrient imbalances is a qualitative indicator of dietary breadth (Lee et al., 2003; Raubenheimer and Simpson, 1999, 2003).

According to the nutritional heterogeneity hypothesis (Simpson et al., 2002), the amount of nutritionally imbalanced food eaten by an herbivore is related to the probability that it will subsequently encounter an equally and oppositely imbalanced food. This probability should be higher in generalist herbivores because they frequently switch between foods and are more mobile. The probability of a specialist herbivore, particularly a less mobile one, encountering a complementary food source is expected to be low. Accordingly, generalists should be more likely to ingest excesses of an imbalanced food. In such cases, the intake array (protein-carbohydrate intake points gathered from a range of imbalanced diets) is expected to be linear with a negative slope. This is termed the fixed proportion (FP) rule (Behmer, 2009). Insects exhibiting a special case of this, termed the equal distance (ED) rule, form an intake array with a slope of -1 (Raubenheimer and Simpson, 1999, 2003). In this case insects are equally likely to ingest excesses of each nutrient being studied and may be regulating total intake through volumetric means, essentially eating the same total amount irrespective of dietary quality. Alternatively, specialists are expected to follow what is termed the closest distance (CD) rule (Lee et al., 2003; Raubenheimer and Simpson, 1997; Simpson and Raubenheimer, 1993) and consume an amount that minimizes the geometric distance from their intake target in nutrient space.

Adult nutritional inputs

Lepidoptera may supplement larval nutrient reserves through adult feeding (Chew and Robbins, 1989; Porter, 1992). Nectar has traditionally been considered a carbohydrate reward for pollinators. Wykes (1952) suggested that the most useful constituents of floral nectar are the energy yielding sugars and that it is a poor source of nitrogen in the form of amino acids or protein. Others suggested that adult Lepidoptera are unable to assimilate amino acids and protein and that the nitrogenous compounds necessary for egg development are derived only from larval reserves (Davey, 1965; Engelmann, 1970). Due to this, a great deal of optimal foraging theory has been applied to nectarivores, following the presumption that they are simply maximizing caloric intake as opposed to selecting for other nutrients (Waldbauer and Friedman, 1991). Carbohydrate intake significantly affects butterfly fitness parameters (Bauerfeind and Fischer, 2005; Boggs and Ross, 1993; Fischer et al., 2004; Hill, 1989; Hill and Pierce, 1989; Murphy et al., 1983; O'Brien et al., 2004), and butterflies may be able to utilize different sugars to a varying degrees (Romeis and Wackers, 2002). The carbon of nonessential amino acids in eggs may be primarily derived from adult carbohydrate intake (O'Brien et al., 2005; O'Brien et al., 2002). Total adult nectar consumption can affect both butterfly fecundity (Boggs and Ross, 1993; Hainsworth et al., 1991; Mevi-Schutz and Erhardt, 2003a) and longevity (Mevi-Schutz and Erhardt, 2005).

Gilbert (1972) first noted that butterflies in the neotropical genus *Heliconius* remove amino acids and protein from pollen and that females collected more pollen than males. He suggested that amino acids and proteins play a role in their reproductive and pollination biology. Dunlap-Pianka et al. (1977) determined that amino acids in the diet derived from pollen feeding sustained egg production and greatly increased longevity for *H. charithonia* relative to those not allowed pollen. Furthermore, essential amino acids are transferred from pollen to *H. charithonia* eggs (O'Brien et al., 2003).

Amino acids are the second most abundant compound in floral nectar after sugars (Gardener and Gillman, 2002), and plant species typically pollinated by butterflies tend to have higher concentrations of amino acids in floral nectar than those principally pollinated by bees, birds, etc. (Baker and Baker, 1973, 1973a; Baker and Baker, 1977; Baker and Baker, 1985; Baker and Baker, 1986, 1990). This suggests that insects regulate nutrient intake on nectars and that butterflies may exhibit selection for increased nitrogenous intake from nectars compared to those nectarivores with alternative nitrogenous resources. Floral amino acid complements (components and ratios thereof) exhibit a great degree of intraspecific constancy, and Baker and Baker (1977) suggested that amino acids may contribute to the “taste” of particular nectars. A constant taste may aid floral visitors in recognizing nectar resources and would assist in maintaining reliable pollen transfer between flowers of the same plant species (Gardener and Gillman, 2002). Erhardt (1991) suggested that higher nectar amino acid content may give plants a selective advantage by keeping their pollinators alive longer, thereby increasing the chances of cross-pollination. Additionally, butterfly preference for nectars rich in amino acids may select for those individual plants with amino acid-rich nectar, thereby increasing their representation in the gene pool. Preferences for the presence of nectar-born amino acids, if genetically based, may spread and become fixed within evolutionary time scales if indeed amino acids confer higher reproductive success to discerning butterflies. Conversely, if there is no selective pressure for amino acid preference, and no selective pressure placed upon plants to provide amino acid rich nectar, the question remains why this correlation is seen in nature.

Butterfly preferences for amino acid-rich nectars

Meeting the demands for reproduction often involves a combination of larval and adult-derived nutrients and energy for many insects (Boggs, 1981a). Understanding the selective pressures affecting acquisition of different nutrients needed for survival and reproduction in Lepidoptera is often complex due to the striking difference between larval and adult food sources (Boggs, 1990). It is well known that nutritional resource quality available for herbivores may vary greatly within and among plant species (Bernays and Chapman, 1994; Schoonhoven et al., 2006; Schowalter, 2006). Nectar quality (eg. carbohydrate and amino acid content) available for nectarivores, however, may also vary both within and among plant species (Baker and Baker, 1983; Corbet and Delfosse, 1984; Gardener and Gillman, 2001; Gottsberger et al., 1990; Langenberger and Davis, 2002; Lanza et al., 1995; Nicolson and Nepi, 2005; Percival, 1961; Petanidou et al., 1996; Plowright, 1981), and can even vary greatly among nectaries of the same flower (Herrera et al., 2006).

Since the research by Baker and Baker, several studies have tested whether butterflies detect and select for the presence of amino acids in nectar. Butterflies exhibited preference for nectars containing amino acids in the majority of studies (Alm et al., 1990; Erhardt and Rusterholz, 1998; Hawn and Lanza, 2004; Mevi-Schutz and Erhardt, 2003b, 2004; Mevi-Schutz et al., 2003), and in most cases only females exhibited preference. This is consistent with the hypothesis that the nutritional hurdle in meeting the amino acid requirements for oviposition is one born by females. Janet Lanza and collaborators have suggested that amino acid preference correlates with the sex deriving fitness benefits (*pers. comm.*). Female preferences for nectar derived amino acids differ due to larval nutrition (Mevi-Schutz and Erhardt, 2003b; Mevi-Schutz et al., 2003) and even mating history (Mevi-Schutz and Erhardt, 2004), indicating that adults can adjust their foraging decisions based on nutritional requirements. Furthermore, these results suggest that the presence of amino acids in nectar may not be affecting butterfly preference due to nectar taste (which would affect both sexes), but due to differential nutritional demands between the sexes (e.g. requirements for egg production and oviposition). Lepidopterans may undergo compensatory feeding in response to nutrient content (Lavoie and Oberhauser, 2004; Slansky and Wheeler, 1992) or pathogen stress (Lee et al., 2006) within the larval stage, yet few studies have specifically addressed compensatory feeding in the adult stage in response to larval diet in these insects (Mevi-Schutz and Erhardt, 2003b).

Effects of nectar-born amino acids on butterfly fitness

Adult feeding habits and the value of adult nutrients in egg production are correlated with age-specific fecundity among several butterfly species (Boggs, 1986; Chew and Robbins, 1989; Erhardt and Rusterholz, 1998). With age, species with no or little adult feeding exhibit a quick decline in age-specific fecundity. Those with more substantial adult feeding appear to utilize these sources more towards egg production and exhibit a longer plateau of age-specific fecundity (Boggs, 1987; Erhardt and Rusterholz, 1998). For butterflies with nitrogen rich adult food or relatively long adult lifespans, resources for egg production may not be limited by larval reserves (Chew and Robbins, 1989; Erhardt and Rusterholz, 1998). The value of adult nutrients in egg production is not only determined through adult feeding habits but also the timing of egg maturation, as adult nutritional intake may potentially play a role in production of unyolked eggs (Boggs, 1986; Erhardt and Rusterholz, 1998; Jervis et al., 2005).

As previously discussed, the nutritional demands of egg production may be reflected in sex-specific amino acid preferences (Alm et al., 1990; Erhardt and Rusterholz, 1998; Mevi-Schutz and Erhardt, 2003b, 2004; Mevi-Schutz et al., 2003). Following this, several studies have focused on the effects of amino acids in the female adult diet on reproductive fitness (Bauerfeind and Fischer, 2009; Hill, 1989; Hill and Pierce, 1989; Mevi-Schutz and Erhardt, 2003a, 2005; Murphy et al., 1983; Romeis and Wackers, 2002), but most have found no marked benefit on fecundity parameters (Hill, 1989; Hill and Pierce, 1989; Mevi-Schutz and Erhardt, 2003a; Murphy et al., 1983). Mevi-Schutz and Erhardt (2005), however, demonstrated that adult derived amino acids had a compensatory effect on *Araschnia levana* fecundity. Females reared on low quality larval diet (based on leaf-nitrogen content) were smaller and deposited fewer eggs than females from high quality larval diet when supplied sugar alone, but did not differ in egg production when provided sugar and amino acids. These results corresponded to earlier results indicating that females developing on a low quality larval diet exhibited a higher preference for nectar containing amino acids (Mevi-Schutz and Erhardt, 2003b). The use of old (low quality) versus new (high quality) leaf growth in these studies may confound the interpretation that effects observed in these studies were due to differences in nitrogen content of the two larval diets because old and new leaf growth differ in structural (undigestible carbohydrate content) and chemical (plant secondary metabolites) respects as well as nutritional (Bernays and Chapman, 1994; Shoonhoven et al., 2006). The use of defined artificial diets allows one to manipulate particular nutrients of interest to specifically address the effects of larval dietary deficiencies on fitness and the ability of adults to compensate through acquisition of similar resources.

Mate Choice

Female mate choice should evolve when males vary in the quality or quantity of benefits provided to females (Thornhill and Alcock, 1983). Male Lepidoptera and Orthoptera often provide females with nutritious spermatophores which can increase female fecundity and longevity (Andersson, 1994; Delisle and Bouchard, 1995; Delisle and Hardy, 1997; Thornhill and Alcock, 1983; Wiklund et al., 1993). Spermatophore size may be positively correlated with male size (Delisle and Hardy, 1997; Oberhauser, 1988; Thornhill and Alcock, 1983; Wiklund and Kaitala, 1995), and thus female Lepidoptera may exhibit mate choice based on male size as a proxy for spermatophore size when discriminating between virgin males. Indeed, larger male *Rothschildia lebeau* Guerin-Meneville (Saturniidae) had a higher probability of being observed mating in the field (Agosta, 2010). Males may also increase fitness by choosing females of higher quality (i.e. potential fecundity) (Clutton-Brock, 2007; Johnstone et al., 1996; Tang-Martinez and Ryder, 2005). Female size may be an indicator of potential fecundity (Honek, 1993; Lederhouse, 1981), and males in many species discriminate between females based on size (Alcock and Gwynne, 1987; Andersson, 1994; Frey et al., 1998; Rutowski, 1982; Thornhill and Alcock, 1983).

Suboptimal larval diets often negatively affect pupal mass (Lee et al., 2004a; Lee et al., 2002; Lindroth et al., 1991). Pupal mass may be strongly correlated with female fecundity (Awmack and Leather, 2002; Honek, 1993), and adult body mass and wing length in many species decreases in individuals under larval food stress (Nylin and Gotthard, 1998). Adult diet also affects butterfly fitness (Bauerfeind and Fischer, 2005; Beck, 2007; Boggs and Ross, 1993; Geister et al., 2008; Lederhouse et al., 1990; Lewis and Wedell, 2007; Mevi-Schutz and Erhardt, 2005; Romeis and Wackers, 2002), though butterfly size (wing length) is affected by larval diet alone. Amino acids acquired during adult feeding may increase female fecundity (Dunlap-Pianka et al., 1977; Mevi-Schutz and Erhardt, 2005), as well as male longevity (Beck, 2007) and virility (Lederhouse et al., 1990). While larval diet may affect the ability of individuals to secure matings by altering size, it is unknown whether adult diet does so.

Study Organism

The Painted Lady butterfly *Vanessa cardui* L. (Nymphalidae) is arguably the most widespread butterfly in the world, earning it the alternate name the Cosmopolitan (Opler and Krizek 1984). Larvae of *V. cardui* are highly polyphagous, feeding on over 100 different species of plants from over 25 families (Williams, 1970). Favored plants include those from the families Asteraceae especially in the genus *Cirsium (Ferris and Brown, 1981; Scott, 1986),* Malvaceae, Fabaceae, and Boraginaceae (Opler and Krizek, 1984). There does not appear to be any particular chemical compound(s) shaping this species' host range (Ellis and Bowers, 1998), and in times when preferred hostplants are consumed or missing *V. cardui* is known to attack cultivated crops including soybean, alfalfa, lettuce, and more (Comstock, 1927; Scott, 1986; Zhang, 1994). Adults avidly feed on floral nectar, often visiting thistles, blazing star, iron-weed, etc., while visiting milkweeds, buttonbush and privet on lesser occasion (Opler and Krizek 1984). Adults may also feed on aphid hondeydew (Scott, 1986).

V. cardui is known for its migratory tendencies with large migrations northwards during spring and small migrations south during the fall (Ellis and Bowers, 1998; Scott, 1986). Continuous broods may occur in areas with a warm, dry climate such as the extreme southwest of California where breeding has been recorded in mid-winter (Williams, 1970). Larvae undergo five instars before pupating and animals do not exhibit diapause during any life stage (Williams, 1970). Field observations have shown that larvae move within and between species while feeding (Bernays and Minkenberg, 1997). Larvae displaced from a hostplant display no induced preference for what they were previously feeding upon, accepting the first hosplant encountered (Garrigan, 1994).

Painted lady adults live for a relatively long time (>2 mo., pers. obs.). Therefore, adult *V. cardui* females have sufficient opportunity to acquire resources for development of soma and eggs through nectar feeding or mating. Indeed, females do not eclose with mature eggs, which means *V. cardui* have an ‘ovigeny index’ (OI) of 0. The ovigeny index is calculated as the number of mature eggs at eclosion divided by the lifetime potential fecundity and quantifies the variation in the degree of early life concentration of egg production, and also variation in initial reproductive effort (Jervis et al., 2005). When the ovigeny index is close to zero, resources acquired by adult foods or male nuptial gifts should be the most likely sources allocated to oogenesis while larval resources should account for a small proportion of the allocated resources, and species may also exhibit ‘income’ breeding (Jervis et al., 2005). *V. cardui* do not mate until 3 days after eclosion (pers. obs.) and females may mate up to 5 times (pers. obs.).

Larvae can be easily reared on a number of commercial artificial diets obtained from either Carolina Biological Supply Co. (Burlington, NC) (Ellis and Bowers, 1998) or Bio-Serv Entomology Division (Frenchtown, NJ). Butterflies perform well on commercial *Manduca sexta* L. (Lepidoptera: Sphingidae) diet obtained from Bio-Serv and readily lay copious amounts of eggs on *Lantana camara* L. (Verbenaceae) leaves, *Malva parviflora* leaves, or on moistened cotton wicks. Larvae also feed readily on a defined artificial diet (Thompson and Redak, 2005; Thompson et al., 2005a, 2005b) modified from that developed for *M. sexta* (Ahmad et al., 1989), making them an ideal model insect to use for examining the effects of variable nutrition on fitness parameters.

Objectives

The broad objective of this dissertation research was to gain insight into how larval and adult nutrition affect butterfly fitness and whether adult foraging behaviors are mediated by larval nutrition. I specifically addressed the effects of carbohydrates and nitrogenous resources (protein for larvae and nectar-born amino acids for adults) by utilizing defined artificial diet for larval rearing (Thompson and Redak, 2005; Thompson et al., 2005a, 2005b), and a defined artificial nectar mimic for adult nutrition based on the complement of sugars and amino acids present in *Lantana camara* (Verbenaceae) floral nectar. I determined whether *V. cardui* larvae display dietary self-selection and what the rules of compromise are when larvae are constrained on suboptimal diet (Chapter II). I then addressed whether adults alter adult nectar preferences or feeding behavior in response to variations in larval nutrition (Chapter III). In Chapter IV I measured the effects of variable larval and adult nutrition on female fecundity and longevity. Lastly, I determined if larval and adult nutrition affect *V. cardui* mate choice (Chapter V).

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CHAPTER II

DIETARY SELF-SELECTION AND RULES OF COMPROMISE BY 5TH INSTAR *VANESSA CARDUI* L. (LEPIDOPTERA: NYMPHALIDAE).

SUMMARY

Dietary self-selection and rules of compromise for the intake of protein (P) and digestible carbohydrate (C) were examined for 5th instar *Vanessa cardui* L. (Lepidoptera: Nymphalidae) larvae. Six fat-free diet pairs were presented in a choice trial to determine the ‘intake target’ of the larvae, and 7 fat-free single diets differing in dietary nutrient ratio were presented to larvae in no-choice trials to determine the rules of compromise they exhibit when constrained to a singular, sub-optimal dietary source. In choice-trials, larvae regulated nutrient intake to near a 1P:1C ratio, exhibiting tighter regulation of protein than carbohydrate. Furthermore, larvae from different diet pair treatments did not differ in pupal mass or stadium duration. Relative to a 1P:1C nutrient ratio, larvae on no-choice diets consumed less total diet on increasingly protein-biased diets and more on increasingly carbohydrate-biased diets. Total nutrient consumption remained the same as nutrient ratio became increasingly protein-biased, but increased as nutrient ratio became increasingly carbohydrate-biased. Differences in carbohydrate consumption were much greater between dietary nutrient ratios than differences in protein consumption. When accounting for initial larval mass and total consumption, dietary nutrient ratio affected pupal mass. Pupal mass decreased as nutrient ratio was shifted off of 1P:1C, but decreased to a greater extent when this was shifted toward carbohydrate than when shifted toward protein. When accounting for nutrient consumption, dietary nutrient ratio affected pupal mass, with protein consumption significantly affecting and carbohydrate consumption not affecting pupal mass. Dietary nutrient ratio significantly affected stadium duration, but this shift was more pronounced when nutrient ratio was shifted toward carbohydrate than toward protein. Regulation to near 1P:1C is consistent with

results found for other Lepidoptera, and the rule of compromise exhibited by *V. cardui* is consistent with that expected for a generalist herbivore.

INTRODUCTION

Mixing food items improves performance over that for feeding on any single food item for many species of generalist herbivores (Bernays et al., 1994). Generalist herbivores may acquire fitness benefits through feeding on several plant species by improved regulation of nutrient intake (Lee et al., 2002; Pulliam, 1975) or reduced intake of defensive plant chemicals (Freeland and Janzen, 1974; Singer et al., 2002). It is well established that animals and insects regulate nutrient intake to optimize performance and growth (Raubenheimer and Simpson, 1997; Waldbauer and Friedman, 1991), while a suboptimal diet can adversely affect caterpillar growth, development, survivorship, and fecundity (Awmack and Leather, 2002). Most research into nutritional quality and self-selection behaviors has focused on the ratio of protein to digestible carbohydrate (P:C) available to insects. This ratio is a chief aspect of dietary quality that significantly impacts performance. Research with caterpillars has shown that those individuals allowed to self-select a dietary ratio of P:C generally have better growth, development, and fecundity than those fed dilute, or either protein- or carbohydrate-biased suboptimal diets (Lee et al., 2004a; Lee et al., 2002; Lee et al., 2003; Lee et al., 2004b; Raubenheimer et al., 2005; Telang et al., 2001; Thompson and Redak, 2005; Waldbauer et al., 1984).

One powerful method used to study the regulation of multiple nutritional components in insect feeding and their subsequent effects on insect performance is the geometric framework, developed and presented convincingly by Simpson and Raubenheimer (Raubenheimer and Simpson, 1997, 1999; Simpson and Raubenheimer, 1993b, 1995). In the terms of the geometric framework regulation ensures that an organism consumes a balance of nutrients, called the “nutrient intake target” (Simpson and Raubenheimer, 1993b), which are optimal for growth and development. Intake targets are dynamic and may change during development, which reflects the nutrient requirements for different stages of growth (Raubenheimer and Simpson, 1999). Intake targets may change due to shifting physiological demands such as increased physical activity (Raubenheimer and Simpson, 1999), or costs associated with fighting off pathogens (Lee et al., 2006b). Regulating nutrient intake to an optimal point may benefit an herbivore for reasons beyond purely nutritional. The deterrent properties of a defensive compound may be mediated by its interaction with the nutritional content of a food, and regulating intake to an optimal point has been found to lessen the effects of plant defensive compounds on an herbivore (Simpson and Raubenheimer, 2001). Nutritional regulation may be disabled to the detriment of the insect. In cases of parasitism, nutritional regulation behaviors may be abandoned (Thompson and Redak, 2005) thereby benefitting the parasitoid (Thompson and Redak, 2008).

When animals are constrained to a single food having the appropriate ratio of nutrients, they need only to regulate consumption to reach the intake target. However, when constrained to a nutritionally unbalanced food, the animal will be unable to reach the intake target and will have to compromise to assure its minimum nutritional requirements are met. While following “rules of compromise” insects will suffer deficiencies and/or excesses of nutrients while attempting to optimize nutrient intake. These rules reflect the costs and benefits of over and under ingesting specific nutrients (Raubenheimer and Simpson 1997, 1999) and which rules the insects follow when faced with nutrient imbalances is a qualitative indicator of dietary breadth (Lee et al., 2003; Raubenheimer and Simpson, 1999, 2003).

According to the nutritional heterogeneity hypothesis (Simpson et al., 2002), the amount of nutritionally imbalanced food eaten by an herbivore is related to the probability that it will subsequently encounter an equally and oppositely imbalanced food. This probability should be higher in generalist herbivores because they frequently switch between foods and are more mobile. The probability of a specialist herbivore, particularly a less mobile one, encountering a complementary food source is expected to be low. Accordingly, generalists should be more likely to ingest excesses of an imbalanced food. In such cases the intake array is expected to be linear with a negative slope. This is termed the fixed proportion (FP) rule (Behmer, 2009). Insects exhibiting a special case of this, termed the equal distance (ED) rule, form an intake array with a slope of -1 (Raubenheimer and Simpson, 1999, 2003). In this case insects are equally likely to ingest excesses of each nutrient being studied and may be regulating total intake through volumetric means (i.e they consume equal amounts of diet, regardless of nutrient ratio). Alternatively, specialists are expected to follow what is termed the closest distance (CD) rule (Lee et al., 2003; Raubenheimer and Simpson, 1997; Simpson and Raubenheimer, 1993b) and consume an amount that minimizes the geometric distance from their intake target in nutrient space.

To date, eight caterpillar species have been studied with respect to their regulation of protein and digestible carbohydrate. These consist of four specialists, including the grass specialist *Spodoptera exempta* (Lee et al., 2003; Lee et al., 2004b), the tree specialist *Malacosoma disstria* (Despland and Noseworthy, 2006), two forb specialists *Heliothis subflexa* and *Manduca sexta* (Lee et al., 2006a; Thompson and Redak, 2005), as well as four generalists; *Heliothis virescens*, *Spodoptera littoralis*, *Spodoptera exigua*, and *Heliothis zea* (Lee et al., 2006a; Lee et al., 2002; Merkx-Jacques et al., 2008; Telang et al., 2001; Waldbauer et al., 1984). Of those studied, only one showed no independent regulation of protein and digestible carbohydrate (Despland and Noseworthy, 2006). Of note is that all of these Lepidopterans are ‘moths’, and represent only three families (Noctuidae, Lasiocampidae, Sphingidae). While beneficial to study closely related species, such as comparing generalist versus specialist nutritional regulation and rules of compromise (Lee et al., 2006a), researchers should strive to include broader representatives within the Lepidoptera as well.

I examined nutritional regulation for 5th instar larvae of the painted lady butterfly *Vanessa cardui* L. (Lepidoptera: Nymphalidae). This species is a highly polyphagous herbivore, feeding on over 100 different species of plants (Williams, 1970) from over 25 families. Favored plants include those from the families Asteraceae, especially in the genus *Cirsium* (Ferris and Brown, 1981; Scott, 1986), Malvaceae, Fabaceae, and Boraginaceae (Opler and Krizek, 1984). There does not appear to be any particular chemical compound(s) shaping this species' host range (Ellis and Bowers, 1998). It is arguably the most widespread butterfly in the world, earning it the alternate name as the Cosmopolitan (Opler and Krizek 1984). In times when the preferred host plants are consumed (i.e. missing), it is known to attack cultivated crops including soybean, alfalfa, lettuce, sunflower, and more (Comstock, 1927; Zhang, 1994). Field observations have shown that larvae move within and between species while feeding (Bernays and Minkenberg, 1997). Furthermore, larvae displaced from a host plant display no induced preference for what they were previously feeding upon, accepting the first host plant subsequently encountered (Garrigan, 1994).

The objectives of the present studies were tripartite: to 1) determine if larvae exhibit dietary self-selection during development, 2) determine what rule of compromise they display when forced off their nutrient target, and 3) determine if sexes differ in their intake targets or rules of compromise. I predicted that *V. cardui* larvae would exhibit dietary self-selection approximately equal to a 1P:1C nutrient intake target, yet slightly biased toward protein as has been found for other Lepidoptera (Lee et al., 2006a; Lee et al., 2002; Lee et al., 2003; Lee et al., 2004b; Merkx-Jacques et al., 2008; Simpson and Raubenheimer, 1993b; Telang et al., 2001; Thompson and Redak, 2005; Waldbauer et al., 1984). Since *V. cardui* is a generalist herbivore, I expected that larvae would display the fixed proportion (FP) rule of compromise (Behmer, 2009; Raubenheimer and Simpson, 2003), as predicted by the nutritional heterogeneity hypothesis (Lee et al., 2003; Simpson et al., 2002). I expected sexes to not differ in self-selection or rule of compromise, as there is no sexual dimorphism exhibited by *V. cardui*. These experiments allow an understanding of the optimal nutritional conditions for 5th instar *V. cardui* and the extent of their homeostatic regulation of protein and carbohydrate intake. Furthermore, this research elucidates whether *V. cardui* behaves as would be expected for a generalist herbivore and independently confirms through performance criteria (Raubenheimer and Simpson, 1997) whether the true intake target corresponds to the defended point.

MATERIALS AND METHODS

Insect Culture

I maintained a colony of *Vanessa cardui* from > 300 original eggs purchased from Shady Oak Butterfly Farm (Brooker FL) continuously under controlled conditions in the lab. I fed larvae a commercial *Manduca sexta* L. (Lepidoptera: Sphingidae) diet (BioServ Entomology Division, Frenchtown, NJ) and reared them individually in clear plastic cups (3 cm H x 4 cm ID) under a L16:D8, 24:24 °C L/D, 70% r.h. cycle in a Percival Scientific environmental chamber (Model: I-30BLL, Series 101, Perry, IA). I hung pupae in a 37W x 36L x 48H cm communal eclosion cage and transferred adults to a 58W x 74L x 81H cm flight cage after emergence. I gave adults a constant food source of sucrose + honey water and an oviposition source of fresh *Lantana camara* L. (Verbenaceae) leaves. I took eggs from leaves daily, soaked them in 2.5% bleach solution for 2 minutes, and placed them within a 100 x 15mm plastic Petri dish on wetted #1 Whatman® filter paper in the environmental chamber to hatch. I subjected eggs to the same light, temperature, and RH regimen as caterpillars.

General Methods for rearing experimental larvae and data collection

I reared larvae as defined under ‘insect culture’ until they reached the final (5th) instar. Upon moulting to the final instar, I weighed experimental larvae and transferred them individually to clean clear plastic cups (3 cm H x 4 cm ID) and provided them with chemically defined diet which contained casein and sucrose as the only digestible sources of protein (P) and carbohydrate (C), respectively. Lepidopteran larvae consume 94-98% of all food during the penultimate and final larval instars (Merkx-Jacques et al., 2008). *V. cardui* consume 98% of total ingested food during the last two instars and 76% of total ingested food during the final instar alone (Poston et al., 1978). The maintenance diet used here was a modification of one initially created for the larvae of *M. sexta* (Ahmad et al., 1989). All diets used were equivalent in total energy, as protein and carbohydrate are approximately equivalent in energy released during metabolism (West et al., 1970). All diets presented had a total combined content of 120g casein and sucrose per liter. Diets also contained 20g agar, 850ml water, 14g Wesson’s salt mixture, 4g cholesterol, 5g ascorbic acid, .25g inositol, 2g sorbic acid, 2g methylparabenzoate, 1g choline chloride, 10ml vitamin mixture, and 20ml formaldehyde per liter.

I changed diet and weighed each larva every 2 days until red frass was present, indicating the larva had voided gut contents and would soon pupate. I weighed all diet blocks on a Sartorius Research microbalance (Model: R200D, Goettingen, Germany) to the nearest 0.01 mg before presentation. I estimated initial dry mass of diet using a previously determined wet/dry mass regression for each diet formulation. At each diet change, I collected diet blocks and frass, dried them to constant mass at 65C and re-weighed them. I calculated dry mass eaten per diet block as initial dry mass presented minus dry mass remaining after feeding. To calculate protein and carbohydrate eaten per diet I multiplied the amount of diet eaten by the proportions of each macronutrient present in the diet. I determined stadium duration and mortality and identified gender 24 hrs post-pupation by noting the sex mark located on the 9th abdominal sternite (Scott, 1986). I then dried pupae to a constant mass as described for diet and weighed them to determine pupal mass.

Experimental Protocol: Dietary Self-Selection

I randomly assigned fifteen newly moulted 5th instar larvae to 1 of 6 different diet pair treatments. Each diet pair contained two defined fat-free diets, individually having the same combined amount of casein and sucrose (120g/L) but consisting of different nutrient ratios. Independently, each diet was imbalanced in P:C content, but together they were complementary (encompassed the nutrient target). Therefore individually these diets blocks were insufficient for reaching the nutrient target, but by regulating consumption from both diet blocks in the pair, larvae were able to freely regulate nutrient intake as to reach their nutrient target. I selected food pairings to give a between-treatment progression in average macronutrient content for which the larvae were exposed, ranging from average protein- to carbohydrate-biased diets. These treatments were as follows: 100P:20C vs. 50P:70C, 100P:20C vs. 40P:80C, 80P:40C vs. 50P:70C, 70P:50C vs. 40P:80C, 80P:40C vs. 20P:100C, 70P:50C vs. 20P:100C. The estimation of the intake target is greatly strengthened by having multiple pairings of different combinations of complementary foods (Raubenheimer and Simpson, 1999; Simpson and Raubenheimer, 1995). If the same point in nutrient space is defended by all treatment groups, it confirms that the intake point is reached through homeostatic regulation of the nutrients being tested (Raubenheimer and Simpson, 1997). I presented diet blocks next to one another within each cup and turned trays 180° and rearranged them every 24hrs to account for positional effects within the rearing chamber. I took measurements as described under ‘general methods for rearing experimental larvae and data collection’.

Experimental Protocol: Rules of compromise

I repeated this protocol twice and combined results after comparisons in SAS indicated no significant differences between trials. I randomly assigned ten larvae to 7 different diet treatments. I constrained each larva to a single defined diet block containing a specified ration of protein and digestible carbohydrate, yet all diets contained the same combined amount of casein + sucrose (120g/L). Larvae constrained to each diet were therefore unable to independently regulate protein and carbohydrate consumption. They were only able to consume more or less of the diet to meet their nutrient requirements. Dietary nutrient ratios were as follows: 5P:1C, 3P:1C, 2P:1C, 1P:1C, 1P:2C, 1P:3C, 1P:5C, where ratios are relative to the amount of casein and sucrose in the stock formulation; that is 1P:1C is equivalent to 60g l^{-1} casein and 60g l^{-1} sucrose. I took measurements as described under 'general methods for rearing experimental larvae and data collection'. I calculated total nutrient consumption from protein and carbohydrate consumption using Pythagoras's theorem (Raubenheimer and Simpson, 2003).

Statistical Methods

I principally employed analysis of covariance (ANCOVA) using newly moulted 5th instar larval mass as the covariate to see whether initial larval weight between treatments affected nutrient consumption and growth (Horton and Redak, 1993; Raubenheimer and Simpson, 1992). I analyzed assimilation efficiency by ANCOVA, incorporating initial mass and total consumption as covariates and consumption-frass as the response variable (Horton and Redak, 1993). There was a significant covariate effect of initial mass on most variables and therefore most data are presented as least square means ± standard error. I used the Shapiro-Wilk "W" test and normal probability plots to assess normality and homogeneity of variance. All data met assumptions of normality, except pupation date, which I analyzed using the Kruskall-Wallis nonparametric alternative to ANOVA.

RESULTS

Choice trial

Selection of nutritional P:C ratio

The ‘intake target’ is the amount and balance of nutrients required by the insect for optimal growth and development throughout the feeding period investigated. During the experiment, 5 larvae out of a total of 90 (1 each from treatments 100P:20C vs. 40P:80C, 70P:50C vs. 40P:80C, 80P:40C vs. 20P:100C and 2 from 80P:40C vs. 50P:70C) died and were excluded from analyses. Larvae in the choice trial regulated nutrient intake to roughly a 1P:1.09C ratio when macronutrient consumption was averaged among all treatments, consuming 0.11922 ± 0.00214 g protein and 0.12965 ± 0.00371 g carbohydrate. Dietary self-selection by larvae is more clearly seen when displayed in a bivariate plot (Raubenheimer and Simpson, 1999, 2003), which simultaneously displays protein and carbohydrate consumption. Each point in two-dimensional space depicts the total intake of the nutrients by larvae on each diet pair throughout the final stadium (Figure 2.1). Initial mass of larvae significantly affected total, carbohydrate, and protein consumption, as well as pupal mass (Tables 2.1, 2.2). Total consumption did not differ among diet pairings, averaging 0.34895 ± 0.00679g throughout the final stadium (Table 2.1, Figure 2.2). Consumption from the individual diet blocks differed between treatments in a pattern as expected. As average nutrient ratio in the diet pairing became protein-biased, larvae consumed less of the protein-biased block and increased consumption of the carbohydrate-biased block. Additionally, caterpillars increased consumption from the protein-biased block and decreased consumption from the carbohydrate-biased block as average nutrient ratio became carbohydrate-biased (Figures. 2.3, 2.4). This demonstrates that larvae in each treatment were able to independently regulate ingestion of dietary protein and carbohydrate in tandem. Diet pairing affected total carbohydrate consumption but did not affect total protein consumption (Table 2.1, Figure 2.1), indicating tighter regulation of protein than carbohydrate intake.

Effects of diet pairing on fitness parameters

Pupal mass did not differ between diet pairings, averaging 0.09743 ± 0.00132g (Table 2.2; Figure 2.5). There was no effect of diet pairing on assimilation efficiency (P = 0.1144). Furthermore, there were no differences in larval mortality or in stadium duration by diet pairing (Kruskall-Wallis P = 0.3973), with average stadium duration lasting 5.14 ± 0.04 days. Both protein and carbohydrate consumption affected pupal mass, with protein consumption having a much greater effect on pupal mass (Table 2.3).

Differences between sexes

When corrected for beginning body mass, males displayed a greater dietary assimilation efficiency than did females (P = 0.0108). No other measured variables differed between sexes.

No-choice trials

Effect of dietary nutrient ratio on consumption

Larvae were constrained to single diets containing a specified ratio of protein to carbohydrate. Accordingly, nutrient intake varied in fixed proportions equal to the nutrient ratios of the diet blocks that larvae were confined to. The combination of total consumption by larvae constrained to each diet formed an intake array that reflected feeding responses to variations in nutrient ratios. A bivariate plot of protein and carbohydrate consumption revealed that total intake points formed a linear intake array with a negative slope of -3.63108 (R^2 = 0.8958; P = 0.0012, Figure 2.6).

Dietary nutrient ratio affected total consumption, as well as total nutrient intake (Table 2.4, Figures 2.7, 2.8). Total consumption decreased as dietary nutrient ratio was shifted toward protein but increased as nutrient ratio was shifted toward carbohydrate (Figure 2.7). Total nutrient intake remained the same on increasingly protein-biased diet blocks, but increased as nutrient ratio became carbohydrate-biased (Figure 2.8). The differences in carbohydrate consumption were much greater between dietary nutrient ratios than differences in protein consumption with an F-value over three times as large (Table 2.4), indicating less variation in protein- than carbohydrate consumption among treatment groups. Essentially, insects suffered greater deficiencies and excesses of carbohydrate than deficiencies and excesses of protein.

Effects of dietary nutrient ratio on fitness parameters

Dietary nutrient ratio affected pupal mass after accounting for initial larval mass and total consumption (Table 2.5). Pupal mass decreased as nutrient ratio was shifted off of 1P:1C, but to a greater extent when dietary nutrient ratio was shifted toward carbohydrate-biased diets than when shifted toward protein-biased diets (Figure 2.9). Dietary nutrient ratio also affected pupal mass after accounting for initial larval mass and nutrient consumption (Table 2.6). While protein consumption significantly affected pupal mass, carbohydrate consumption did not (Table 2.6). Stadium duration was significantly effected by dietary nutrient ratio (Kruskall-Wallis $P < 0.0001$). Stadium duration increased as nutrient ratio diverged from 1P:1C, but more drastically when nutrient ratio was shifted toward carbohydrate (Figure 2.10). Dietary nutrient ratio significantly affected assimilation efficiency (Figure 2.11). Assimilation efficiency decreased as nutrient ratio diverged from 1P:1C, but this drop in efficiency was more pronounced when nutrient ratio became carbohydrate-biased.

Differences between sexes

Males consumed more and therefore had greater carbohydrate, protein, and total nutrient intake than females on the 1P:1C ratio diet ($P = 0.0312$), yet sexes did not differ in total consumption on any other diet. Although males had greater consumption on 1P:1C diet, females had greater pupal mass when accounting for beginning mass and total consumption ($P=0.0464$).

When accounting for beginning mass and total consumption, there was a trend for females to have a greater pupal mass than males on 2P:1C diet (P=0.0548).

DISCUSSION

The geometric framework offers a powerful methodology to use in studies of pre- and post-ingestive nutritional regulation behaviors. Using this approach, I was able to determine the nutrient intake target, as well as the effects of and interactions between dietary protein and carbohydrate levels and nutrient consumption for 5th instar *V. cardui* larvae.

Self-selection of an intake target for protein and digestible carbohydrate

My results demonstrate that 5th instar *V. cardui* caterpillars select a near equal P:C intake target and defend it despite nutritional variability. Generalist caterpillar species studied to date are characterized by selection of a protein-biased diet, such as *H. virescens* (80P:20C), *S. littoralis* (24P:18C), and *S. exigua* (22P:20C) (Lee et al., 2002; Merkx-Jacques et al., 2008; Simpson et al., 2002; Telang et al., 2001; Waldbauer et al., 1984). Oligo- and monophagous species, by comparison, such as *M. sexta*, *H. subflexa*, and *S. exempta*, select diets with either equal or slightly carbohydrate-biased ratios, and it has be theorized that these specialists evolved selection behaviors that match the nutrient content of their host plants (Lee et al., 2006a; Lee et al., 2004b; Thompson and Redak, 2005). Only one Lepidopteran studied to date, *Malacosoma disstria*, has not shown independent regulation of protein and digestible carbohydrate intake (Despland and Noseworthy, 2006). The authors of this study proposed that this was due to a close phenology with host budding and competition in this gregarious tree-feeding caterpillar which should reduce the benefits of regulation. *V. cardui* that were allowed to self-select in choice trials regulated to a nutrient intake ratio approximating 1P:1.09C, resembling selection more akin to specialist than generalist species.

Regulation of intake when constrained to diets of varying P:C ratio

The pattern displayed here by 5th instar *V. cardui* forms a linear intake array that supports the prediction that *V. cardui* exhibits the FP rule of compromise expected of a generalist herbivore (Behmer, 2009). This, along with high survivorship on all treatments, suggests that 5th instar *V. cardui* can grow well on diets that are highly variable in their ratios of macronutrients. Furthermore, my data suggest that *V. cardui* consume excesses of carbohydrate to a much greater extent than protein, indicating that protein intake may be under tighter regulation than carbohydrate intake. This is supported by the dietary self-selection studies where diet pairing affected carbohydrate but not protein consumption.

Compensatory feeding on carbohydrate-biased diets by *V. cardui* is similar to that found for many generalist caterpillar species including *S. littoralis* that counteract protein limitations by increased consumption, which may lead to increased pupal lipid accumulation apparently resulting from excess carbohydrate intake (Lee et al., 2002; Lindroth et al., 1991; Telang et al., 2001; Thompson et al., 2003). This is unlike the feeding strategy found for other generalist caterpillars, including *H. virescens* and *S. exigua*, in which larvae reduced nutrient intake on carbohydrate-biased diets (Lee et al., 2006a; Lee et al., 2002; Merkx-Jacques et al., 2008). Food switching behavior may be mediated by threshold trehalose levels in the hemolymph (Friedman et al., 1991; Thompson, 2003; Thompson et al., 2005a), as well as hemolymph amino acid levels (Simpson and Raubenheimer, 1993a). One explanation for the feeding behavior exhibited by larva constrained to varying P:C ratio diets is that *V. cardui* may have relatively high hemolymph trehalose and/or low hemolymph amino acid thresholds, which are implicated in directing feeding behavior (Bede et al., 2007; Simpson and Raubenheimer, 1993a). However, if this was the case one might expect caterpillars to self-select an intake target in choice trials with a P:C ratio further skewed towards carbohydrate than was found. An alternative, though not mutually exclusive, explanation to the feeding behavior exhibited by *V. cardui* deals with post-ingestive processing. This species may exhibit the observed behaviors of compromise due to an ability to process excesses of carbohydrate to a much greater extent than excesses of protein.

Effects of P:C ratio on larval performance

Final mass, estimated as dry pupal mass, indicates that a diet consisting of roughly equal amounts of protein and carbohydrate is the most suitable for growth of 5th instar *V. cardui*. Pupal mass was reduced on protein-biased diets relative to equal ratio diets, although to a lesser extent than when constrained to carbohydrate-biased diets. Other Lepidopteran species (e.g. *S. eridania*, *S. littoralis*, *S. exempta*, and *M. sexta*) can acquire lipids through the deamination of excess amino acids from dietary protein and subjection of their carbon skeletons to gluconeogenesis (Lee et al., 2004a; Lee et al., 2002; Lee et al., 2003; Thompson et al., 2003). More research is needed to determine if *V. cardui* are able to use excess dietary protein in the same fashion. Nonetheless, for many of these species caterpillar pupal mass is lower on protein-biased diets relative to equal ratio diet. This possibly reflects the increased energy requirements for processing excess nitrogenous wastes (Karowe and Martin, 1989; Nation, 2002) and for amino acid deamination and subsequent subjection to gluconeogenesis (Lee et al., 2002; Lee et al., 2004b). By comparison, *S. exigua* caterpillars reared on protein-biased diets have the same pupal size and lipid reserves as those reared on a diet containing more digestible carbohydrates, and appear to be extremely efficient at processing excess amino acids as well as carbohydrate utilization (Merkx-Jacques et al., 2008). Increased carbohydrate-intake may be converted and laid down as increased lipid accumulation (Lee et al., 2002; Lindroth et al., 1991; Telang et al., 2001). It has been proposed that excess carbohydrates may be respired through increased metabolic rate (Zanotto et al., 1993), although this ‘wastage respiration’ has yet to be demonstrated (Zanotto et al., 1997).

Further studies are needed to clarify the post-ingestive mechanisms of nutrient regulation in *V. cardui*.

Survival was similar among treatments, indicating that *V. cardui* can survive well on variable P:C ratios, although studies have shown absolute mortality when larvae are reared on diets lacking either protein or carbohydrate (pers. obs.). Other studies have shown both the presence, (Karowe and Martin, 1989; Merkx-Jacques et al., 2008; Raubenheimer et al., 2005) and absence (Lee et al., 2004a; Lee et al., 2002; Lee et al., 2004b) of significant mortality costs associated with carbohydrate-biased diets. The absence of significant mortality costs in this study and others may be due to focusing on the final instar and therefore not being able to detect cumulative effects (Merkx-Jacques et al., 2008).

There are trade-offs in Lepidopteran larval growth between the need to accumulate sufficient resources, and rapid development which may lead to increased reproductive cycles during a season and limits exposure to hazards (Price et al., 1980; Williams, 1999). While *V. cardui* final stadium duration increased as dietary nutrient ratio became either protein- or carbohydrate-biased, this increase was more considerable on carbohydrate-biased diets. Delayed development appears to be a general outcome of protein limitation (Despland and Noseworthy, 2006; Lee et al., 2006a; Lee et al., 2002; Lee et al., 2003; Lee et al., 2004b; Lindroth et al., 1991; Merkx-Jacques et al., 2008; Raubenheimer et al., 2005; Telang et al., 2001; Thompson et al., 2005a). In one extreme case many final instar *H. zea* lived for more than 30 days without molting before eventually dying after constantly losing weight when constrained to diet lacking protein (Waldbauer et al., 1984). *V. cardui* also exhibits extremely extended stadium duration (> 14 days) when fed a diet lacking protein, losing weight before finally succumbing to death (pers. obs.).

Differences between sexes

It is interesting to note that males in choice trials had higher assimilation efficiencies than females but did not differ in any other respect, while males constrained to an optimal nutrient ratio had greater consumption than females but did not differ in assimilation efficiency. One might expect that greater assimilation efficiency and the same consumption, or greater consumption and similar assimilation efficiency, would lead to larger males than females. Males, however, did not differ from females in pupal mass in choice trials although they had similar consumption and greater assimilation efficiency than females. Furthermore, when accounting for total consumption, males were smaller than females in pupal mass when constrained to 1P:1C diet even though males consumed more than females and had similar assimilation efficiencies. This may indicate that males have increased physiological demands than females and, while sequestering more nutrients from the food, may be losing them to subsequent increased metabolism. In such a case, females may actually be more efficient at storing sequestered nutrients than males.

Comparing choice and no-choice trials

Larvae were allowed to regulate nutrient consumption in choice trials, yet there appears to be a fitness cost over those feeding on a single optimal diet. While it has been stated that the selected intake target must lie along the intake array (Thompson and Redak, 2005), the self-selected point fell outside the 95% confidence limits of the intake array as determined by simple regression. Larvae allowed to self-select had lower nutrient intake and pupal mass than those on the 1P:1C diet from the no-choice study (pupal mass 0.097433 + 0.001316 vs 0.106849 + 0.002429 T-test P = 0.001932). Larvae allowed to self-select did not differ in pupal mass from those on 2P:1C or 1P:2C diets. Larvae in choice trials had moderately shorter stadium durations than those on the 1P:1C no-choice diet although this was not significant. Reduced intake and mass may reflect a cost associated with decision making in dietary switching that may be due to increased wandering (Waldbauer et al., 1984). Learning may add to fitness by increasing foraging efficiency by decreasing incidences of random food sampling (Bernays and Bright, 2005). While measurements such as pupal mass are often strongly correlated with potential fecundity (Awmack and Leather, 2002; Honek, 1993), further studies are needed to clarify if smaller pupal mass is a true indicator of reduced fitness in these butterflies.

Conclusions

These studies show that 5th instar *V. cardui* caterpillars are adept at regulating their protein and carbohydrate intake when allowed to independently adjust feeding between two complementary diets. In choice-trials, caterpillars regulated intake to a near equal ratio of protein and carbohydrate, and were more effective at regulating protein intake than carbohydrate intake. The fact that *V. cardui* larvae are so effective at regulating protein intake counters the general finding that caterpillars do not regulate protein intake as tightly as locusts (Bernays et al., 2004; Thompson, 2003). Furthermore, 5th instar larvae compensated for protein deficiency better than carbohydrate deficiency in no-choice trials, in contrast to the general finding that caterpillars compensate more effectively for carbohydrate deficiency than for protein deficiency (Friedman et al., 1991; Simpson et al., 1990). Larvae exhibited compensatory feeding on protein-deficient diets and terminated consumption on protein-biased diets while suffering large deficiencies in carbohydrate intake. Carbohydrate-biased diets negatively affected caterpillar fitness to a greater degree than protein-biased larval diets, even though larvae suffered less protein deficiency on carbohydrate biased diets than carbohydrate deficiency on protein-biased diets. Results from both choice and no-choice trials demonstrate the importance of dietary protein on larval fitness for this species. Further studies are needed to assess the post-ingestive regulation of protein and carbohydrate by *V. cardui*, thus adding an important level of interpretation to the research presented here. Chapters II and III examine the effects of larval dietary nutrient ratio on adult feeding preferences and fitness parameters in this species.

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TABLES AND FIGURES

Table 2.1 ANCOVA summary demonstrating the effects of dietary pairing on total consumption, carbohydrate consumption, and protein consumption over the fifth instar of *V. cardui* larvae maintained on chemically defined diet pairs after accounting for initial 5th instar larval mass.

Dependent variable	**df**	**Mean square**	***F***	***P***
Total Diet Consumption (g)				
Diet pair	5	0.0040	1.29	=0.2749
Covariate (Initial mass g)	1	0.0594	19.30	<0.0001
Error	78	0.0031		
Carbohydrate Consumption (g)				
Diet pair	5	0.0032	3.45	=0.0072
Covariate (Initial mass g)	1	0.0115	12.44	=0.0007
Error	78	0.0009		
Protein Consumption (g)				
Diet pair	5	0.0006	1.95	=0.0962
Covariate (Initial mass g)	1	0.0045	15.24	=0.0002
Error	78	0.0003		

Table 2.2 ANCOVA summary demonstrating the effects of dietary pairing on pupal mass of *V. cardui* after accounting for initial 5th instar larval mass and differences in total consumption.

Dependent variable	**df**	**Mean square**	***F***	***P***
Dry Pupal Mass (g)				
Diet pair	5	0.0001	2.18	=0.0645
Covariate (Total diet consumption g)	1	0.0006	12.82	=0.0006
Covariate (Initial mass g)	1	0.0042	95.30	<0.0001
Error	77	0.0000		

Table 2.3 ANCOVA summary demonstrating the effects of dietary pairing and nutrient consumption on pupal mass of *V. cardui* after accounting for initial 5th instar larval mass.

Dependent variable	**df**	**Mean square**	***F***	***P***
Dry Pupal Mass (g)				
Diet pair	5	<0.0001	1.30	=0.2734
Covariate (Initial mass g)	1	0.0004	13.59	=0.0004
Covariate (Carbohydrate consumption g)	1	0.0005	17.17	<0.0001
Covariate (Protein consumption g)	1	0.0036	131.62	<0.0001
Error	76	<0.0001		

Table 2.4 ANCOVA summary demonstrating the effects of dietary nutrient ratio on total consumption, protein consumption, and carbohydrate consumption over the fifth instar of *V. cardui* larvae maintained on chemically defined diet pairs after accounting for initial 5th instar larval mass.

Dependent variable	**df**	**Mean square**	***F***	***P***
Total Consumption (g)				
Dietary nutrient ratio	6	0.0200	3.76	=0.0018
Covariate (Initial mass g)	1	0.0019	0.35	=0.5533
Covariate (Interaction)	6	0.0083	1.56	=0.1659
Error	118	0.0053		
Protein Consumption (g)				
Dietary nutrient ratio	6	0.0267	78.11	<0.0001
Covariate (Initial mass g)	1	0.0016	4.73	=0.0316
Error	118	0.0003		
Carbohydrate Consumption (g)				
Dietary nutrient ratio	6	0.3966	269.09	<0.0001
Covariate (Initial mass g)	1	0.0001	0.03	=0.8563
Error	118	0.0015		

Table 2.5 ANCOVA summary demonstrating the effects of dietary nutrient ratio on pupal mass of *V. cardui* after accounting for initial 5th instar larval mass and differences in total consumption.

Dependent variable	**df**	**Mean square**	***F***	***P***
Dry Pupal Mass (g)				
Dietary nutrient ratio	6	0.0039	70.83	<0.0001
Covariate (Initial mass g)	1	0.0011	20.88	<0.0001
Covariate (Total consumption g)	1	0.0028	51.91	<0.0001
Error	123	0.0001		

Table 2.6 ANCOVA summary demonstrating the effects of dietary nutrient ratio on pupal mass of *V. cardui* after accounting for initial 5th instar larval mass and differences in nutrient consumption between diets.

Dependent variable	**df**	**Mean square**	***F***	***P***
Dry Pupal Mass (g)				
Dietary nutrient ratio	6	0.0020	41.04	<0.0001
Covariate (Initial mass g)	1	0.0007	14.29	=0.0002
Covariate (Protein consumption g)	1	0.0017	34.68	<0.0001
Covariate (Carbohydrate consumption g)	1	<0.0001	0.44	=0.5074
Error	131	0.0001		

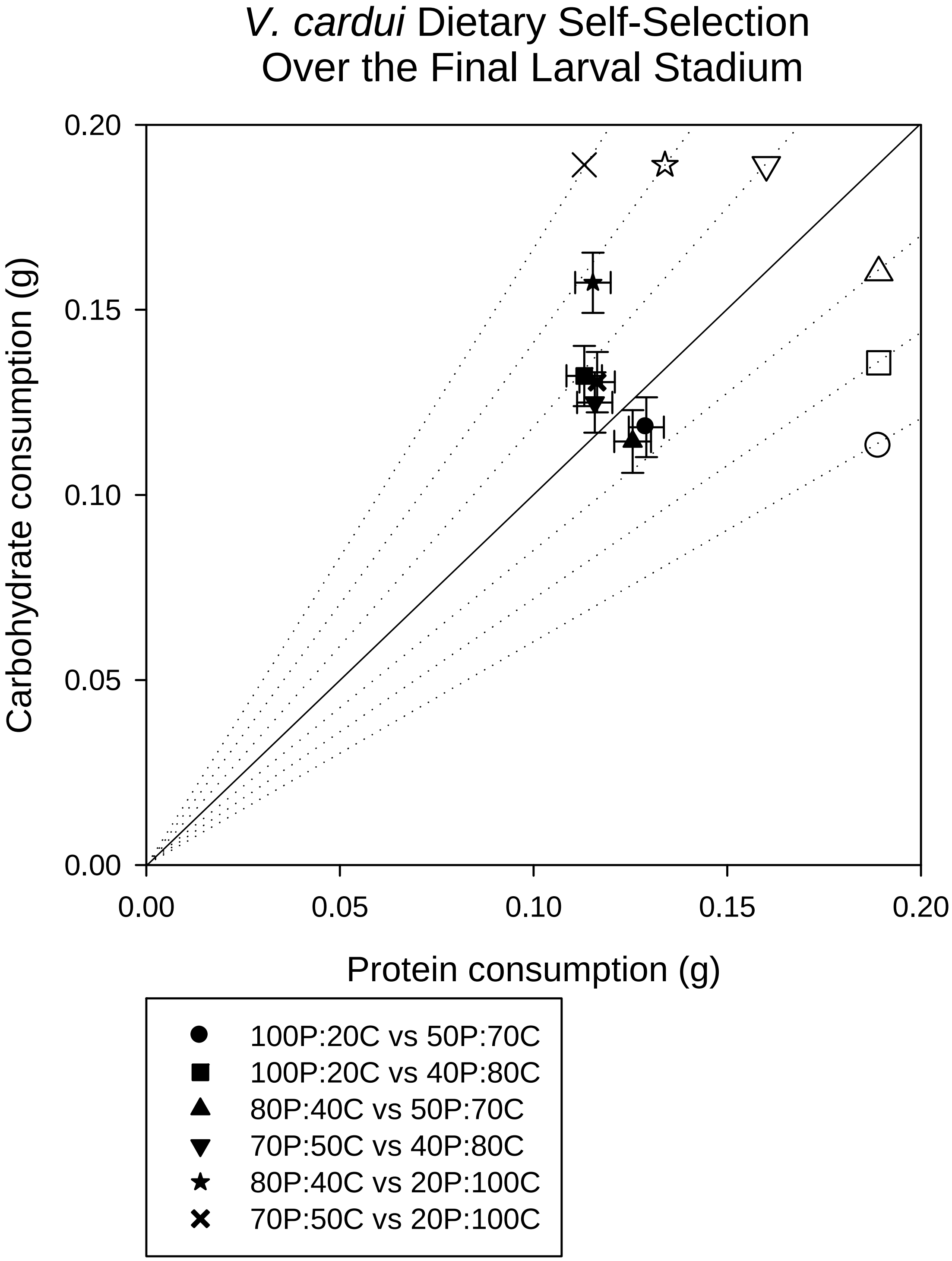

Figure 2.1 Bivariate least squares means ± S.E.M. for selected intake of protein and carbohydrate by 5th instar *V. cardui* for each of the dietary pairings. Solid symbols represent dietary selection by larvae. Open symbols denote the random feeding rails that larvae would feed along if they fed equally from each diet in each pairing.

Figure 2.2 Effects of dietary pairing on total combined dry mass consumption by 5th instar *V. cardui* larvae maintained on 6 defined diet pairings ranging from average protein bias on the left to average carbohydrate bias on the right. Each diet pair contained one protein biased diet block and one carbohydrate biased diet block. Dietary nutrient ratios are listed as g/L. Bars show least squares means ± S.E.M. Significant differences ($P<0.05$) among diet pairings are denoted by different letters.

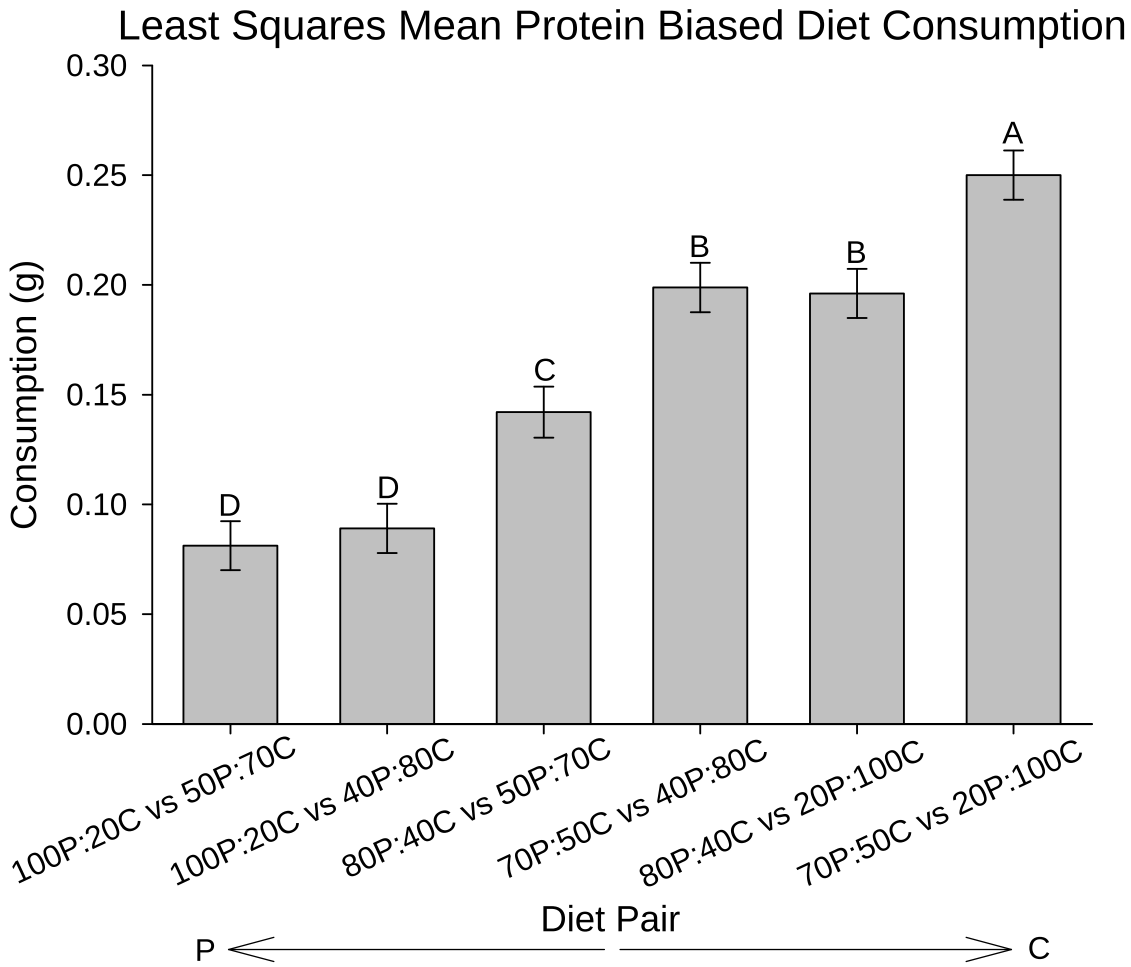

Figure 2.3 Effects of dietary pairing on dry mass consumption from the protein biased diet block by 5th instar *V. cardui* larvae maintained on 6 defined diet pairings ranging from average protein bias on the left to average carbohydrate bias on the right. Each diet pair contained one protein biased diet block and one carbohydrate biased diet block. Dietary nutrient ratios are listed as g/L. Bars show least squares means ± S.E.M. Significant differences (P<0.05) among diet pairings are denoted by different letters.

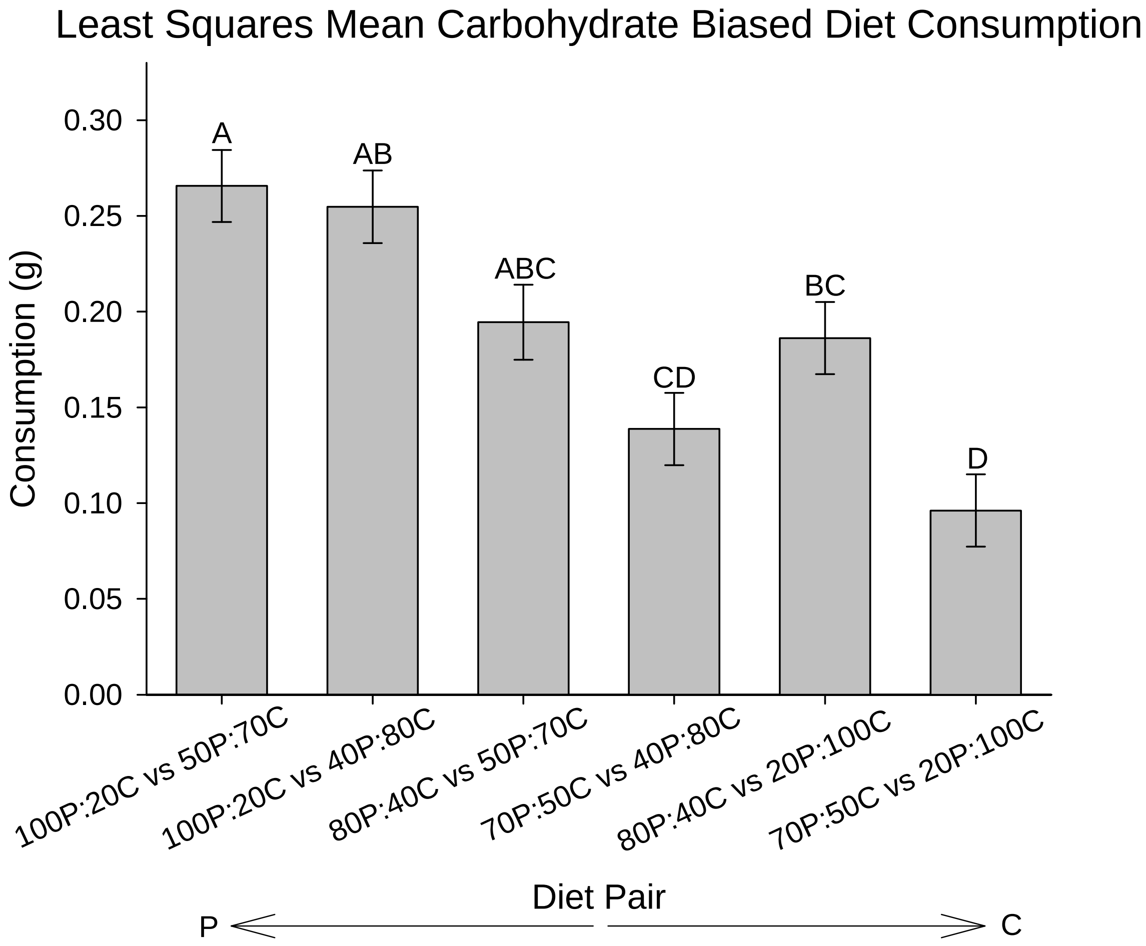

Figure 2.4 Effects of dietary pairing on dry mass consumption from the carbohydrate biased diet block by 5th instar *V. cardui* larvae maintained on 6 defined diet pairings ranging from average protein bias on the left to average carbohydrate bias on the right. Each diet pair contained one protein biased diet block and one carbohydrate biased diet block. Dietary nutrient ratios are listed as g/L. Bars show least squares means ± S.E.M. Significant differences (P<0.05) among diet pairings are denoted by different letters.

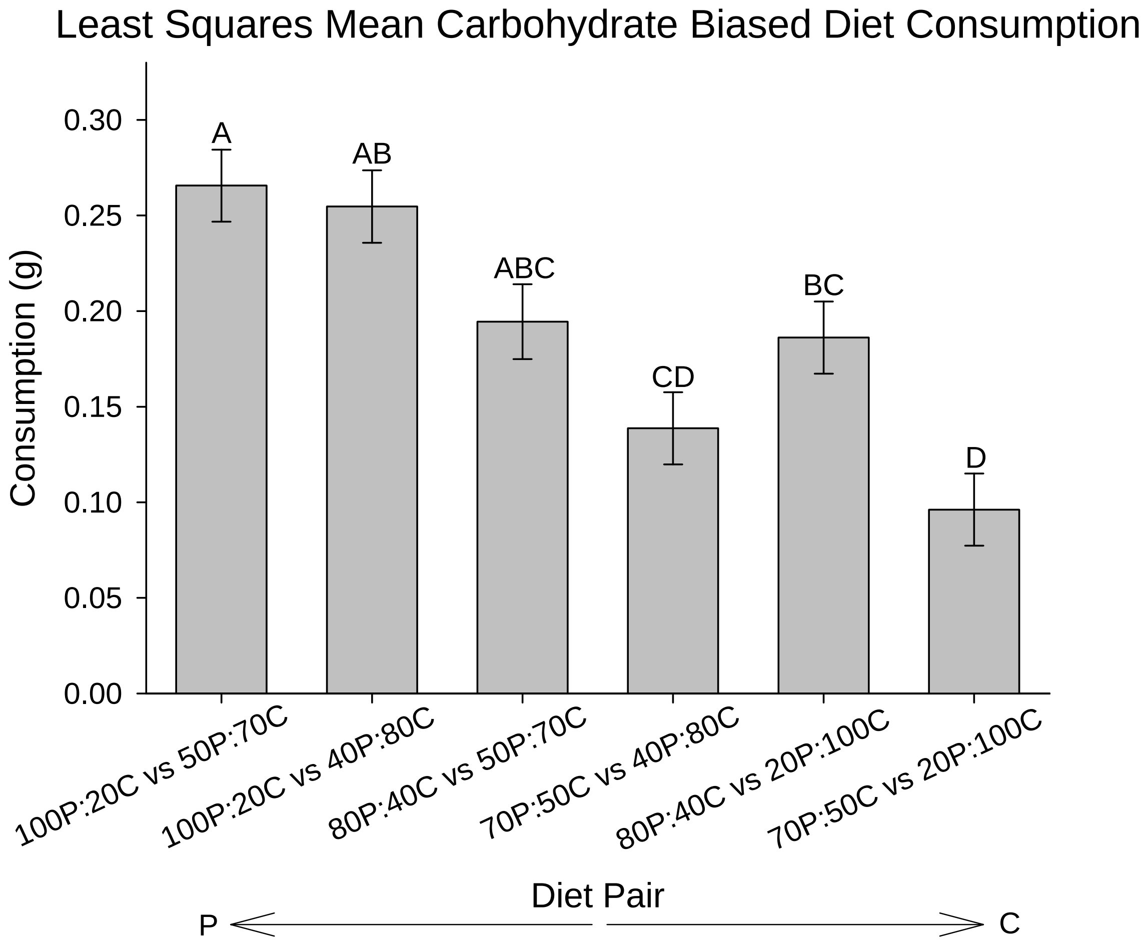

Figure 2.5 Effects of dietary pairing on dry pupal mass of *V. cardui* maintained on 6 defined diet pairings throughout the 5th instar. Diet pairings ranged from average protein bias on the left to average carbohydrate bias on the right. Each diet pair contained one protein biased diet block and one carbohydrate biased diet block. Dietary nutrient ratios are listed as g/L. Bars show least squares means ± S.E.M. Significant differences (P<0.05) among diet pairings are denoted by different letters.

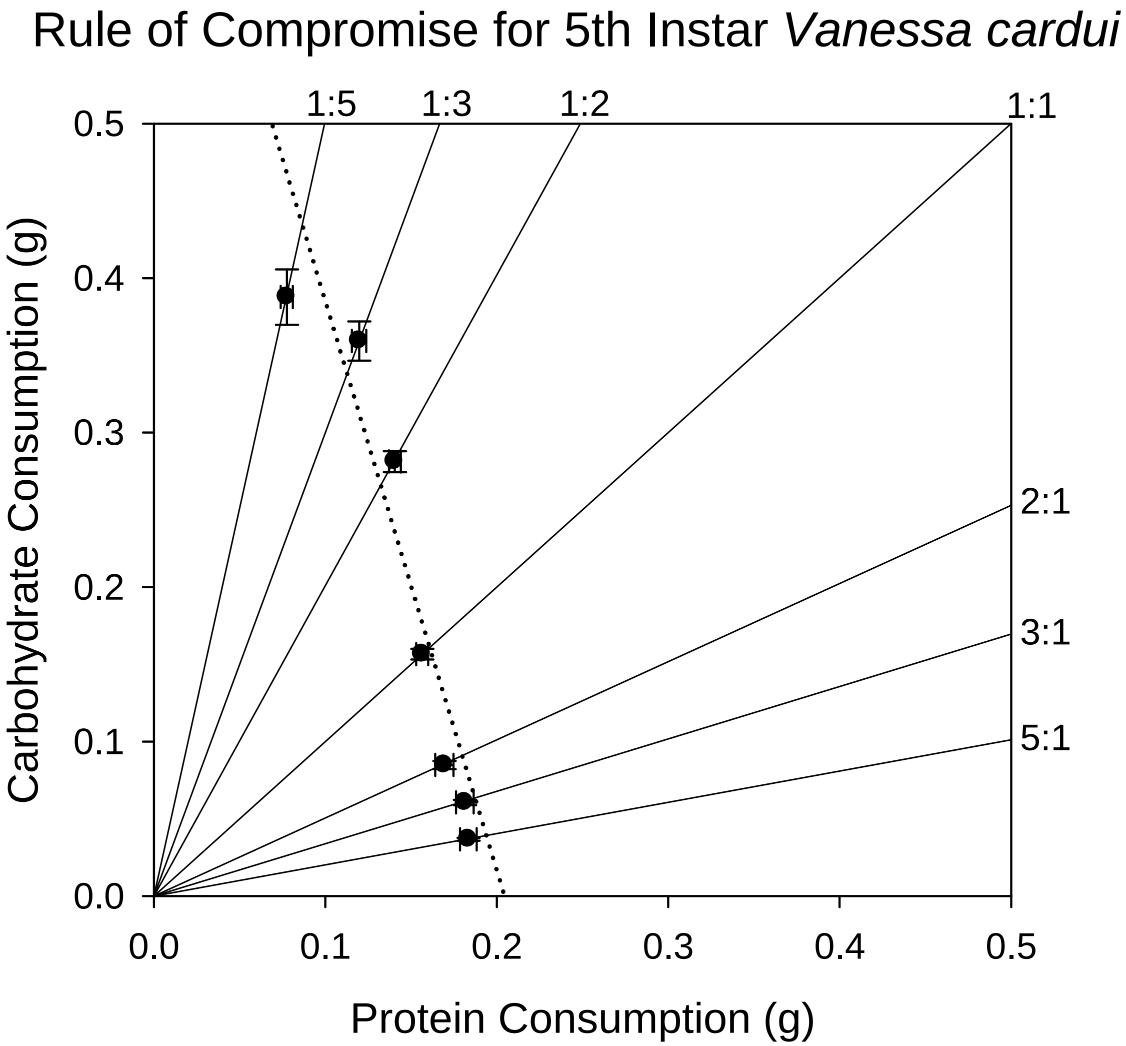

Figure 2.6 Bivariate means ± S.E.M. for intake of protein and carbohydrate by 5th instar *V. cardui* when constrained to 7 defined diets. Dietary nutrient ratios ranged from 5P:1C to 1P:5C, denoted at the end of each rail. Nutrient ratios are relative to the amount of casein and sucrose in the stock formulation; that is 1P:1C is equivalent to 60g l^{-1} casein and 60g l^{-1} sucrose. Total intake points formed a linear intake array with a negative slope of -3.63108 (R^2 = 0.8958; *P* = 0.0012).

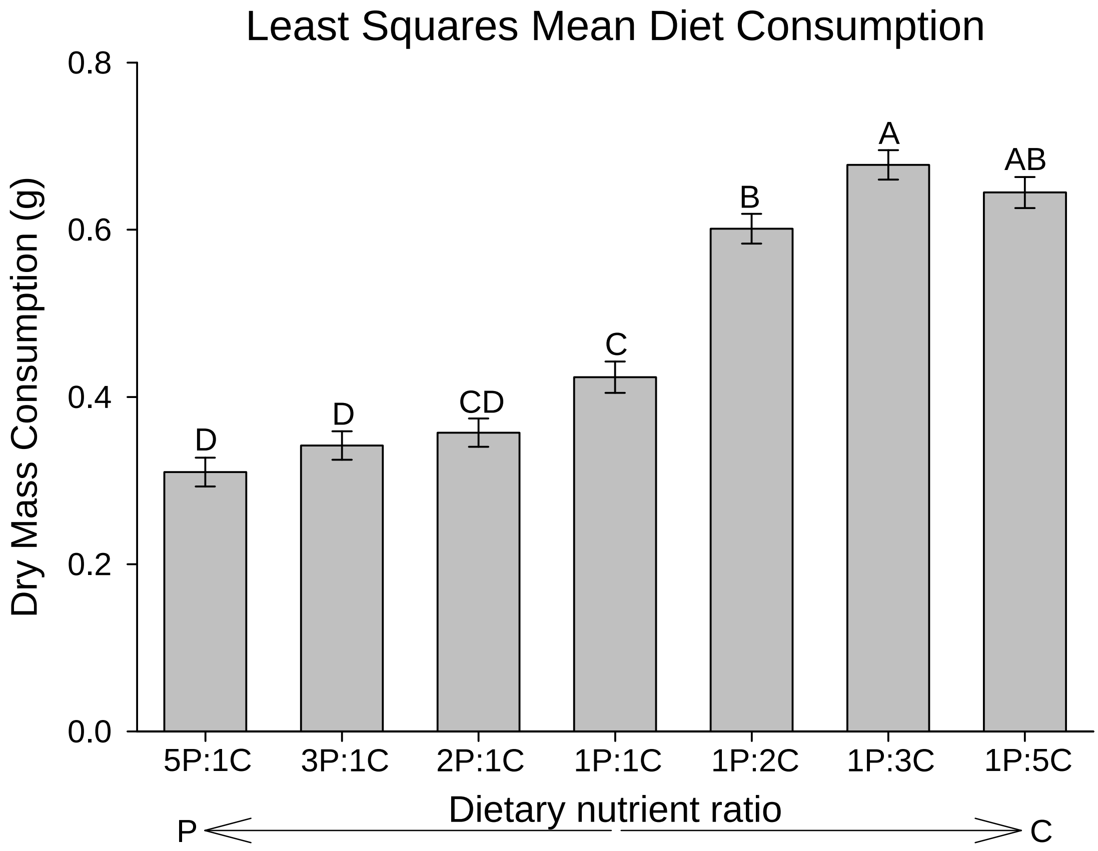

Figure 2.7 Effects of dietary nutrient ratio on total dry mass consumption by 5th instar *V. cardui* larvae maintained on 7 defined diets ranging from protein bias on the left to carbohydrate bias on the right. Nutrient ratios are relative to the amount of casein and sucrose in the stock formulation; that is 1P:1C is equivalent to 60g l^{-1} casein and 60g l^{-1} sucrose. Bars show least squares means ± S.E.M. Significant differences ($P<0.05$) among dietary nutrient ratios are denoted by different letters.

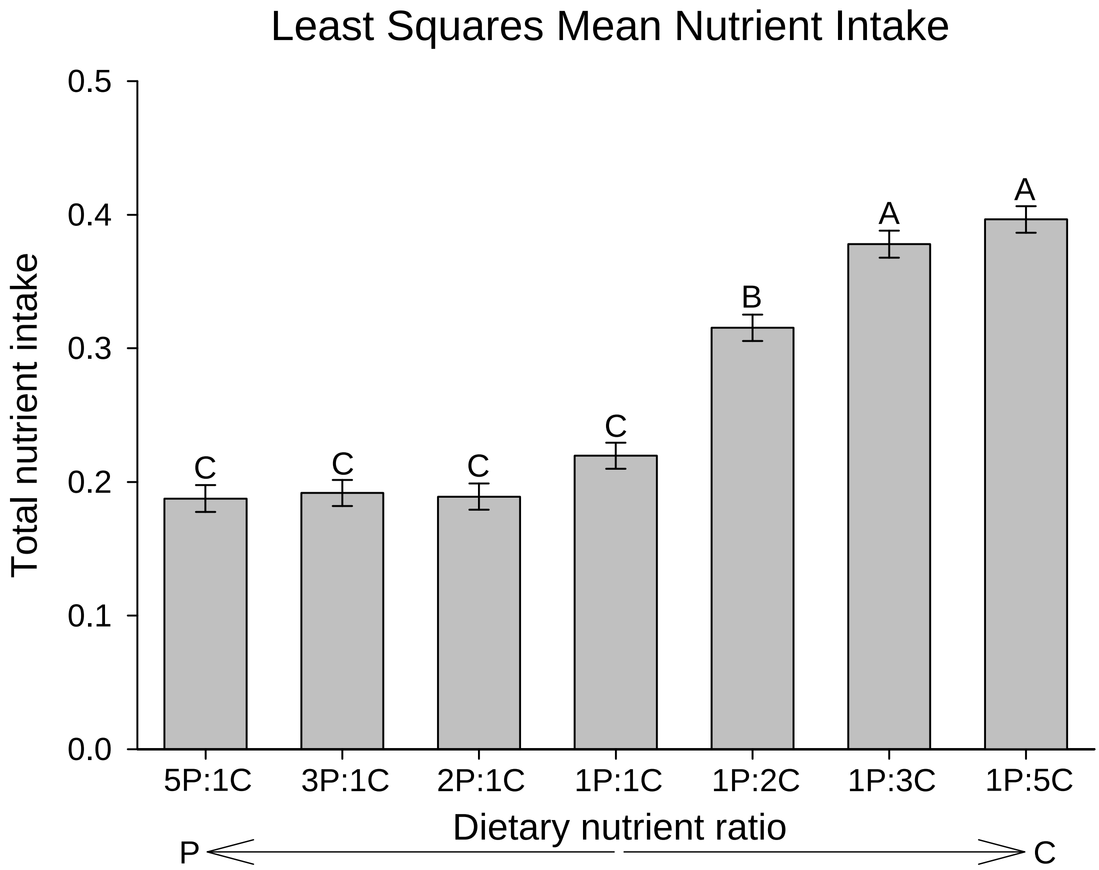

Figure 2.8 Effects of dietary nutrient ratio on total nutrient intake of 5th instar *V. cardui* larvae maintained on 7 defined diets ranging from protein bias on the left to carbohydrate bias on the right. Total nutrient consumption was calculated from protein and carbohydrate consumption using Pythagoras's theorem. Nutrient ratios are relative to the amount of casein and sucrose in the stock formulation; that is 1P:1C is equivalent to 60g l^{-1} casein and 60g l^{-1} sucrose. Bars show least squares means ± S.E.M. Significant differences ($P<0.05$) among dietary nutrient ratios are denoted by different letters.

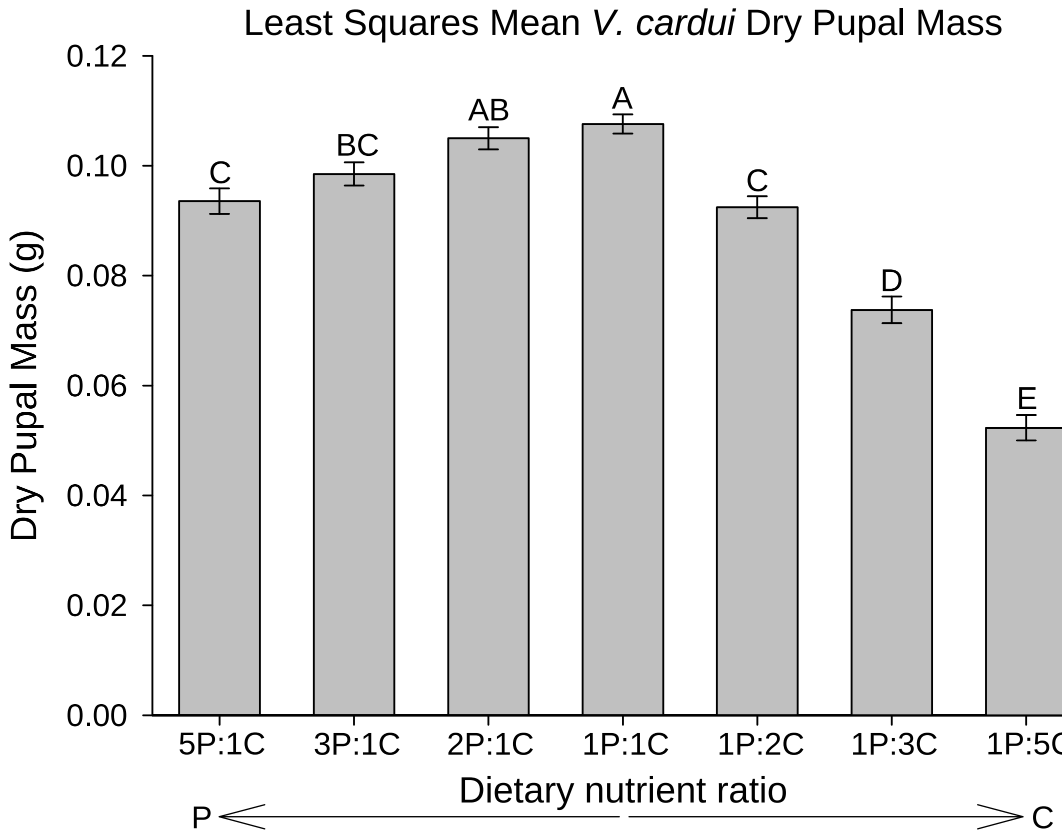

Figure 2.9 Effects of dietary nutrient ratio on the dry pupal masses of 5th instar *V. cardui* larvae maintained on 7 defined diets ranging from protein bias on the left to carbohydrate bias on the right. Nutrient ratios are relative to the amount of casein and sucrose in the stock formulation; that is 1P:1C is equivalent to 60g l^{-1} casein and 60g l^{-1} sucrose. Bars show least squares means ± S.E.M. Significant differences ($P<0.05$) among dietary nutrient ratios are denoted by different letters.

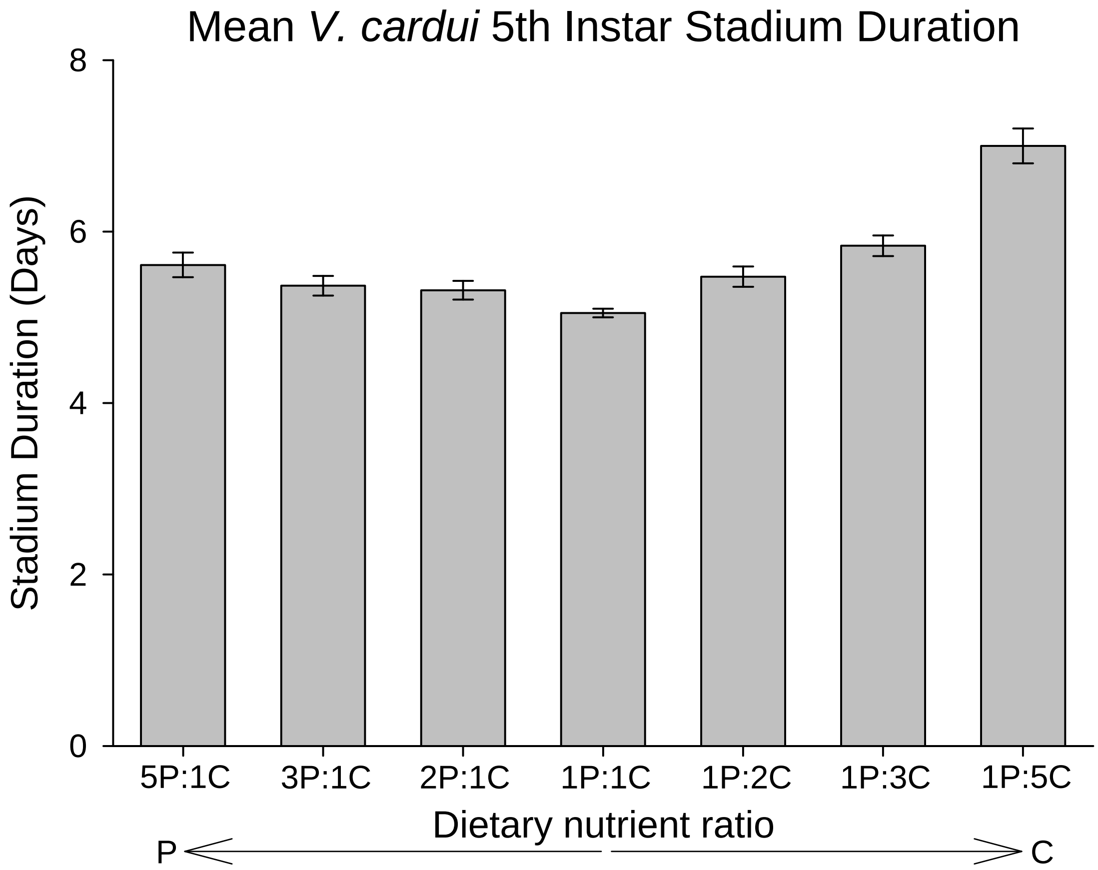

Figure 2.10 Effects of dietary nutrient ratio on the mean stadium duration of 5^{th} instar *V. cardui* larvae maintained on 7 defined diets ranging from protein bias on the left to carbohydrate bias on the right. Nutrient ratios are relative to the amount of casein and sucrose in the stock formulation; that is 1P:1C is equivalent to 60g l^{-1} casein and 60g l^{-1} sucrose. Bars show least squares means ± S.E.M. Significant differences ($P<0.05$) among dietary nutrient ratios are denoted by different letters.

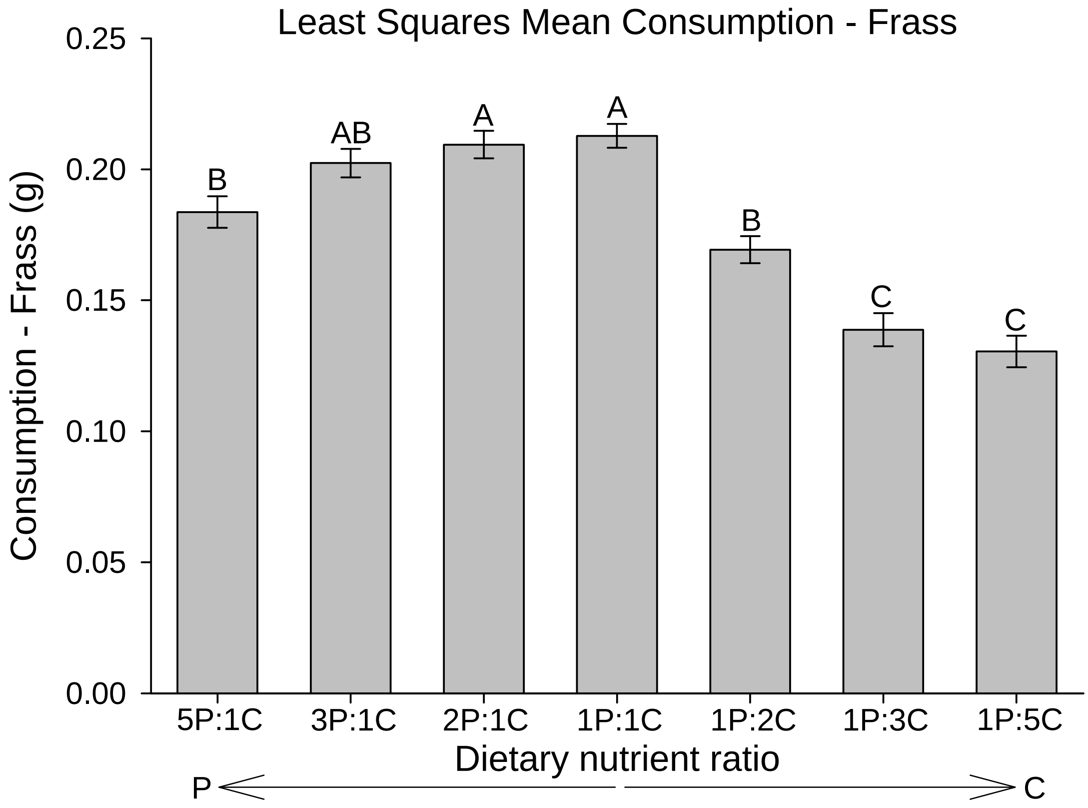

Figure 2.11 Effects of dietary nutrient ratio on the assimilation efficiency of 5th instar *V. cardui* larvae maintained on 7 defined diets ranging from protein bias on the left to carbohydrate bias on the right. Assimilation efficiency was analyzed by ANCOVA, incorporating initial mass and total consumption as covariates and consumption-frass as the response variable. Nutrient ratios are relative to the amount of casein and sucrose in the stock formulation; that is 1P:1C is equivalent to 60g l^{-1} casein and 60g l^{-1} sucrose. Bars show least squares means ± S.E.M. Significant differences ($P<0.05$) among dietary nutrient ratios are denoted by different letters.

CHAPTER III

EFFECTS OF LARVAL DIETARY NUTRITION ON ADULT BUTTERFLY (*VANESSA CARDUI* L. [LEPIDOPTERA: NYMPHALIDAE]) NECTAR PREFERENCE AND CONSUMPTION.

ABSTRACT

Adult butterflies may exhibit feeding preferences for nectars containing amino acids, and these preferences may be affected by larval nutrition. Fifth instar *Vanessa cardui* L. (Lepidoptera: Nymphalidae) initially were constrained to feed upon defined artificial diets that were either protein-biased, of equal-ratio protein to carbohydrate, or carbohydrate-biased. Adult butterflies arising from these larvae were then evaluated for initial probing responses towards floral nectar mimics containing sugars alone or containing sugars plus amino acids. Chi-square analyses revealed that female butterflies reared on carbohydrate-biased diet were significantly more likely to probe towards the sugar and amino acid nectar mimic than towards the sugar-only mimic. Females reared on either the equal-ratio protein:carbohydrate or protein-biased diet and males were equally likely to probe towards either nectar solution. In a separate set of experiments, adults were offered a choice between artificial flowers either containing sugar or sugar plus amino acid nectar mimic. Nectar consumption was significantly affected by experimental trial for females, and by trial and larval diet for males. Both sexes consumed more nectar during trial one than trial two. Males reared on protein-biased diet consumed more nectar than those reared on carbohydrate-biased or equal-ratio protein:carbohydrate diet. Proportion consumption of sugar and amino acid nectar was affected by larval diet for both sexes during trial one, but not during trial two. Female butterflies reared on carbohydrate-biased diet exhibited higher initial preference for sugar and amino acid nectar. Animals, however, did not differ in proportion of sugar and amino acid nectar consumption due to larval diet in consistent patterns between experimental trials. The absence of consistent differences in proportion sugar and amino acid nectar

consumption among butterflies from different larval diets suggests that butterflies do not alter feeding behaviors to account for differences in larval nutrition.

INTRODUCTION

Lepidopteran larvae may undergo compensatory feeding in response to nutrient content (Lavoie and Oberhauser, 2004; Slansky and Wheeler, 1992) or pathogen stress (Lee et al., 2006). Larval nutritional resources can vary greatly within and between plant species (Bernays and Chapman, 1994; Schoonhoven et al., 2006; Schowalter, 2006). Adult nutritional resources, (eg. the carbohydrate and amino acid content of nectar) also vary within and among plant species (Baker and Baker, 1983; Corbet and Delfosse, 1984; Gardener and Gillman, 2001; Gottsberger et al., 1990; Herrera et al., 2006; Langenberger and Davis, 2002; Lanza et al., 1995; Nicolson and Nepi, 2005; Percival, 1961; Petanidou et al., 1996; Plowright, 1981).

Nectar is generally regarded as an energetic reward for pollinators in the form of carbohydrates. Butterfly-pollinated plant species, however, tend to have greater concentrations of amino acids in floral nectar than those pollinated primarily by birds, bees, etc. (Baker and Baker, 1973, 1973a; Baker and Baker, 1977; Baker and Baker, 1985; Baker and Baker, 1986, 1990). Several studies have demonstrated that butterflies prefer nectars containing amino acids (Alm et al., 1990; Erhardt and Rusterholz, 1998; Goverde et al., 2008; Hawn and Lanza, 2004; Mevi-Schutz and Erhardt, 2002, 2003b, 2004; Mevi-Schutz et al., 2003). In most cases, only female butterflies display preference for the presence of amino acids (Alm et al., 1990; Erhardt and Rusterholz, 1998; Goverde et al., 2008; Mevi-Schutz and Erhardt, 2002, 2003b, 2004; Mevi-Schutz et al., 2003). These preferences for nectar derived amino acids may differ due to larval nutrition, mating history, and nutritional demands brought on by oogenesis (Mevi-Schutz and Erhardt, 2003b, 2004; Mevi-Schutz et al., 2003). Sex-specific amino acid preferences are likely due to the nutritional demands of egg production (Alm et al., 1990; Erhardt and Rusterholz, 1998;

Mevi-Schutz and Erhardt, 2003b, 2004; Mevi-Schutz et al., 2003), and amino acids acquired by adult feeding can increase female fecundity (Mevi-Schutz and Erhardt, 2005).

The objectives of this study were to determine if: 1) the generalist insect *Vanessa cardui* L. (Lepidoptera: Nymphalidae) exhibits feeding preferences between solutions containing only sugars, or those containing a mixture of sugars and amino acids, and 2) if differences in larval protein and carbohydrate nutrition affects adult *V. cardui* foraging decisions (i.e. alters total adult consumption or preference among nectars containing or lacking amino acids). *V. cardui* was used as a model organism for this study, because it is easily maintained in colony, larvae readily feed on defined artificial diets (Chapter II), and adults avidly feed on nectar (Opler and Krizek, 1984; Scott, 1986). Females eclose without mature eggs and adults live for a relatively long time (>2 mo., pers. obs.). Therefore, adults have sufficient opportunity to acquire resources through nectar feeding.

I expected female *V. cardui* to exhibit preference for sugar and amino acid nectar versus sugar-only nectar, while males would not exhibit feeding preference, because only females in the majority of species studied to date have exhibited sugar and amino acid nectar preference (Alm et al., 1990; Erhardt and Rusterholz, 1998; Goverde et al., 2008; Mevi-Schutz and Erhardt, 2002, 2003b, 2004; Mevi-Schutz et al., 2003). I also predicted that larval nutrition would affect the proportion of sugar and amino acid nectar consumption by females due to other butterflies adjusting foraging decisions based on the nitrogen content of leaves on which they were reared (Mevi-Schutz and Erhardt, 2003b; Mevi-Schutz et al., 2003). I did not expect butterflies from different backgrounds to differ in total nectar consumption, as larval diet did not affect daily consumption for the butterfly *Araschnia levana* L. (Nymphalidae) (Mevi-Schutz and Erhardt, 2005).

MATERIALS AND METHODS

Insect culture

A colony of *Vanessa cardui* originating from > 300 original eggs purchased from Shady Oak Butterly Farm (Brooker FL) was continuously maintained under controlled conditions in the lab. Larvae were fed a commercial *Manduca sexta* diet (BioServ Entomology Division, Frenchtown, NJ) and reared individually in clear plastic cups (3 cm H x 4 cm ID) under a 16:8 L/D, 24:24 ^{o}C D/N, 70% RH cycle in a Percival Scientific environmental chamber (Model: I-30BLL, Series 101, Perry, IA). Upon pupation, individuals were hung in a 37W x 36L x 48H cm communal eclosion cage. Upon emergence, adults were transferred to a 58W x 74L x 81H cm flight cage. Adults were provided a constant food source of sucrose and honey water and an oviposition source of moistened braided cotton rolls (Richmond Dental). Eggs were removed from cotton rolls daily, soaked in 2.5% bleach solution for 2 minutes, and placed within a 100 x 15mm plastic Petri dish on moistened #1 Whatman® filter paper. Dishes containing eggs were held in an environmental chamber at the above conditions until eggs hatched.

Methods for rearing experimental larvae

Larvae were reared as defined under 'insect culture' until they reached the final (5th) instar. *V. cardui* consume 98% of total ingested food during the last two instars and 76% of total ingested food during the final instar alone (Poston et al., 1978). Upon moulting to the final instar, larvae were weighed and randomly assigned to one of three dietary treatments. Larvae were placed individually in clean clear plastic cups (3 cm H x 4 cm ID) and provided with one of three chemically defined diets that contained casein and sucrose as the only digestible sources of protein and carbohydrate, respectively. The experimental treatment diets were a modification of one initially created for the larvae of *M. sexta* (Ahmad et al., 1989). Diets had nutrient ratios of 5 parts casein to 1 part sucrose (protein-biased), equal parts casein and sucrose (equal-ratio), and 1 part casein to 5 parts sucrose (carbohydrate-biased). Results from Chapter II demonstrated that diets of unequal nutrient ratios negatively affected larval growth and development, while the equal-ratio diet was optimal for larval growth and development. All experimental diets contained a total combined content of 120g casein and sucrose per liter and were equivalent in the total energy provided; protein and carbohydrate are approximately equivalent in energy released during metabolism (West et al., 1970). Diets also contained 20g agar, 850ml water, 14g Wesson's salt mixture, 4g cholesterol, 5g ascorbic acid, .25g inositol, 2g sorbic acid, 2g methylparabenzoate, 1g choline chloride, 10ml vitamin mixture, and 20ml formaldehyde per liter. Treatment diets were exchanged every 3 days until pupation occurred. Twenty-four hours after pupation, insects were weighed on a Sartorius Research© microbalance (Model: R200D, Goettingen, Germany) to the nearest .01 mg. Sex was determined by noting the sex mark located on the 9th abdominal sternite (Scott, 1986). Individual pupae were hung in 7cm x 10cm clear plastic tubing until eclosion. Adults were then subjected to nectar preference trials or nectar consumption trials (see methods below).

Nectar solutions

Studies evaluating adult nectar preference and consumption used nectar mimics containing the ratios and amounts of sugars and amino acids present in *Lantana camara* L. (Verbenaceae) nectar (Alm et al., 1990). Using *L. camara* nectar mimic has several advantages (Alm et al., 1990; Erhardt and Rusterholz, 1998): 1) the sugar and amino acids composition are known, 2) *L. camara* is frequently visited by butterflies, including *V. cardui*, 3) the floral nectar amino acid concentration is high (16µmoles/ml, corresponding to 9 to 10 on the histidine scale), and 4) multiple studies have used this nectar mimic (Alm et al., 1990; Erhardt, 1992; Erhardt and Rusterholz, 1998; Lanza et al., 2003; Mevi-Schutz and Erhardt, 2002, 2003a) allowing results to be compared. Two mimics were used in these studies. The full *L. camara* nectar mimic (denoted as sugar and amino acid) contained 187.25 g sucrose, 58.0 g glucose, and 57.0 g fructose as well as 0.064 g alanine, 0.032 g arginine, 0.056 g asparagine, 0.048 g glutamic acid, 0.136 g glutamine, 0.178 g glycine, 0.256 g proline, 0.144 g serine, 0.080 g threonine, 0.040 g tyrosine, and 0.016 g valine per liter water (Alm et al., 1990). The other nectar solution (denoted as sugar) lacked amino acids.

Initial nectar preference trials

In order to determine if larval dietary nutrient ratio affects adult feeding preference when given a choice between nectar containing only sugar and nectar containing sugar and amino acids, adults derived from each experimental larval diet (carbohydrate-biased, equal-ratio, protein-biased) were subjected to initial nectar preference trials 24 hrs after eclosion. *V. cardui* are able to discriminate between nectars when in simultaneous tarsal contact with two solutions (Hainsworth, 1989). When simultaneously evaluating a choice of two nectars with the tarsi, adults will feed preferentially upon one of the solutions. Therefore, to evaluate butterfly nectar preferences, tarsi of adults were simultaneously placed in contact with both nectar mimics, and the initial probing direction was noted. Two Petri dish covers (72 mm ID x 7 mm H) were filled with nectar solution. One dish was filled with the sugar-only solution while the other was filled with the sugar plus amino acid solution. The solutions were placed side by side, and an individual butterfly was held between them so that tarsi from one side of the animal were in contact with the sugar solution while tarsi from the other side of the body were simultaneously in contact with sugar and amino acid solution. It was then noted whether initial proboscis extension was toward one solution or the other. The nectar solutions were alternated regularly to account for positional effects.

Nectar consumption trials

Upon eclosion, 30 adults (15 of each sex) from each of the three larval diets were placed into individual flight cages consisting of clear plastic water bottles (22.86cm x 12.7cm x 12.7cm) filled 2 cm high with plaster of Paris to assure a level lower surface. Each cage was surrounded by individual white paper "blinders" to minimize outside visual inputs and normalize visual input within a cage. Each individual butterfly was simultaneously presented with both nectar mimics. Nectar consumption was measured through the use of a flower-mimic apparatus that presented two artificial flowers at the same height. Flowers (29 mm D) were constructed out of red cardstock using a floral craft punch and a single hole punch to create an inner opening (6 mm D) (Figure 3.1). One apparatus was placed within each cage. Cardstock flowers were affixed with hot glue to the ends of clear vinyl tubing that was attached to graduated pipettes with precision of measurement to 10µl. One pipette per apparatus was filled with sugar-only nectar, while the other was filled with sugar and amino acid nectar. Cardstock flowers were dipped into the respective nectar solutions to which they represented and allowed to dry for two days prior to securing to the plastic tubing at the beginning of the experiment. This assured that butterflies would be able to taste each nectar mimic when alighting upon the flowers, thereby initiating a probing response.

Butterfly consumption was measured over three, 3-day feeding periods. Nectar levels were filled at the beginning of each period and checked at the end of every three days. The amount of each nectar solution consumed by each individual butterfly was calculated as the difference between the levels measured in each pipette at the beginning and end of each three day feeding period. A control cage was set up without the addition of a butterfly to determine amount of evaporative loss for each feeding period. Cages were rotated 180^0 at the end of each feeding period to minimize effects of vertical visual input. Nectar consumption over the three feeding periods was combined for analysis. Two experimental trials were performed.

Statistical Methods

Adult feeding preferences were analyzed with Chi-square analyses by larval dietary ratio and sex to determine if initial nectar preference differed from random (SAS version 9.2. 2007. SAS Institute Inc. Cary, NC, USA.). Two-way analysis of covariance with pupal mass as the covariate was used to determine the effects of trial and larval dietary nutrient ratio on total nectar consumption and proportion of sugar and amino acid nectar consumption. Females were analyzed separately from males. When a significant treatment effect was detected, differences among treatment means were determined using the Ryan-Einot-Gabriel-Welsch Multiple Range Test. Nectar preference was examined using a one sample t-test to determine if the proportion consumed of sugar and amino acid nectar differed significantly from 50%. An alpha level of 0.05 was used for all statistical tests.

RESULTS

INITIAL NECTAR PREFERENCE

Females reared on the carbohydrate-biased diet were significantly more likely to probe towards the sugar and amino acid nectar solution, while females reared on protein-biased or equal-ratio larval diet did not differ in their choice of nectar rewards (Table 3.1). Males showed no preference with respect to nectar solutions provided regardless of the larval dietary nutrient ratio on which they were reared (Table 3.1).

NECTAR CONSUMPTION TRIALS

Effects of trial and larval diet on total nectar consumption

When two-way ANCOVA with pupal mass as a covariate was initially performed, no significant effect of the covariate or effects of covariate interactions with treatments were shown (Table 3.2). With the lack of covariate effects, a subsequent two-way analysis of variance with trail and larval diets as main effect treatments was performed using nectar consumption as the dependent variable. Trial affected female total consumption, and both trial and larval diet affected male total consumption (Table 3.3). Both females and males consumed significantly more nectar during trial one than during trial two (Figures 3.2A, 3.3A). Females reared on protein-biased diet consumed significantly more nectar than those reared on carbohydrate-biased diet (Figure 3.2B). Males reared on protein-biased diet consumed significantly more nectar than those reared on carbohydrate-biased or equal-ratio diets (Figure 3.3B).

Effects of trial and larval diet on proportion sugar and amino acid nectar consumption

When two-way ANCOVA with pupal mass as a covariate was initially performed, no significant effect of the covariate or effects of covariate interactions with treatments were shown (Table 3.4). Consequently, a subsequent two-way analysis of variance was performed using proportion sugar and amino acids nectar consumption as the dependent variable and trial and larval diet as the main effect factors. The proportion of sugar and amino acid nectar consumed by females was unaffected by experimental trial and larval diet, but there was a significant interactive effect of trial and larval diet on this variable (Table 3.5). Females reared on equal-ratio diet consumed a significantly lower proportion of sugar and amino acid nectar than those reared on carbohydrate-biased or protein-biased diet during trial one; however, females from different larval diet backgrounds did not differ in proportion sugar and amino acid consumption during trial two (Figure 3.4C). The proportion of sugar and amino acid nectar consumed by males was unaffected by experimental trial and larval diet; and again, there was an interactive effect of experimental trial and larval diet on this variable (Table 3.5). Males reared on equal-ratio diet consumed a significantly lower proportion of sugar and amino acid nectar than those reared on carbohydrate-biased diet during trial one; males did not differ in proportion sugar and amino acid nectar consumption during trial two, regardless of larval diet on which they were reared (Figure 3.5C).

Effect of larval diet on nectar preference

One-sample t-tests revealed that females reared on equal-ratio diet consumed significantly lower proportions of sugar and amino acid nectar than expected due to random feeding during trial one. The proportion of sugar and amino acid nectar consumed by females did not differ from that expected due to random feeding during trial two (Figure 3.6). Males reared on carbohydrate-biased larval diet consumed significantly higher proportions of sugar and amino acid nectar than expected during trial one. The proportion of sugar and amino acid nectar consumed did not differ from that expected due to random feeding during trial two (Figure 3.7).

DISCUSSION

INITIAL NECTAR PREFERENCE

Only female butterflies reared on carbohydrate-biased diet were found to display initial probing preference for sugar and amino acid solution. In contrast, the likelihood of a male probing toward sugar or sugar and amino acid solution was essentially equal regardless of dietary nutrient ratio on which males were reared. The results of this study support previous results that demonstrated that only females displayed selection for the presence of amino acids (Alm et al., 1990; Erhardt and Rusterholz, 1998; Goverde et al., 2008; Hill and Pierce, 1989; Mevi-Schutz and Erhardt, 2002, 2003b, 2004; Mevi-Schutz et al., 2003; Rusterholz and Erhardt, 2000). Furthermore, the results of observations on initial probing responses indicate that *V. cardui* female preference for sugar and amino acid nectar may be mediated by dietary history. Similar patterns were shown with the butterflies *A. levana* and *Coenonympha pamphilus* L. (Satyridae) (Mevi-Schutz and Erhardt, 2003b; Mevi-Schutz et al., 2003).

NECTAR CONSUMPTION TRIALS

Effects of trial and larval diet on total nectar consumption

The covariate of *V. cardui* pupal mass did not significantly affect total nectar consumption in the current study nor did adult emergence mass affect daily nectar intake for *A. levana* (Mevi-Schutz and Erhardt, 2005). Hainsworth et al. (1991) found a significant positive correlation between *V. cardui* body mass and meal size. Analysis of variance indicated that females reared on protein-biased larval diet consumed greater quantities of nectar than those reared on carbohydrate-biased larval diet. In contrast, female *A. levana* daily consumption was unaffected by larval diet when animals were constrained to larval diets of low or high leaf quality (Mevi-Schutz and Erhardt, 2005), suggesting that total adult nectar consumption may be affected by larval nutrition for some species, but not others. Males in this study consumed more nectar when reared on protein-biased diet than when reared on equal-ratio or carbohydrate-biased diet. Since butterflies reared on protein-biased diet consume significantly less carbohydrate than their optimal carborhydrate intake target (Chapter II), increased nectar consumption may indicate compensatory feeding by adults for carbohydrates. Energy acquired from nectar feeding is vital for the manufacture of eggs (O'Brien et al., 2004), and is used in the synthesis of nonessential amino acids (O'Brien et al., 2005; O'Brien et al., 2002). Boggs and Ross (1993) found that daily egg production and lifespan were directly correlated with daily intake for *Speyeria mormonia* Biosduval (Nymphalidae) fed ad libitum while Mevi-Schutz and Erhardt revealed that total consumption affected total fecundity for *Lasiommata megera* L. (Nymphalidae) (2003a) and that daily amount of nectar consumed by female *Araschnia levana* L. (Nymphalidae) affected longevity (2005). Previous research with *V. cardui* revealed that egg production was linearly dependent on amount of ingested sucrose (Hainsworth et al., 1991). While carbohydrates are important in the manufacture of eggs by females, males also require ample carbohydrates for the energetically expensive maintenance of territories as well as pursuit and courtship of females. In nature *V. cardui* males perch in open areas and will rapidly pursue females that fly through their territory (Scott, 1986).

Effects of trial and larval diet on proportion sugar and amino acid nectar consumption

Proportion sugar and amino acid nectar was unaffected by the covariate of pupal mass, regardless of sex. This is contrary to results found for *A. levana*, in which proportion consumption of sugar and amino acid nectar decreased with increasing pupal mass (Mevi-Schutz and Erhardt, 2003b). Females reared on equal-ratio diet consumed a lower proportion of sugar and amino acid nectar than those reared on carbohydrate-biased or protein-biased diet during trial one. Those from different larval diets did not differ in proportion sugar and amino acid consumption during trial two. Males reared on equal-ratio diet consumed a lower proportion of sugar and amino acid nectar than those reared on carbohydrate-biased larval diet during trial one, but did not differ in proportion sugar and amino acid consumption during trial two. The combined results of both experimental trials indicate that *V. cardui*, regardless of sex, does not alter feeding between nectars either containing or lacking amino acids due to larval nutrition. This is in contrast to what has been found for *A. levana*, which exhibited increased preference for amino acid rich nectar when reared on leaves low in nitrogen content (Mevi-Schutz and Erhardt, 2003b). Furthermore, *C. pamphilus* females reared on fertilized plants exhibited a significant decrease in preference for amino acid rich nectar than those reared on unfertilized plants (Mevi-Schutz et al., 2003). Female flesh flies *Sarcophaga bullata* Parker (Diptera:Sarcophagidae) exhibited preference for sugar and amino acid nectar over sugar nectar, but only if animals were deprived of alternate sources of protein (Rathman et al., 1990). These results indicate that larval nutrition can affect the preference for amino acid rich nectar and that increased amino acid preferences may result due to deficiencies in protein intake.

Effect of larval diet on nectar preference

Larval diet did not consistently affect *V. cardui* nectar preference between experimental trials. Females reared on equal-ratio diet consumed a significantly lower proportion of sugar and amino acid nectar than expected during trial one, but not during trial two. Furthermore, males reared on the carbohydrate-biased larval diet consumed a greater proportion of sugar and amino acid nectar than expected during trial one, but not during trial two. Results of both experimental trials, when taken together, indicate that *V. cardui* do not exhibit a strong preference for nectars containing amino acids, regardless of nutrient ratio of the larval diet. It is unclear why nectar consumption patterns did not reflect initial nectar preferences. Butterflies preferred nectars containing amino acids when simultaneously tasting both nectar solutions, but artificial flowers were separated. If animals preferred one nectar solution to the other, yet were unable to learn which flower contained the preferred solution, then consumption trials may not reflect this preference. It should be noted that the task of associating the preferred solution with the correct flower would be made difficult in this experimental setup due to the identical visual presentation of the two nectar sources. Future studies may address whether the presentation of nectar solutions using different visual cues would provide different results.

In conclusion, I found that initial nectar preferences by *V. cardui* were affected by larval dietary ratio on which butterflies were reared for females, but not for males. This is consistent with previous research which indicates that females, but not males, select for nectars containing amino acids. This altered feeding response appears to be limited to initial probing responses. Butterflies from different larval diets did not display any consistent preference as estimated by nectar consumption for or avoidance of *L. camara* nectar mimic containing amino acids between experimental trials, although this may have been due to animals being unable to associate the preferred nectar with the correct source. To test this hypothesis, future studies should present nectar solutions using different visual cues to aid in butterfly learning.

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TABLES AND FIGURES

Table 3.1 Summary of Chi-square analyses demonstrating the effects of larval dietary nutrient ratio on butterfly probing responses toward solutions containing either sugars only (S), or sugars + amino acids (SA).

Initial Probing Direction				
Larval diet	N	Frequency		*P*
Females		S	SA	
Carbohydrate-biased	28	7	21	**0.0082**
Equal-ratio	22	9	13	0.3938
Protein-biased	21	13	8	0.2752
Males				
Carbohydrate-biased	37	18	19	0.8694
Equal-ratio	33	16	17	0.8618
Protein-biased	33	19	14	0.3841

Table 3.2 ANCOVA Effects of trial and larval dietary nutrient ratio on total consumption with all treatment effects and interactions included.

Dependent Variable	df	Mean Square	*F*	*P*
Total Nectar Consumption				
Females				
Trial	1	0.00100	0.13	0.7161
Larval Diet	2	0.00180	0.24	0.7875
Trial x Larval Diet	2	0.00375	0.50	0.6092
Pupal Mass	1	0.00347	0.46	0.4986
Pupal Mass x Trial	1	0.00325	0.43	0.5126
Pupal Mass x Larval Diet	2	0.00211	0.28	0.7562
Pupal Mass x Trial x Larval Diet	2	0.00380	0.51	0.6048
Error	68	0.00751		
Males				
Trial	1	0.00301	0.47	0.4944
Larval Diet	2	0.00463	0.73	0.4874
Trial x Larval Diet	2	0.00950	1.49	0.2327
Pupal Mass	1	0.00920	1.44	0.2339
Pupal Mass x Trial	1	0.00096	0.15	0.6997
Pupal Mass x Larval Diet	2	0.00474	0.74	0.4797
Pupal Mass x Trial x Larval Diet	2	0.00903	1.41	0.2496
Error	75	0.00639		

Table 3.3 ANOVA Effects of trial and larval diet on total nectar consumption.

Dependent Variable	df	Mean Square	*F*	*P*
Total Nectar Consumption				
Females				
Trial	1	0.23148	32.35	<0.0001
Larval Diet	2	0.01964	2.75	0.0708
Trial x Larval Diet	2	0.00254	0.35	0.7029
Error	74	0.00716		
Males				
Trial	1	0.19019	29.16	<0.0001
Larval Diet	2	0.05948	9.12	0.0003
Trial x Larval Diet	2	0.00761	1.17	0.3164
Error	81	0.00652		

Table 3.4 ANCOVA Effects of trial and larval diet on proportion sugar and amino acid nectar consumption with all treatment effects and interactions included.

Dependent Variable	df	Mean Square	*F*	*P*
Proportion Sugar and Amino Acid Nectar Consumption				
Females				
Trial	1	0.00199	0.12	0.7299
Larval Diet	2	0.03871	2.33	0.1046
Trial x Larval Diet	2	0.03380	2.04	0.1381
Pupal Mass	1	0.01266	0.76	0.3853
Pupal Mass x Trial	1	0.00812	0.49	0.4863
Pupal Mass x Larval Diet	2	0.05254	3.17	0.0483
Pupal Mass x Trial x Larval Diet	2	0.03390	2.04	0.1374
Error	68	0.01658		
Males				
Trial	1	0.01482	0.41	0.5219
Larval Diet	2	0.04408	1.23	0.2977
Trial x Larval Diet	2	0.00689	0.19	0.8252
Pupal Mass	1	0.00139	0.04	0.8443
Pupal Mass x Trial	1	0.01382	0.39	0.5362
Pupal Mass x Larval Diet	2	0.04829	1.35	0.2657
Pupal Mass x Trial x Larval Diet	2	0.00871	0.24	0.7847
Error	75	0.03579		

Table 3.5 Effects of trial and larval diet on proportion sugar and amino acid nectar consumption. Reduced models.

Dependent Variable	df	Mean Square	*F*	*P*
Proportion Sugar and Amino Acid Nectar Consumption				
Females				
Trial	1	0.02681	1.48	0.2282
Larval Diet	2	0.01638	0.90	0.4102
Trial x Larval Diet	2	0.08423	4.64	0.0127
Error	74	0.01816		
Males				
Trial	1	0.04080	1.17	0.2831
Larval Diet	2	0.00311	0.09	0.9149
Trial x Larval Diet	2	0.20008	5.73	0.0047
Error	81			

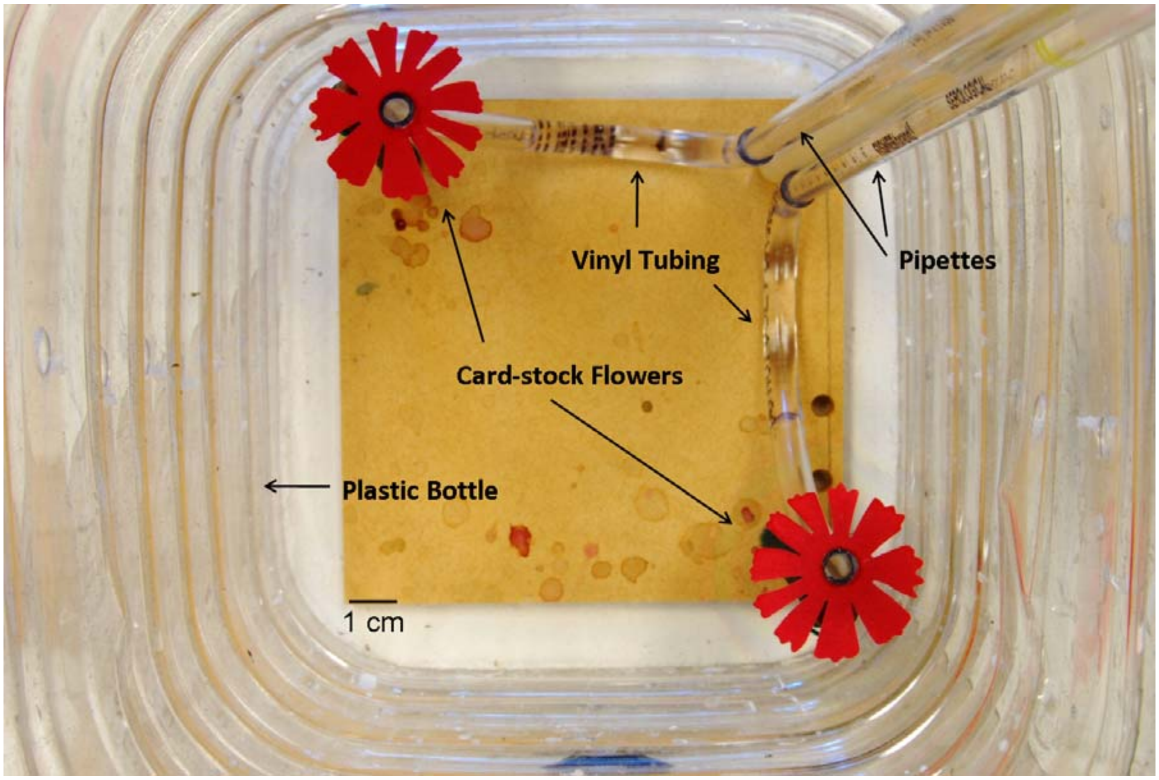

Figure 3.1 Top view of feeding apparatus used in nectar consumption trials. Flowers were created out of red cardstock using a floral crafting punch (3 cm outer diameter). Cardstock flowers were hot glued to the ends of clear plastic vinyl tubing that was attached to graduated pipettes with precision of measurement to 10 µl.

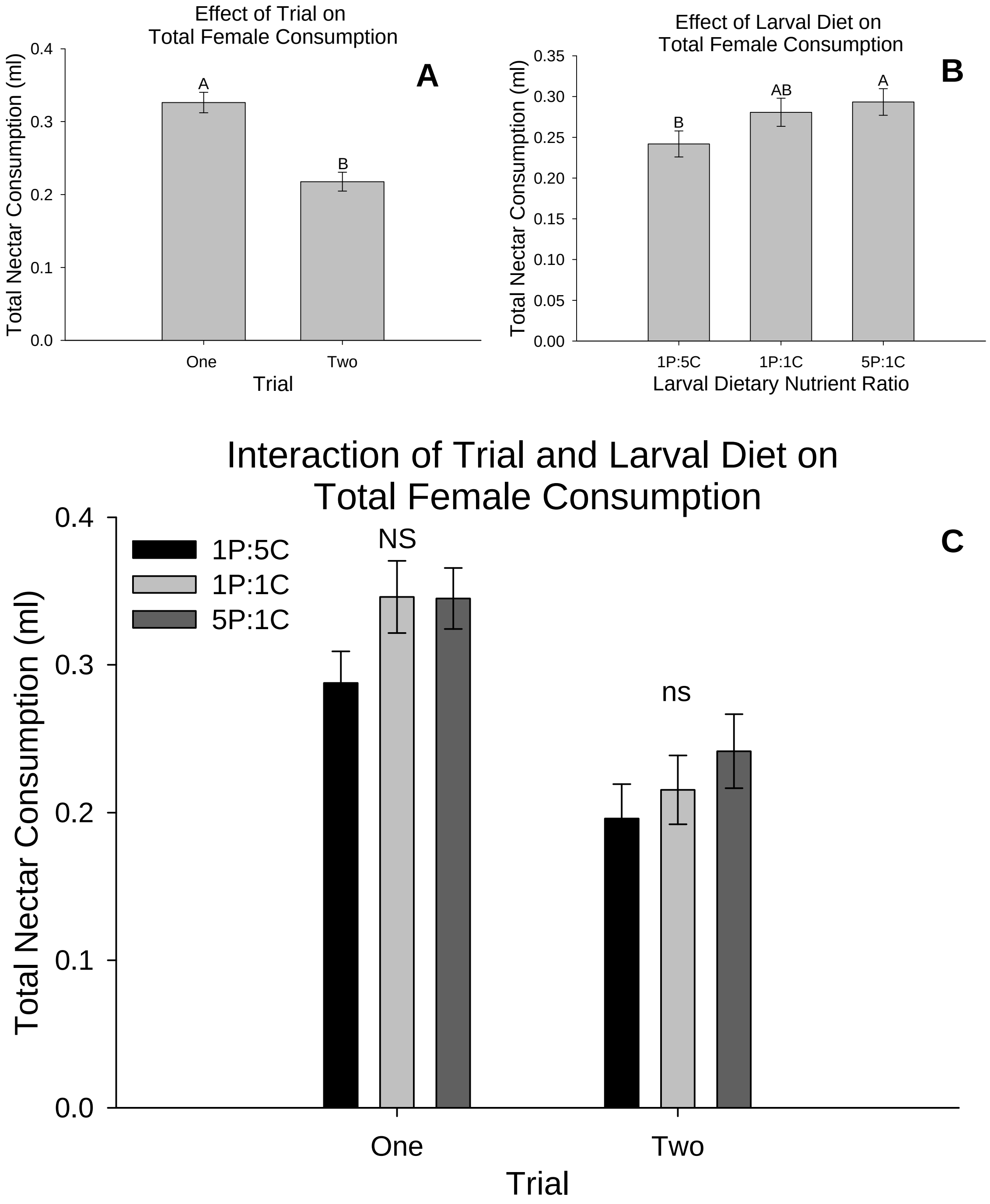

Figure 3.2 Effects of trial (A), larval diet (B), and interaction (C) on total female nectar consumption. Data are least squares means ± 1 S.E. Significant differences are marked by different letters.

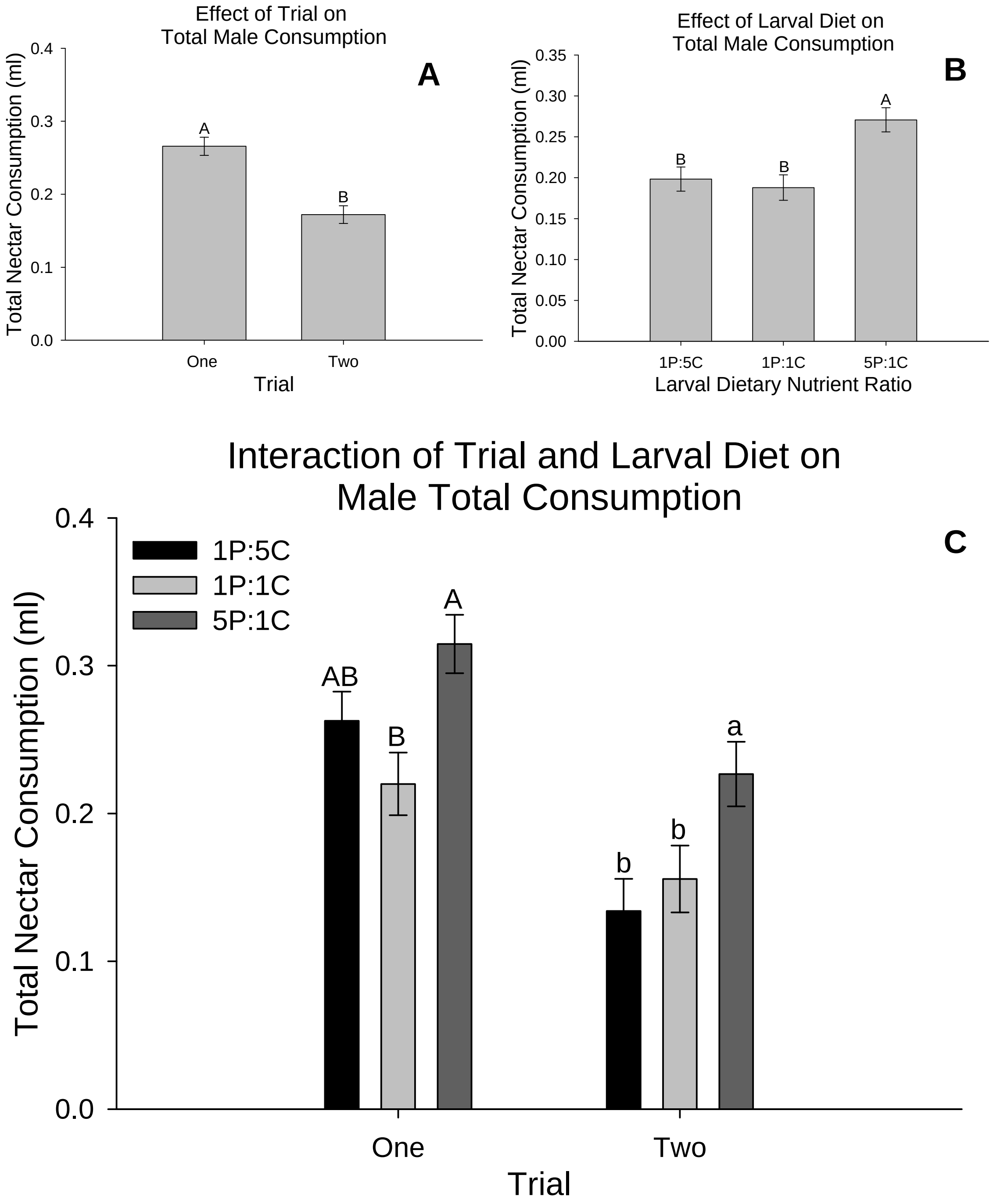

Figure 3.3 Effects of trial, larval diet, and interaction on total male nectar consumption. Data are least squares means ± 1 S.E. Significant differences are marked by different letters.

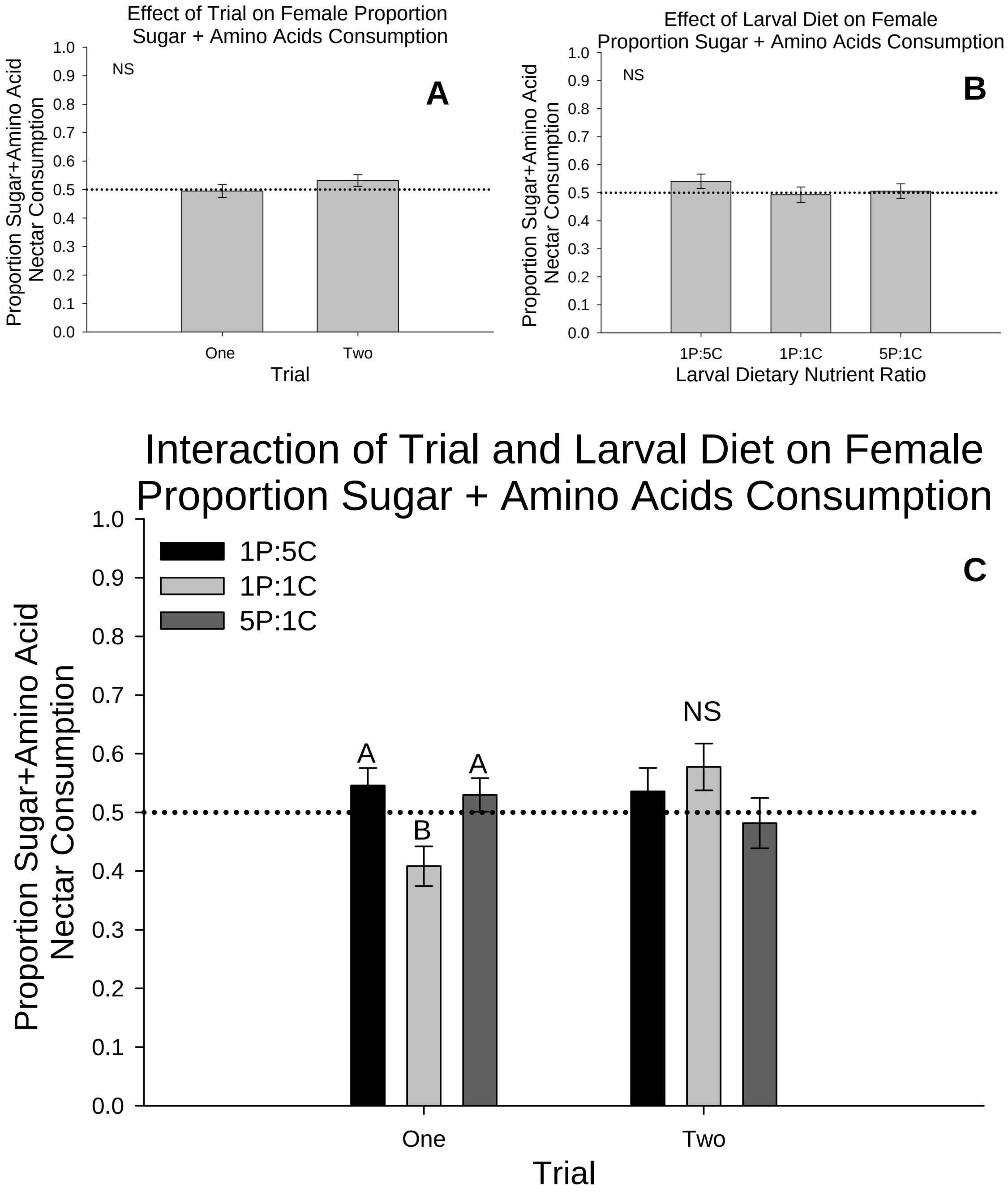

Figure 3.4 Effects of trial (A), larval diet (B), and interaction (C) on female proportion sugar and amino acid nectar consumption. Data are least squares means ± 1 S.E. Significant differences are marked by different letters.

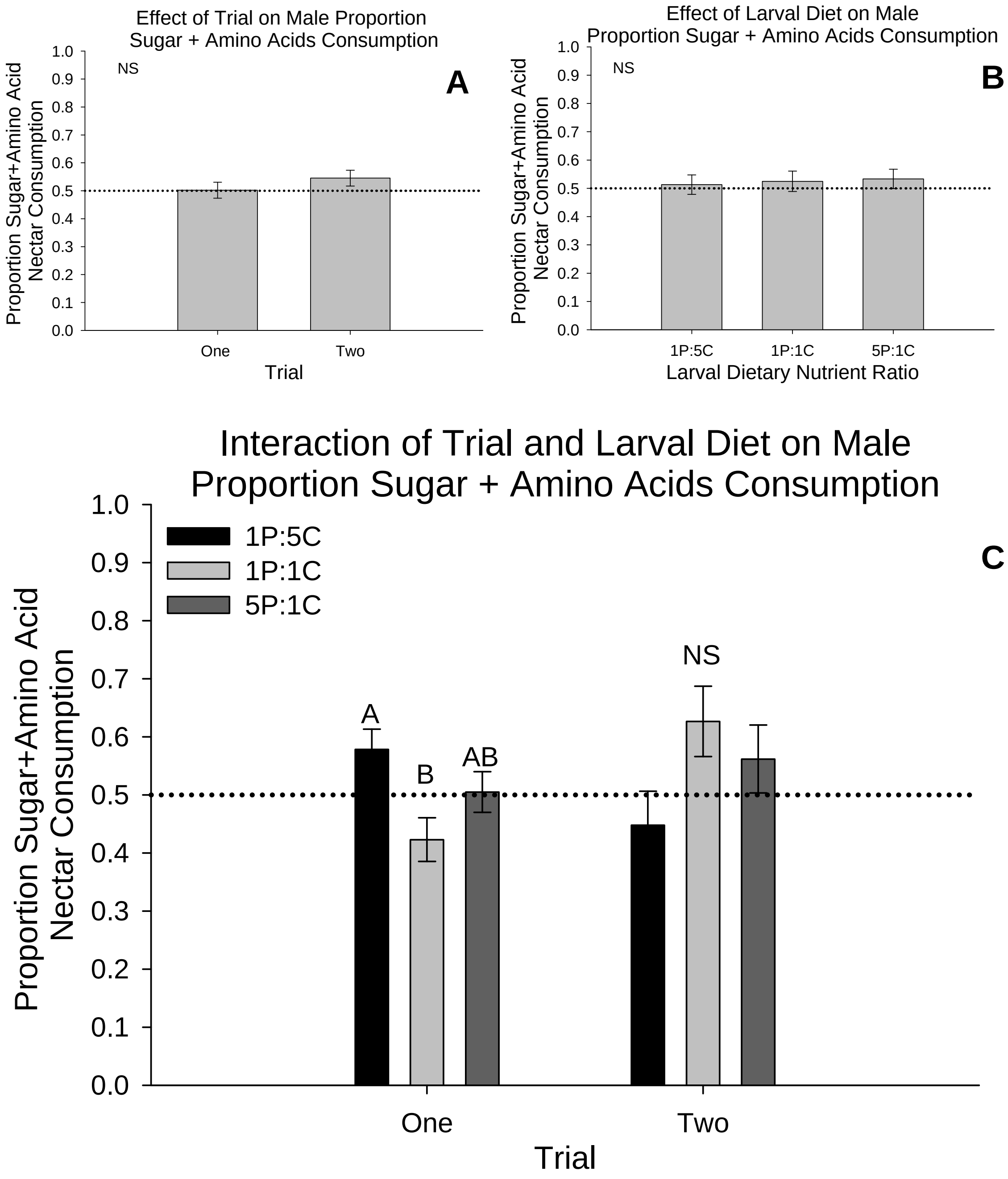

Figure 3.5 Effects of trial (A), larval diet (B), and interaction (C) on male proportion sugar and amino acid nectar consumption. Data are least squares means ± 1 S.E. Significant differences are marked by different letters.

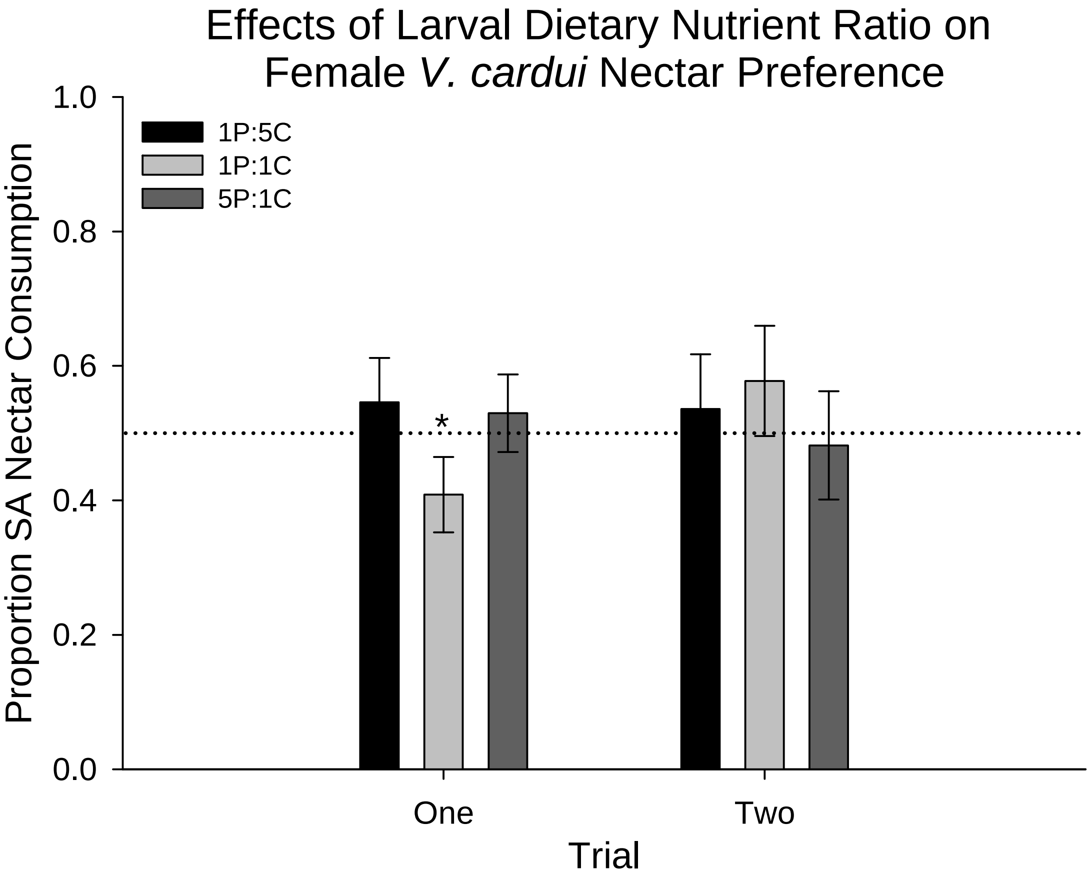

Figure 3.6 Effects of larval diet on female *V. cardui* nectar preference. Data are mean proportion sugar plus amino acid nectar consumption ± 2 S.E. Asterisks denote significant differences from random feeding (dotted line).

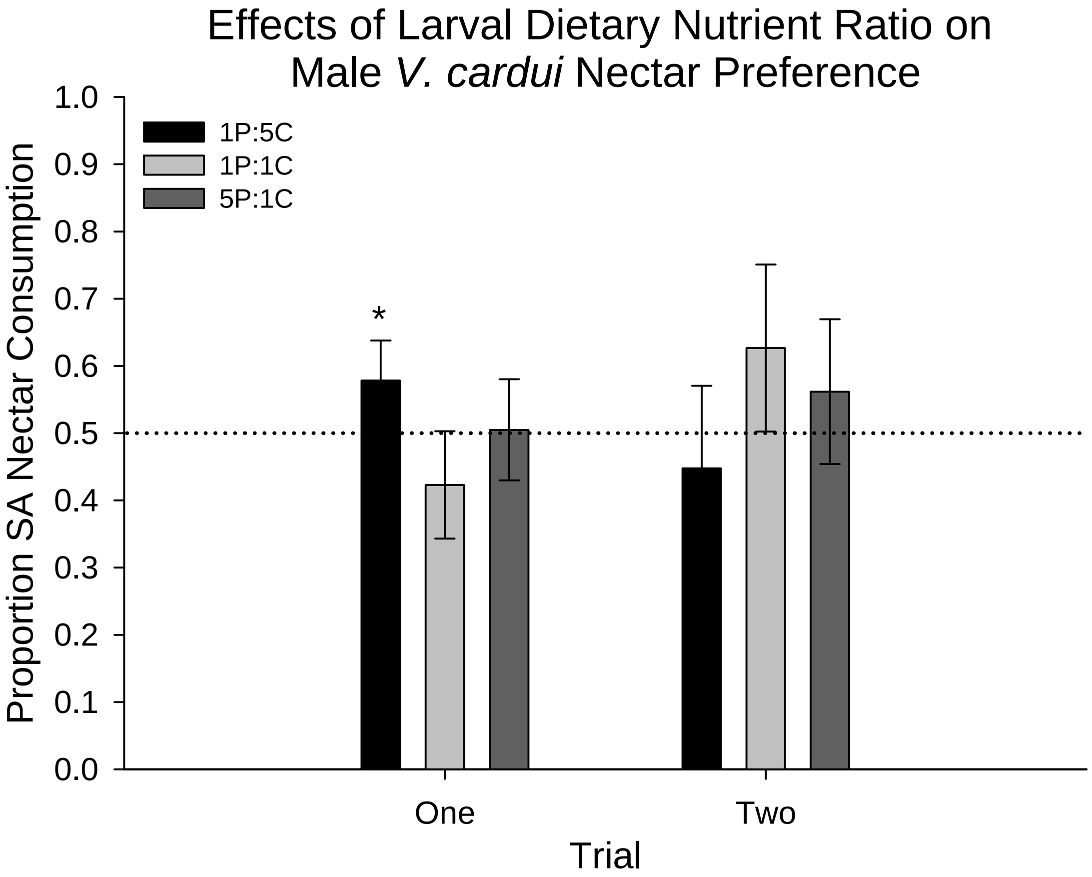

Figure 3.7 Effects of larval diet on male *V. cardui* nectar preference. Data are mean proportion sugar plus amino acid nectar consumption ± 2 S.E. Asterisks denote significant differences from random feeding (dotted line).

CHAPTER IV

THE EFFECTS OF VARIABLE LARVAL AND ADULT NUTRITION ON THE FECUNDITY AND LONGEVITY OF *VANESSA CARDUI* L. (LEPIDOPTERA: NYMPHALIDAE)

SUMMARY

Butterfly fitness is affected by both larval and adult nutrition, but most studies to date only focus on fitness effects of one or the other as opposed to varying larval and adult nutrition in tandem. The aim of this study was to determine the relative importance of larval and adult dietary nutrient intake on *Vanessa cardui* fitness through the use of artificial diets. This study specifically addressed the importance of larval protein and carbohydrate intake, and adult carbohydrate and amino acid intake on overall female fecundity and longevity. I determined that adult nutrition plays a greater role in realized fitness than larval nutrition for *V. cardui*, but that larval dietary history mediates the effects of adult diet. Butterflies maintained egg production for longer when adults were given sugar, whether alone or in combination with amino acids, than when they were not. Butterflies reared on a diet of equal-ratio protein and carbohydrate performed best when provided only sugar as adults while the addition of amino acids negatively affected total egg production.

INTRODUCTION

Lepidopteran fitness studies are often limited to the juvenile stages and do not consider effects of larval nutrition on adult fecundity and longevity (Karowe and Martin, 1989; Lee and Roh, 2010; Lindroth et al., 1991; Morehouse and Rutowski, 2010; Wheeler and Halpern, 1999). Suboptimal larval diets often negatively affect pupal mass (Lee et al., 2004; Lee et al., 2002; Lindroth et al., 1991) (see also Chapter II). Pupal mass may be strongly correlated with fecundity (Awmack and Leather, 2002; Honek, 1993), and adult body mass and wing length in many species decreases in individuals under larval food stress (Nylin and Gotthard, 1998). Adult mass from larval reserves correlates with longevity (Karlsson and Wickman, 1990), and larval dietary restriction can negatively affect butterfly longevity (Boggs and Freeman, 2005). However, a combination of larval and adult-derived nutrients and energy may be used for reproduction in species that consume resources as adults (Boggs, 1981a). Larval reserves should significantly affect Lepidopteran fecundity when adult resource acquisition is low, but not when high (Oberhauser, 1997).

The relationship between size and fecundity is absent in many Lepidoptera (Leather, 1988) and may depend on the amount of adult resources acquired for egg production. Species with no or little adult feeding exhibit a quick decline in age-specific fecundity, while those with more substantial adult feeding appear to utilize these sources towards egg production and exhibit a longer plateau of age-specific fecundity (Boggs, 1987; Erhardt and Rusterholz, 1998). Adult nutritional intake may play a role in production of unyolked eggs (Boggs, 1986; Erhardt and Rusterholz, 1998; Jervis et al., 2005), and may be especially important for Lepidopterans with extended lifespans (Jervis et al., 2005). Carbohydrate intake significantly affects butterfly fitness parameters (Bauerfeind and Fischer, 2005; Boggs and Ross, 1993; Fischer et al., 2004; Hill, 1989; Hill and Pierce, 1989; Murphy et al., 1983; O'Brien et al., 2004), and certain sugars may be more beneficial than others (Romeis and Wackers, 2002). Carbon in nonessential amino acids in eggs may be primarily derived from adult carbohydrate intake (O'Brien et al., 2005; O'Brien et al., 2002). Total adult consumption can affect fecundity (Boggs and Ross, 1993; Hainsworth et al., 1991; Mevi-Schutz and Erhardt, 2003a) and longevity (Mevi-Schutz and Erhardt, 2005).

Butterflies in the neotropical Nymphalid genus *Heliconius* remove amino acids and protein from pollen, and females collect more pollen than males in the field (Gilbert, 1972). Amino acids derived from adult pollen feeding sustain egg production and greatly increase longevity for *H. charithonia* L. (Dunlap-Pianka et al., 1977), and essential amino acids are transferred from pollen to *H. charitonia* eggs (O'Brien et al., 2003). Plant species typically pollinated by butterflies tend to have higher concentrations of amino acids in their floral nectars than those principally pollinated by bees, birds, etc. (Baker and Baker, 1973, 1973a; Baker and Baker, 1977; Baker and Baker, 1985; Baker and Baker, 1986, 1990) which have other dietary sources of nitrogen. Female *Araschnia levana* L. (Nymphalidae) reared on low nitrogen leaves had lower fecundities than females from high nitrogen leaves when fed sugars, but compensated for reduced fecundity when fed sugars and amino acids (Mevi-Schutz and Erhardt, 2005). This indicates that female butterflies can compensate for poor larval nutrition through adult consumption of amino acid rich nectar. Old and new leaf material, however, differ in structural and chemical respects as well as nutritional (Bernays and Chapman, 1994; Shoonhoven et al., 2006).

It is unknown whether larval or adult nutrition affects butterfly fecundity and longevity to a greater degree, and how larval and adult nutrition interact to jointly affect fitness. In light of current knowledge, I expected that butterflies reared on suboptimal diet would have reduced lifetime fecundity and longevity. I expected the beneficial effects of adult sugar and sugar plus amino acids consumption to be greatest when larvae were reared on carbohydrate-biased larval diet, and absent or diminished when butterflies were reared on equal-ratio protein to carbohydrate or protein-biased larval diets. Furthermore, I predicted sugar and amino acids diet would increase butterfly fitness relative to sugar diet when butterflies were reared on carbohydrate-biased larval diet, but that there would be no difference in performance between butterflies fed sugar or sugar plus amino acids nectars when larvae were fed equal-ratio protein to carbohydrate or protein-biased larval diets.

The objective of this study was to determine the relative importance of larval and adult dietary nutrient intake on *V. cardui* fitness. This study addresses the importance of larval protein and carbohydrate intake, and adult carbohydrate and amino acid intake on fecundity (total egg production, duration of egg production, mean daily egg production), and longevity.

MATERIALS AND METHODS

Study Insect

The painted lady butterfly *Vanessa cardui* L. (Lepidoptera: Nymphalidae) was used for this study due to several aspects of larval and adult behavior and biology. Larvae are highly polyphagous and feed on over 100 different species of plants (Williams, 1970), move within and between species while feeding (Bernays and Minkenberg, 1997), and will accept the first hostplant encountered if displaced (Garrigan, 1994). This species does not appear to have a host range shaped by any particular chemical compound(s) (Ellis and Bowers, 1998) and is economically important as a pest on soybean, alfalfa, lettuce, and more (Comstock, 1927; Zhang, 1994). *V. cardui* likely rely on resources acquired during the adult stage for maximum fitness. Newly eclosed females do not have mature eggs present, and mating does not occur until 3 days post-eclosion (pers. obs.). Adults avidly feed on floral nectar (Opler and Krizek 1984), live for a relatively long time (>2 mo., pers. obs.), and may mate up to 5 times (pers. obs.). Therefore, females have ample opportunity to acquire resources for development of soma and eggs through nectar feeding and income breeding.

Insect Culture

A colony of *Vanessa cardui* originating from > 300 original eggs purchased from Shady Oak Butterly Farm (Brooker FL) was continuously maintained under controlled conditions in the lab. Larvae were fed a commercial *Manduca sexta* L. (Lepidoptera: Sphingidae) diet (BioServ Entomology Division, Frenchtown, NJ) and reared individually in clear plastic cups (3 cm H x 4 cm ID) under a 16:8 L/D, 24:24 ^{o}C D/N, 70% RH cycle in a Percival Scientific environmental chamber (Model: I-30BLL, Series 101, Perry, IA). Upon pupation, individuals were hung in a

37W x 36L x 48H cm communal eclosion cage. Upon emergence, adults were transferred to a 58W x 74L x 81H cm flight cage. Adults were provided a constant food source of sucrose and honey water and an oviposition source of moistened braided cotton rolls (Richmond Dental). Eggs were removed from cotton rolls daily, soaked in 2.5% bleach solution for 2 minutes, and placed within a 100 x 15mm plastic Petri dish on moistened #1 Whatman® filter paper. Dishes containing eggs were held in an environmental chamber at the above conditions until eggs hatched.

Rearing experimental larvae

Larvae were reared as defined under 'Insect Culture' until they reached the final (5th) instar. Upon molting to the final instar, larvae were weighed and randomly assigned to one of three dietary treatments. Larvae were placed individually in clean clear plastic cups (3 cm H x 4 cm ID) and provided with one of three chemically defined diets that contained casein and sucrose as the only digestible sources of protein and carbohydrate, respectively. The diet was a modification of one initially created for the larvae of *M. sexta* (Ahmad et al., 1989). All diets presented had a total combined content of 120g casein and sucrose per liter. Diets had nutrient ratios of 5 parts casein to 1 part sucrose, equal-ratio casein and sucrose, and 1 part casein to 5 parts sucrose. Results from Chapter II demonstrated that diets of unequal nutrient ratios negatively affected larval fitness, while the equal-ratio diet was optimal for larval growth and development. All diets used were equivalent in total energy, as protein and carbohydrate are approximately equivalent in energy released during metabolism (West et al., 1970). Diets also contained 20g agar, 850ml water, 14g Wesson's salt mixture, 4g cholesterol, 5g ascorbic acid, .25g inositol, 2g sorbic acid, 2g methylparabenzoate, 1g choline chloride, 10ml vitamin mixture, 4ml linseed oil, and 20ml formaldehyde per liter. Diets were changed every 3 days. Sex was determined 24 hrs post-pupation by noting the sex mark located on the 9th abdominal sternite

(Scott, 1986). Pupae were separated and hung in eclosion chambers according to larval diet and sex.

Adult Diets

Adults were supplied one of four different solutions, consisting either of: 1) water alone, 2) amino acids, 3) sugar, or 4) sugar and amino acids. Sugars and amino acids supplied in solution were based on the ratios and concentrations found in *Lantana camara* L. (Verbenaceae) floral nectar (Alm et al., 1990). Using *L. camara* nectar mimic has several advantages (Alm et al., 1990; Erhardt and Rusterholz, 1998): 1) the sugar and amino acids composition are known, 2) *L. camara* is frequently visited by butterflies, including *V. cardui*, 3) the floral nectar amino acid concentration is high (16µmoles/ml, corresponding to 9 to 10 on the histidine scale), and 4) multiple studies have used this nectar mimic (Alm et al., 1990; Erhardt, 1992; Erhardt and Rusterholz, 1998; Lanza et al., 2003; Mevi-Schutz and Erhardt, 2002, 2003a) allowing results to be compared. The full *L. camara* nectar mimic (denoted as sugar and amino acids) contained 187.25 g sucrose, 58.0 g glucose, and 57.0 g fructose as well as 0.064 g alanine, 0.032 g arginine, 0.056 g asparagine, 0.048 g glutamic acid, 0.136 g glutamine, 0.178 g glycine, 0.256 g proline, 0.144 g serine, 0.080 g threonine, 0.040 g tyrosine, and 0.016 g valine per liter water (Alm et al., 1990).

Effects of nutrition on butterfly fitness

Adult male-female butterfly pairs from each larval diet (protein-biased, equal-ratio, carbohydrate-biased) were randomly assigned to four adult diet treatments. Pairs were assigned to individual clear plastic water bottles (22.86cm x 12.7cm x 12.7cm) filled 2 cm high with plaster of Paris to assure a level lower surface. Males were marked with a circle on the left ventral hind-wing using black non-toxic Sharpie® marker while females remained unmarked. Adult diet was supplied *ad libitum* in Falcon® 35x10mm dishes containing four blue glass marbles to elicit butterfly attraction (Romeis and Wackers, 2002) while preventing butterflies from becoming stuck in solution. Diets were replenished each day and replaced every two days. The combination of 3 larval diet levels (carbohydrate-biased, equal-ratio, protein-biased) and 4 adult diet levels (water, amino acids, sugar, sugar and amino acids) resulted in 12 treatments overall (Table 4.1).

Butterflies were provided substrate of moistened 3cm x 5cm cotton squares (Target Brand®) on which females readily oviposit, thus reducing variability in oviposition due to variation in substrate chemistry, size, or shape. Total egg production, duration of egg production, and daily egg production (calculated as total egg production divided by number of days that eggs were laid) and female longevity were recorded. Only butterfly pairs that produced eggs were included in analyses. Three trials of this experiment were performed.

General Statistical Methods

Three-way analysis of variance was employed to examine the main effects of trial, larval diet, adult diet and interactions on the butterfly fitness variables total egg production, duration of egg production, and daily egg production. The effects of larval and adult diet on total egg production were also examined while accounting for duration of egg production and female longevity by including these as covariates. The effects of larval and adult diet on duration of egg production and daily egg production were also examined while accounting for female longevity. Female longevity was analyzed through Survival Analysis. All data were analyzed using SAS (Version 9.2. 2007. SAS Institute Inc. Cary, NC, USA).

RESULTS

Total Egg Production

Three-way ANOVA revealed that adult diet and the interaction of larval diet and adult diet significantly affected total egg production while trial and larval diet did not (Table 4.2). The model was reduced to include only significant terms (Table 4.3), and post-hoc Tukey-Kramer multiple comparisons revealed that butterflies given sugar or sugar and amino acids solution produced significantly more eggs than those given water or amino acids alone (Figure 4.1B). Animals reared on carbohydrate-biased and protein-biased diets did not significantly differ in total egg production due to adult diet, while those reared on equal-ratio diet produced significantly more eggs when provided sugar than when provided water, amino acids, or sugar and amino acids (Figure 4.1C).

When accounting for duration of egg production and longevity, larval diet, adult diet, and the interaction between larval and adult diet significantly affected total egg production, while trial did not (Table 4.4). The model was reduced to include only significant terms (Table 4.5), and post-hoc Tukey-Kramer multiple comparisons revealed that butterflies reared on carbohydrate-biased diet laid significantly fewer eggs than those reared on equal-ratio or protein-biased diet (Figure 4.2A). Butterflies given either sugar or sugar and amino acids laid significantly more eggs than those given water or amino acids alone (Figure 4.2B). Animals reared on carbohydrate or protein-biased diets did not differ in total egg production regardless of adult diet provided, while those reared on equal-ratio diet laid significantly more eggs when given sugar than when given water, amino acids, or sugar and amino acids (Figure 4.2C).

Duration of egg production

Adult diet significantly affected the duration of egg production while trial and larval diet did not (Table 4.2). Non-significant terms were excluded from the model (Table 4.3), and post-hoc Tukey-Kramer multiple comparisons revealed that animals given sugar or sugar and amino acids solution produced eggs for significantly longer than those given water or amino acids alone (Figure 4.3B). When accounting for female longevity, adult diet significantly affected duration of egg production while larval diet and the interaction of larval and adult diet did not (Table 4.6).

Mean Daily Egg Production

Daily egg production was unaffected by trial, larval diet, and adult diet (Table 4.2). As trial did not affect daily egg production, it was removed from the model and a two-way ANOVA was performed on the effects of larval and adult diet on daily egg production. Daily egg production was unaffected by larval diet, adult diet, and the interaction of larval and adult diet (Table 4.3, Figure 4.4). When accounting for female longevity, adult diet significantly affected daily egg production while larval diet and the interaction of larval and adult diet did not (Table 4.6). Post-hoc Tukey-Kramer multiple comparisons revealed that butterflies given sugar or sugar and amino acids laid significantly more eggs per day than those given water (Figure 4.5B).

Female Longevity

Effects of larval diet

Survival analysis revealed that females reared on protein-biased diet initially died at a slower rate than those reared on carbohydrate-biased and equal-ratio diets (Wilcoxon P=0.0486, Figure 4.6). This trend did not continue throughout the experiment, as females reared on protein-biased diet subsequently died at a greater rate than those reared on carbohydrate-biased and equal-ratio diets, causing survival curves to converge later in the experiment (Log-Rank P = 0.1137, Figure 4.6).

Effects of Adult Diet

Adult diet had a strong effect on female survival throughout the entire experiment (Wilcoxon and Log-Rank $P < 0.0001$). Females given only water or amino acids died at a significantly greater rate than those provided with sugar or sugar and amino acids (Figure 4.7).

Interaction of larval and adult diet

Adult diet had a strong effect on female survival, regardless of the larval diet on which females were reared (Wilcoxon and Log-Rank $P < 0.0001$). Females provided with sugar or sugar and amino acids lived significantly longer than those given water or amino acids alone (Figure 4.8).

DISCUSSION

Total Egg Production

The expectation that larval diet would significantly affect total egg production was unsupported when performing ANOVA due to the strong effect of adult diet on total egg production, mainly through increasing duration of egg production. When duration of egg production and female longevity were accounted for, however, the effect of larval diet on total fecundity became apparent. Insect size poses a constraint on fecundity (Honek, 1993). *V. cardui* reared on carbohydrate-biased diet have lower pupal mass than those reared on equal-ratio and protein-biased diets (Chapters II, III), and butterflies reared on carbohydrate-biased diet produced significantly fewer eggs than butterflies from equal-ratio and protein-biased diets as expected. In contrast, total egg production by butterflies reared on equal-ratio and protein-biased diets did not differ as expected, although butterflies reared on protein-biased larval diet have significantly lighter pupal mass than butterflies reared on equal-ratio diet (Chapters II, III).

Correlations between female size and fecundity have been found for *Pieris rapae crucivora* Boisduval (Pieridae) (Jones et al., 1982; Suzuki, 1978), *Papilio polyxenes* Fabricius (Papilionidae) (Lederhouse, 1981), *Euphydryas editha* Boiduval (Nymphalidae) (Jones et al., 1986), *E. chalcedona* Doubleday (Nymphalidae) (Jones et al., 1986), *Malacosoma disstria* Hübner (Lasiocampidae) (Colasurdo et al., 2009) and *Epirrita autumnata* Borkhausen (Geometridae) (Haukioja and Neuvonen, 1985). The relationship between size and fecundity, however, is often not strong or absent for Lepidopterans (Leather, 1988). Butterfly size did not affect fecundity for *Lasiommata megera* L. (Nymphalidae) (Wiklund and Karlsson, 1984), *Danaus plexippus* L. (Nymphalidae) (Oberhauser, 1997; Svard and Wiklund, 1988), *Pieris napi*

L. (Pieridae) (Wiklund and Kaitala, 1995), *Speyeria mormonia* Edwards (Nymphalidae) (Boggs, 1986) or *Pararge aegeria* L. (Nymphalidae) (Wiklund and Persson, 1983).

Adult *V. cardui* with access to carbohydrates, whether alone or in combination with amino acids laid significantly more eggs than those lacking carbohydrate intake, which supports predictions. Adult access to carbohydrates often increases butterfly fecundity. Female *P. aegeria* provided carbohydrates had increased lifetime fecundity over those given water alone (Karlsson and Wickman, 1990). Fecundity was greater for *Phthorimaea operculella* Zeller (Gelechiidae) fed 10% honey-water solution than for those fed water alone (Jordao et al., 2010). Adult resource stress negatively affected *S. mormonia* fecundity (Boggs and Ross, 1993). Butterflies given amino acids alone did not differ in total egg production from butterflies given water as expected. Lifetime fecundity was not significantly different between *Bicyclus anynana* Butler (Nymphalidae) given amino acids versus those given water alone (Bauerfeind and Fischer, 2005).

I predicted that butterflies reared on carbohydrate-biased diet would benefit from sugar and amino acids versus sugar alone, due to compensatory effects of amino acids on protein deprived butterflies (Mevi-Schutz and Erhardt, 2005). However, butterflies reared on carbohydrate-biased larval diet did not differ in total egg production, regardless of adult diet provided. Adding amino acids to sugars did not increase fecundity above *B. anynana* fed sugars only (Molleman et al., 2008). Romeis and Wackers (2002) found that adult diet significantly affected butterfly fitness, while amino acids had no to little effect. Amino acids did not affect total egg production by *L. megera* (Mevi-Schutz and Erhardt, 2003a), or fecundity or longevity of *Jalmenus evagoras* Hübner (Lycaenidae) (Hill and Pierce, 1989). I expected that butterflies reared on equal-ratio diet would not differ in fecundity between those given sugar or sugar and amino acids. In contrast, females reared on equal-ratio diet laid significantly more eggs when given sugar than when given sugar and amino acids. This suggests that adult intake of amino acids may negatively affect total fecundity for butterflies reared on equal-ratio diet. When *Euploea core corinna* W. S. Macleay (Nymphalidae) were fed sugar and amino acids, fecundity was reduced compared to butterflies fed sugar alone, whether on a 25% or 1% sugar solution (Hill, 1989).

Results of this study are contrary to the predictions that larval reserves should influence fecundity when adult resource intake is low, but not when adult resource contributions are high (Oberhauser, 1997). Larval nutrition affected fecundity when butterflies had access to sugar or sugar and amino acids, but not when adults were constrained to water or amino acids alone. This suggests that *V. cardui* butterflies need adult carbohydrate resources to realize the full potential of their larval reserves. One potential explanation for why adult diet did not affect total egg production when butterflies were reared on carbohydrate-biased diet is that butterflies reared on carbohydrate-biased diet were simply too devoid of protein reserves that females remained unable to increase egg production regardless of adult nutritional intake.

Since consumption was not measured in this study it is possible that sugar and amino acids nectar was less appealing to butterflies than sugar nectar and less was consumed. Total nectar consumption can affect fecundity (Boggs and Ross, 1993; Hainsworth et al., 1991; Mevi-Schutz and Erhardt, 2003a) and longevity (Mevi-Schutz and Erhardt, 2005), and larval diet can alter preference for amino acid enriched nectar (Mevi-Schutz and Erhardt, 2003b; Mevi-Schutz et al., 2003). It is unlikely that butterflies from different larval diets responded differentially to the adult diets provided, however, as *V. cardui* do not exhibit selection between sugar and sugar and amino acids nectar solutions regardless of larval diet on which they are reared (Chapter III). It is uncertain why butterflies reared on protein-biased diet were not negatively affected by sugar and amino acids nectar like butterflies reared on equal-ratio diet, if this difference is physiological. One hypothesis is that being reared on protein-biased diet made butterflies somehow more resistant to the negative effects of amino acids, or that it shifted their physiology to more efficiently utilize nectar derived amino acids. Late instar larvae of the salvinia moth *Samea multiplicalis* Guenee (Pyralidae) on high nitrogen plants had greater nitrogen assimilation efficiencies when reared on high nitrogen plants than when reared on low nitrogen plants during early instars (Taylor, 1989).

Duration of Egg Production

Butterflies did not differ in duration of egg production due to larval diet as predicted. Adult diet affected the duration of egg production as expected, with females provided sugar, whether alone or in combination with amino acids, laying eggs for significantly longer than those lacking carbohydrate intake. This is due to the expectation that animals would survive longer when having access to an adult energy source. Increased female longevity is not the sole factor leading to increased duration of egg production, however, as adult diet still significantly affected duration of egg production when controlling for female longevity.

Mean Daily Egg Production

Contrary to expectations, mean daily egg production was not affected by larval nutrition. When accounting for female longevity, adult nutrition affected mean daily egg production as expected. Butterflies fed sugar or sugar and amino acids solutions produced significantly more eggs per day than those without adult carbohydrate intake.

Female Longevity

Contrary to expectations, larval nutrition did not affect female longevity. Adult grasshoppers *Locusta migratoria* L. (Acrididae) have increased resistance to starvation when reared on carbohydrate-biased diets (Raubenheimer and Simpson, 1997), and larval resource stress reduces adult survival for the butterfly *S. mormonia* (Boggs and Freeman, 2005). Adult derived nutrition may be one of the most important factors affecting fecundity for many

Lepidoptera due to its effect on longevity, with longevity being the single most important factor influencing fecundity (Leather, 1988). As expected, *V. cardui* given sugar or sugar and amino acids solutions survived significantly longer than those without access to carbohydrates. Similarly, adult access to sugars increased longevity for *P. aegeria* (Karlsson and Wickman, 1990), *Pieris brassicae* L. (Pieridae) (Romeis and Wackers, 2002), *B. anynana* (Bauerfeind and Fischer, 2005; Ferkau and Fischer, 2006), and *P. napi* (Ferkau and Fischer, 2006). In contrast, *S. mormonia* longevity is conserved during adult resource stress (Boggs and Ross, 1993). In the current study female *V. cardui* fed sugar and amino acids solution did not differ in longevity from those fed sugar solution. Similarly, no difference in longevity was found between *B. anynana* fed sugar or sugar and amino acids solutions (Molleman et al., 2008).

Some caveats

In this study, adult *V. cardui* were confined to small cages. Differences in fitness were likely dampened compared to what might be seen in larger chambers or in a natural setting. Butterflies under larval or adult resource stress may suffer greater decreases in fitness when flying around to deposit eggs, evade predation, or to find and court mates. Gibbs et al. (2010) found that offspring from female *P. aegeria* forced to fly for three 5 minute intervals on days 0, 4, and 8 of oviposition had lower larval masses and longer development times than those from flight restricted females. It is possible that differential egg provisioning may result from maternal larval or adult resource stress as well, which may exacerbate negative effects of maternal flight stress on offspring survival.

The current study measured four components of female butterfly fitness, including total egg production, duration of egg production, daily egg production, and female longevity. Additional measures such as daily weight loss by butterflies, daily egg mass, fertility, hatchling size, larval growth rate, or larval survival may further elucidate the effects of variable nutritional quality of larval and adult diets on butterfly fitness. For example, larvae hatching from larger eggs may have larger head capsules and therefore may have increased success when feeding on tough foliage (Brady, 1994). Egg size may decreases over time as females age (Gibbs et al., 2010), and it may be informative to determine the effects of larval and adult nutrition on the rate of egg mass decrease over time. In addition to its effects on fecundity and longevity, adult nutrition is positively correlated with egg mass, fertility, and mating success (Boggs, 1987).

Overall, this investigation revealed that adult nutrition is more important in determining realized fitness of *Vanessa cardui* than larval nutrition. Increased fitness by females was primarily due to females with access to carbohydrates as adults, whether alone or in combination with amino acids, living significantly longer than those without access to carbohydrates and maintaining egg production for a longer period of time. This was clearly demonstrated by larval diet affecting total egg production when duration and female longevity were accounted in analyses, while it otherwise did not.

Until recently, most studies on Lepidopteran fitness have dealt with the effects of larval diet on larval performance or alternatively have focused on the effects of adult diet alone on adult fitness parameters, without altering larval and adult nutrition in tandem. This study reveals that the effects of adult diet on butterfly fitness may be mediated by larval diet and challenges the notion that nectar-derived amino acids positively contribute to butterfly fitness. Butterflies provided sugar and amino acids had reduced fecundity in comparison to adults given sugar alone when larvae were reared on equal-ratio diet. The results found in the current study are the first to indicate that nectar-derived amino acids may have negative consequences under certain circumstances, such as when larvae are reared on diet approximating their nutrient targets. Since caterpillars of many species exhibit dietary self-selection approximating their nutrient target (see Chapter II), a negative effect of amino acids on butterfly fecundity may be prevalent in nature.

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TABLES AND FIGURES

Table 4.1 Numbers of caged *V. cardui* male-female pairs per treatment, including pairs that produced eggs and pairs that did not produce any eggs.

Treatment	**Number of Butterfly Pairs**	**Number of Pairs Producing Eggs**	**Number of Pairs Not Producing Eggs**
Carbohydrate-biased / Water	17	13	4
Carbohydrate-biased / Amino Acids	16	13	3
Carbohydrate-biased / Sugar	17	16	1
Carbohydrate-biased / Sugar and Amino Acids	16	15	1
Equal-ratio / Water	22	17	5
Equal-ratio / Amino Acids	22	17	5
Equal-ratio / Sugar	22	19	3
Equal-ratio / Sugar and Amino Acids	22	19	3
Protein-biased/ Water	18	4	14
Protein-biased/ Amino Acids	17	3	14
Protein-biased / Sugar	17	15	2
Protein-biased / Sugar and Amino Acids	17	15	2

Table 4.2 Effects of trial, larval diet, and adult diet on total egg production, duration of egg production, and daily egg production.

Dependent Variable	**df**	**Mean Square**	***F***	***P***
Total Egg Production				
Trial	2	56600.17	0.40	0.6681
Larval Diet	2	314118.57	2.25	0.1100
Adult Diet	3	1625895.68	11.62	<0.0001
Trial*Larval Diet	4	25536.59	0.18	0.9471
Trial*Adult Diet	6	23936.25	0.17	0.9841
Larval Diet*Adult Diet	6	356770.25	2.55	0.0228
Trial*Larval Diet*Adult Diet	10	61768.12	0.44	0.9235
Error	132	139915.19		
Duration of Egg Production				
Trial	2	0.79	0.05	0.9498
Larval Diet	2	1.64	0.11	0.8982
Adult Diet	3	517.88	34.01	<0.0001
Trial*Larval Diet	4	2.46	0.16	0.9574
Trial*Adult Diet	6	1.04	0.07	0.9987
Larval Diet*Adult Diet	6	31.66	2.08	0.0598
Trial*Larval Diet*Adult Diet	10	24.75	1.63	0.1059
Error	132	15.23		
Daily Egg Production				
Trial	2	1252.43	0.88	0.4165
Larval Diet	2	2781.32	1.96	0.1452
Adult Diet	3	1393.77	0.98	0.4038
Trial*Larval Diet	4	1159.33	0.82	0.5171
Trial*Adult Diet	6	679.31	0.48	0.8236
Larval Diet*Adult Diet	6	1361.07	0.96	0.4563
Trial*Larval Diet*Adult Diet	10	700.07	0.49	0.8923
Error	132	1420.60		

Table 4.3 Effects of larval diet, adult diet, and the interaction on total egg production, duration of egg production, and daily egg production. Reduced models.

Dependent Variable	**df**	**Mean square**	***F***	***P***
Total Egg Production				
Larval Diet	2	313479.51	2.48	0.0871
Adult Diet	3	1880073.02	14.87	<0.0001
Larval Diet*Adult Diet	6	402670.21	3.19	0.0056
Error	154	126408.47		
Duration of Egg Production				
Larval Diet	2	5.50	0.37	0.6912
Adult Diet	3	574.11	38.62	<0.0001
Larval Diet*Adult Diet	6	18.50	1.24	0.2866
Error	154	14.87		
Daily Egg Production				
Larval Diet	2	3340.01	2.53	0.0828
Adult Diet	3	1655.98	1.26	0.2917
Larval Diet*Adult Diet	6	1879.10	1.42	0.2085
Error	154	1318.87		

Table 4.4 Effects of trial, larval diet, and adult diet on total egg production, accounting for duration of egg production and female longevity.

Dependent Variable	**df**	**Mean Square**	***F***	***P***
Total Egg Production				
Trial	2	49410.71	0.60	0.5521
Larval Diet	2	510917.98	6.17	0.0028
Trial*Larval Diet	4	96085.97	1.16	0.3314
Adult Diet	3	295679.48	3.57	0.0160
Trial*Adult Diet	6	60844.18	0.74	0.6223
Larval Diet*Adult Diet	6	184812.22	2.23	0.0443
Trial*Larval Diet*Adult Diet	10	40672.14	0.49	0.8930
Covariate (Duration)	1	7090713.85	85.66	<0.0001
Covariate (Female Longevity)	1	2213304.04	26.74	<0.0001
Error	124	82773.66		

Table 4.5 Effects of larval diet, and adult diet on total egg production, accounting for duration of egg production and female longevity. Reduced model.

Dependent Variable	**df**	**Mean Square**	***F***	***P***
Total Egg Production				
Larval Diet	2	413942.25	5.28	0.0061
Adult Diet	3	295905.21	3.77	0.0121
Larval Diet*Adult Diet	6	269753.97	3.44	0.0033
Covariate (Duration)	1	7071882.09	90.15	<0.0001
Covariate (Female Longevity)	1	1929883.82	24.60	<0.0001
Error	146	78445.66		

Table 4.6 Effects of larval diet and adult diet on the duration of egg production and daily egg production, accounting for female longevity. Reduced model.

Dependent Variable	**df**	**Mean Square**	***F***	***P***
Duration of Egg Production				
Larval Diet	2	2.66	0.23	0.7928
Adult Diet	3	54.95	4.81	0.0032
Larval Diet*Adult Diet	6	11.11	0.97	0.4462
Covariate (Female Longevity)	1	465.35	40.72	<0.0001
Error	147	11.43		
Daily Egg Production				
Larval Diet	2	2779.33	2.20	0.1139
Adult Diet	3	4685.38	3.72	0.0129
Larval Diet*Adult Diet	6	1983.63	1.57	0.1587
Covariate (Female Longevity)	1	11513.35	9.13	0.0030
Error	147	1260.50		

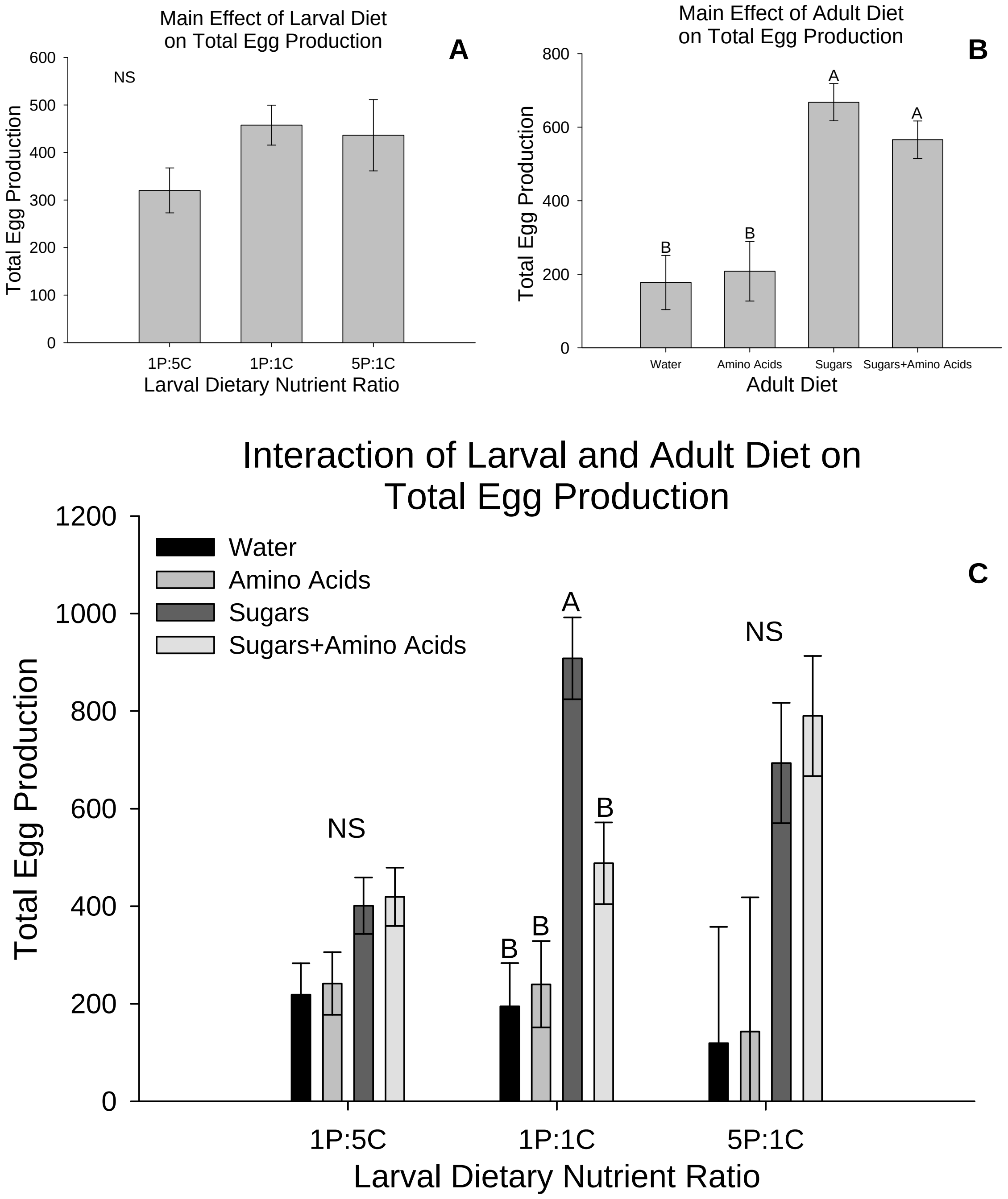

Figure 4.1 Main effects of larval dietary nutrient ratio (A), adult diet (B), and interaction (C) on total egg production by single male-female *Vanessa cardui* butterfly pairs. 1P:5C, carbohydrate-biased; 1P:1C equal-ratio; 5P:1C protein-biased. Data are least squares means ± SE. Significantly different means are represented by different letters.

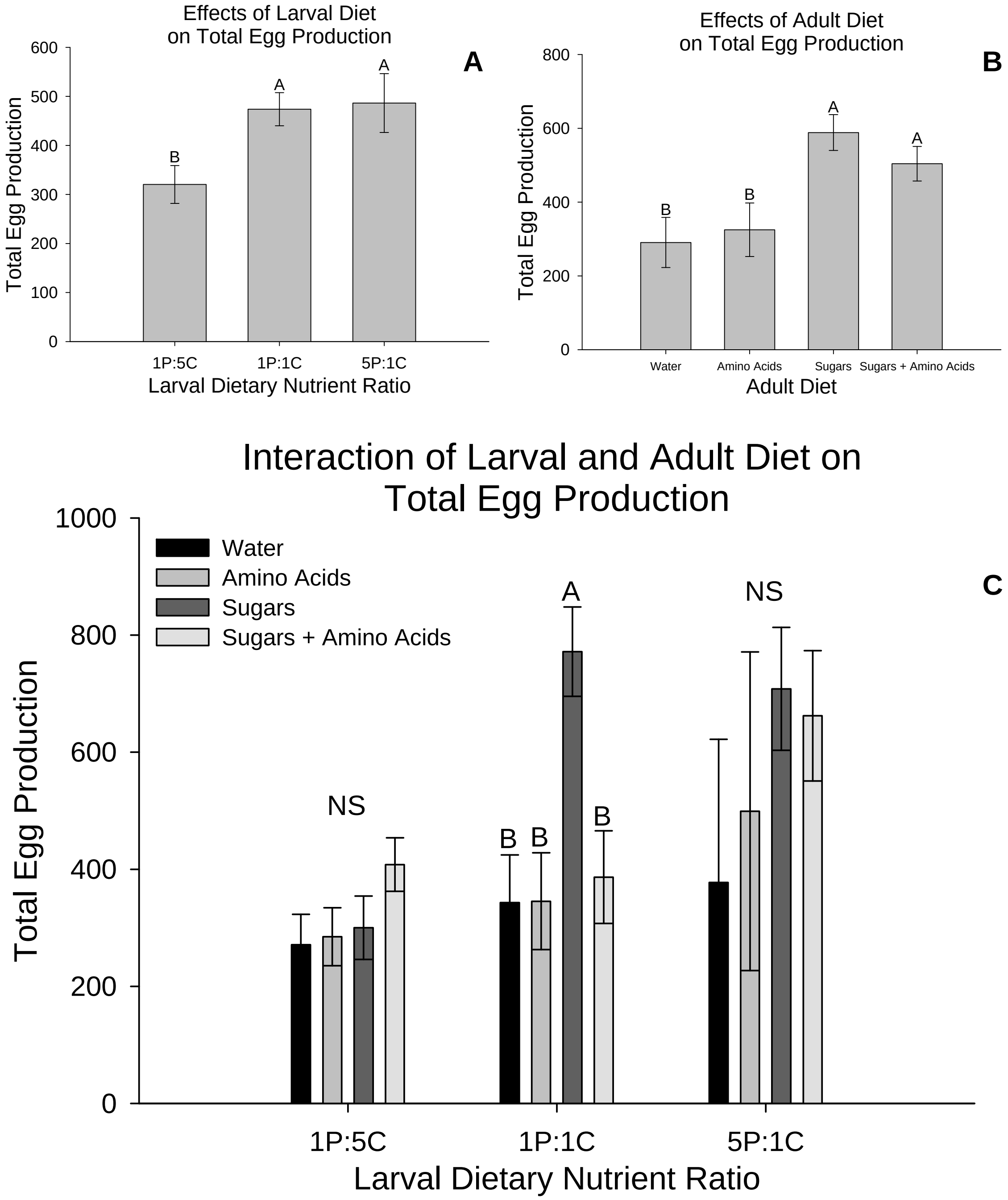

Figure 4.2 Effects of larval dietary nutrient ratio (A), adult diet (B), and interaction (C) on total egg production by single male-female *Vanessa cardui* butterfly pairs, accounting for duration of egg production and female longevity. 1P:5C, carbohydrate-biased; 1P:1C equal-ratio; 5P:1C protein-biased. Data are least squares means ± SE. Significantly different means are represented by different letters.

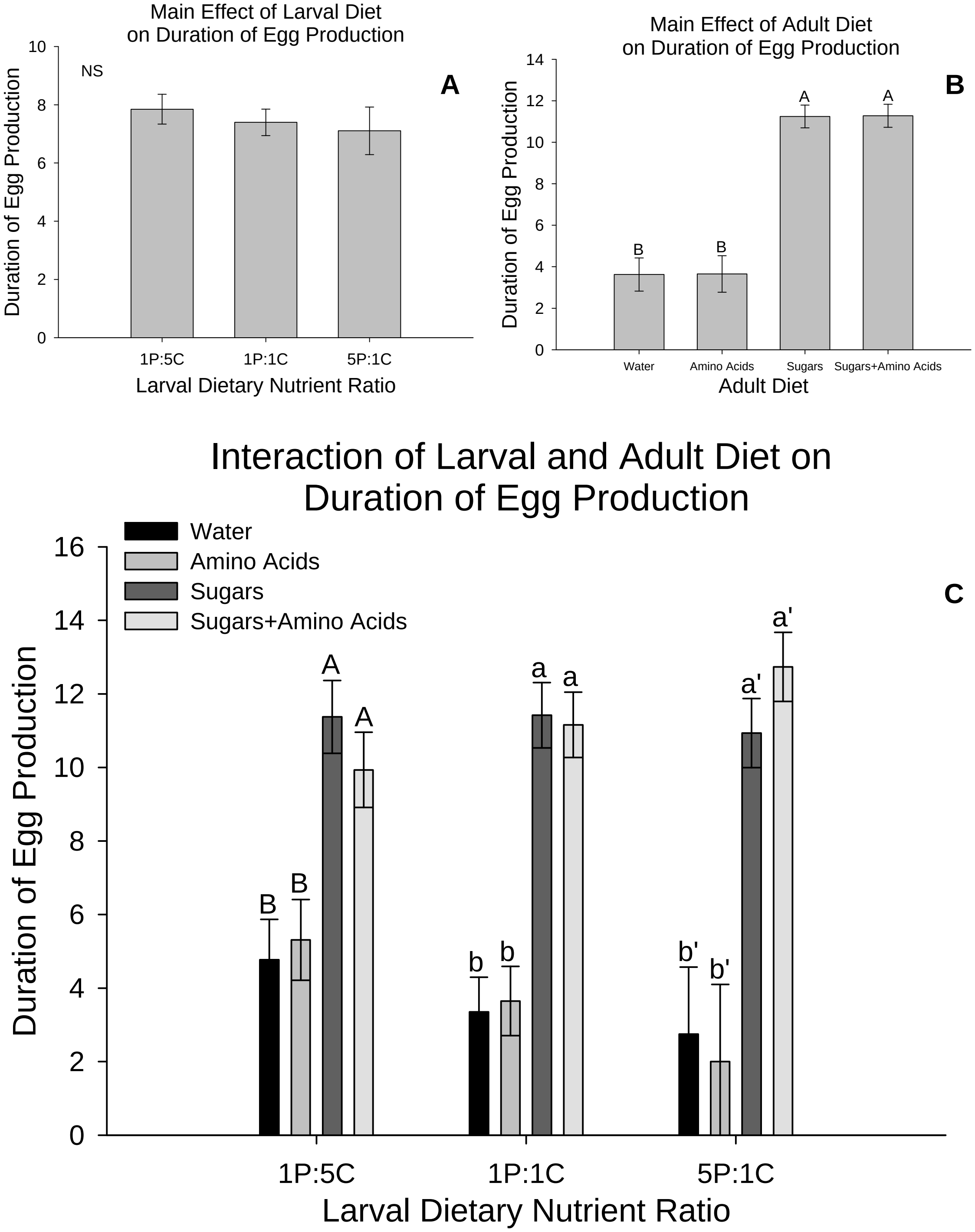

Figure 4.3 Main effects of larval dietary nutrient ratio **(**A**)**, adult diet **(**B**)**, and interaction **(**C**)** on the duration of female *Vanessa cardui* egg production. 1P:5C, carbohydrate-biased; 1P:1C equal-ratio; 5P:1C protein-biased. Data are least squares means ± SE. Significantly different means are represented by different letters.

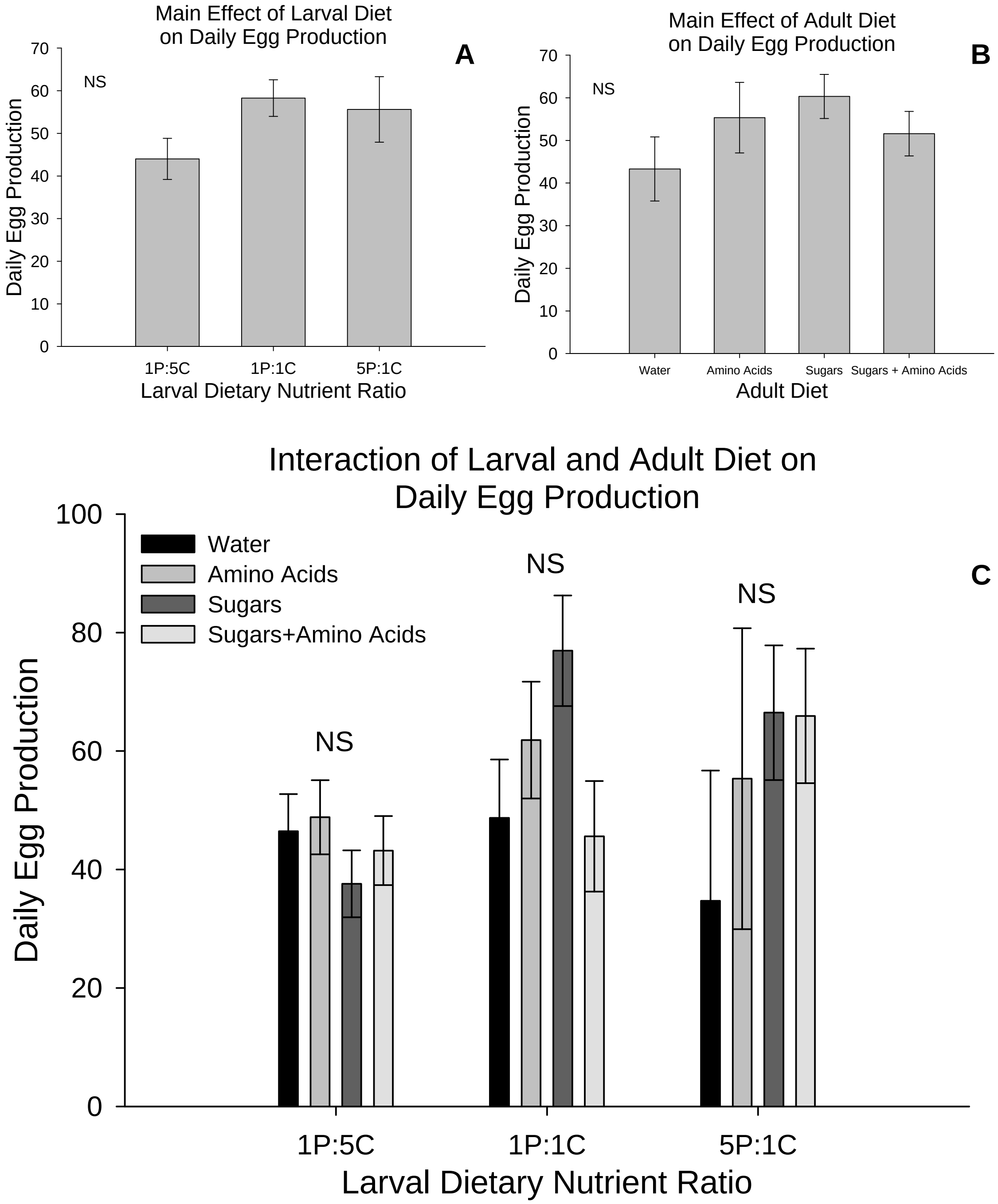

Figure 4.4 Main effects of larval dietary nutrient ratio (A), adult diet (B), and interaction (C) on daily egg production by female *Vanessa cardui*. 1P:5C, carbohydrate-biased; 1P:1C equal-ratio; 5P:1C protein-biased. Data are least squares means ± SE. Significantly different means are represented by different letters.

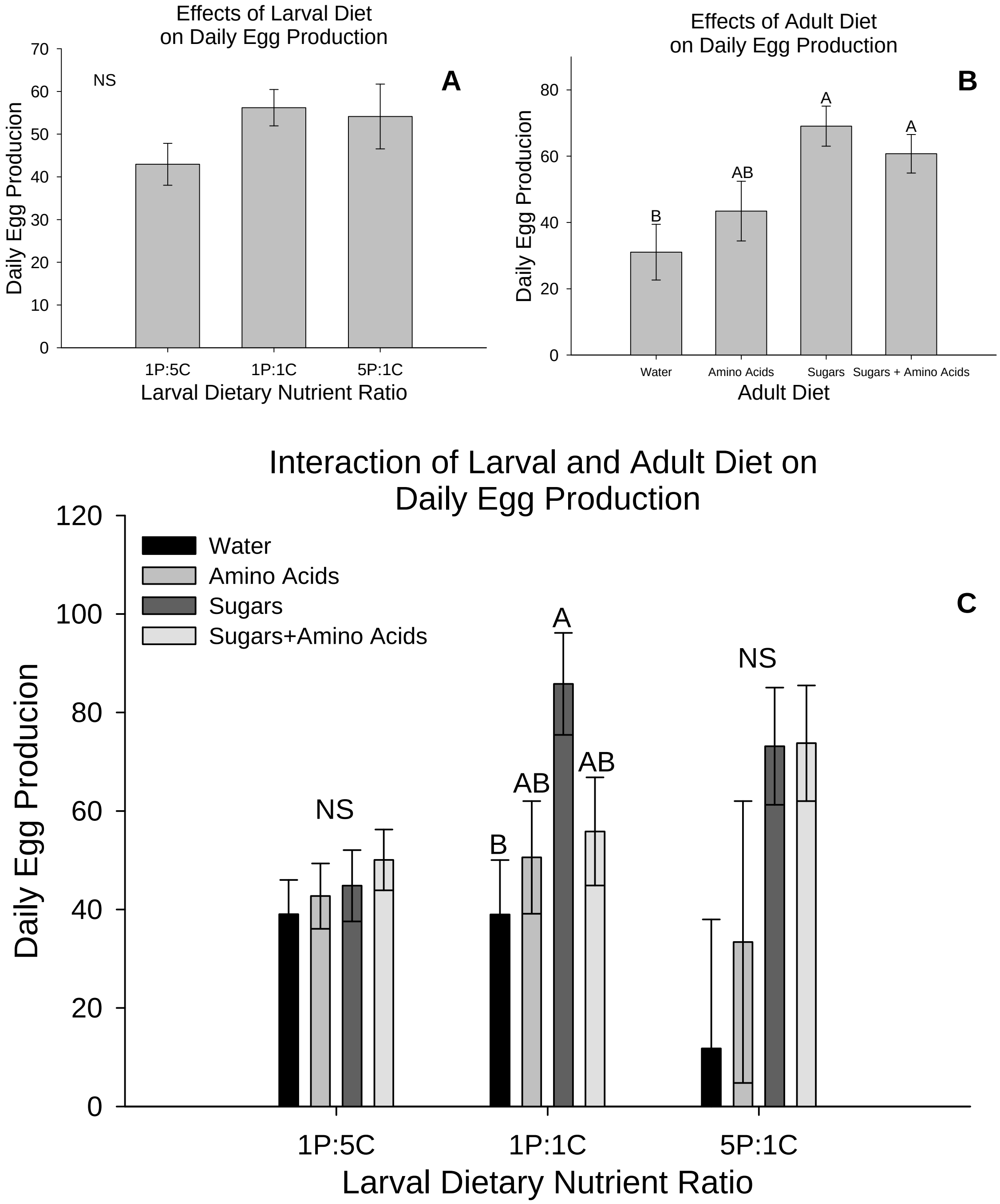

Figure 4.5 Effects of larval dietary nutrient ratio (A), adult diet (B), and interaction (C) on daily egg production by female *Vanessa cardui*, accounting for female longevity. 1P:5C, carbohydrate-biased; 1P:1C equal-ratio; 5P:1C protein-biased. Data are least squares means ± SE. Significantly different means are represented by different letters.

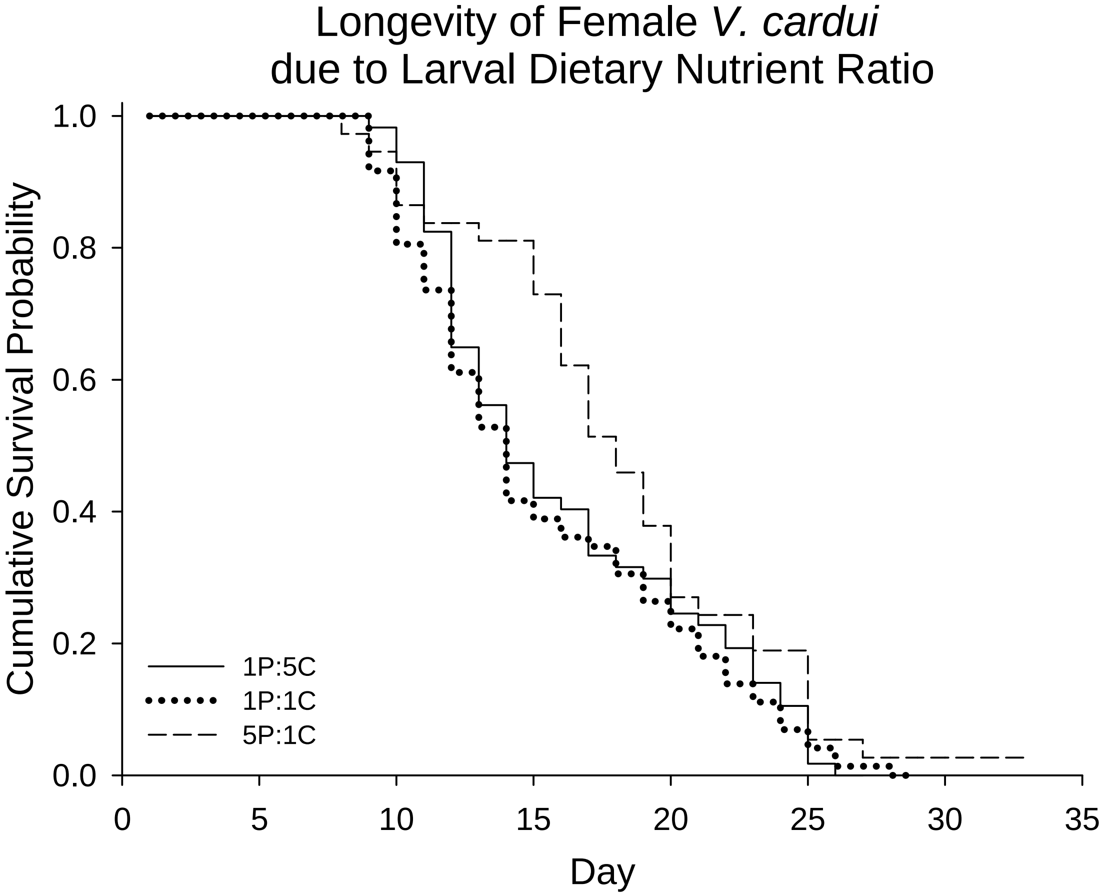

Figure 4.6 Cumulative survival probabilities of female *V. cardui* fed on different larval dietary nutrient ratios, regardless of adult diet provided. 1P:5C, carbohydrate-biased; 1P:1C equal-ratio; 5P:1C protein-biased. Wilcoxon $P = 0.0486$, Log-Rank $P = 0.1137$

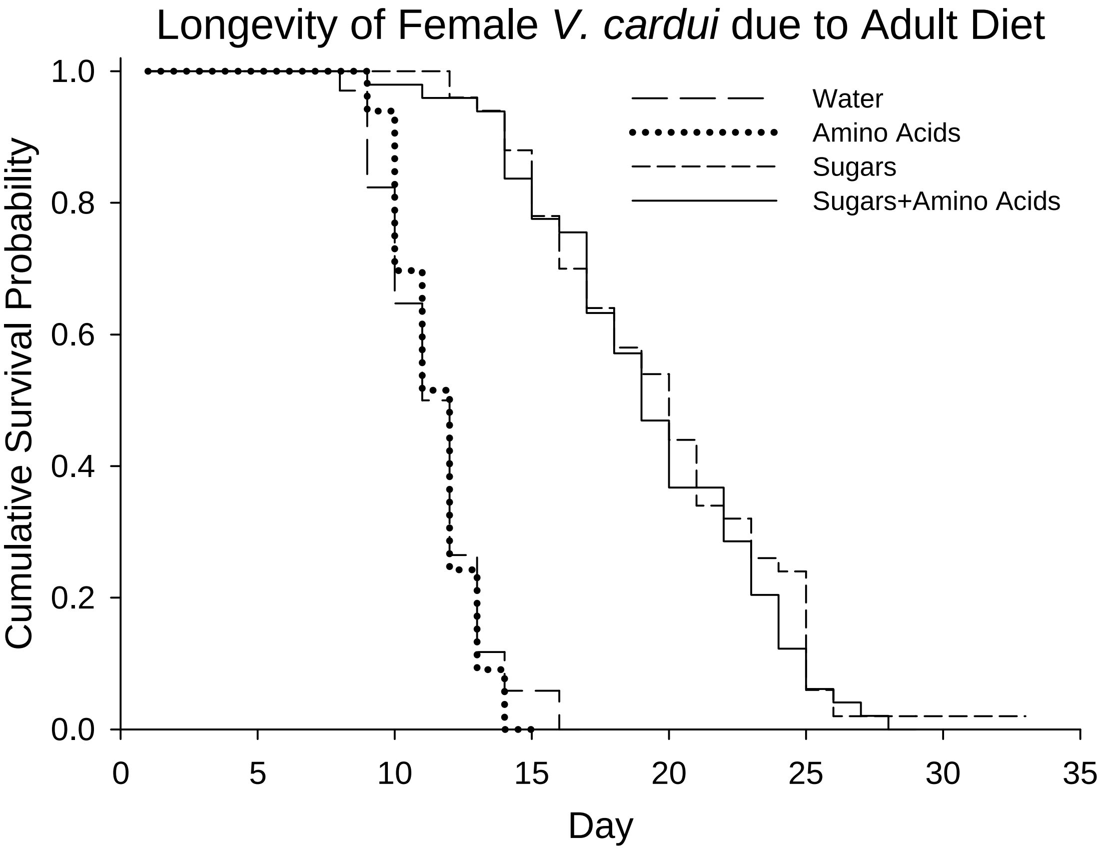

Figure 4.7 Cumulative survival probabilities of female *V. cardui* fed different adult diets, regardless of larval dietary nutrient ratio on which they were reared. Wilcoxon and Log-Rank $P < 0.0001$

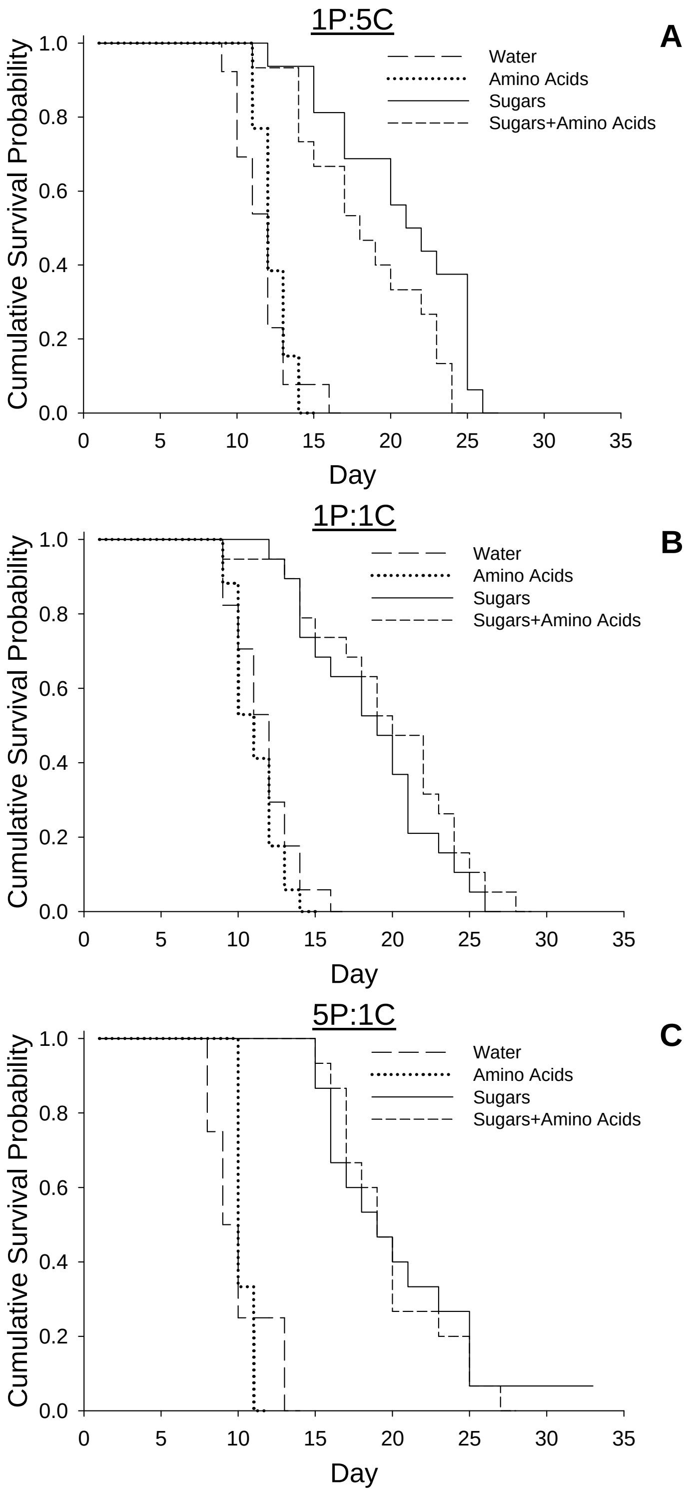

Figure 4.8 Cumulative survival probabilities of female *V. cardui* fed different adult diets, grouped within larval diet treatments. (A) 1P:5C, carbohydrate-biased; (B) 1P:1C, equal-ratio; (C) 5P:1C, protein-biased. Wilcoxon and Log-Rank $P < 0.0001$ for all.

CHAPTER V

EFFECTS OF LARVAL AND ADULT NUTRITION ON *VANESSA CARDUI* L. (LEPIDOPTERA: NYMPHALIDAE) MATE CHOICE

ABSTRACT

Butterflies should select between potential mates that differ in quality (i.e. nutrients offered, potential fecundity), and size may indicate potential quality. However, only larval nutrition affects size while adult nutrition can also affect mate quality. The objective of this study was to determine if *V. cardui* exhibits a preference between potential mates when given a choice of individuals differing in larval and adult diets. Larval diet significantly affected adult size; butterflies reared on carbohydrate-biased diet were significantly smaller than those reared on protein-biased diet. The likelihood that a potential mate was chosen was affected by the adult diet, but not the larval diet. Adults provided both sugar and amino acids were more likely to be chosen by the opposite sex than those given sugar alone. Both male and female butterflies presented with a choice of potential mates were similarly selective. This study indicates that *V. cardui* do not discriminate between potential mates due to size. These results are the first to indicate that adult diet affects mate choice and support the hypothesis that amino acids acquired during the adult stage positively affect fitness.

INTRODUCTION

Thornhill and Alcock (1983) predicted that female mate choice should evolve when males vary in the quality or quantity of benefits provided to females. Male Lepidopterans and Orthopterans often provide females with nutritious spermatophores which can increase fecundity and longevity (Andersson, 1994; Delisle and Bouchard, 1995; Delisle and Hardy, 1997; Thornhill and Alcock, 1983; Wiklund et al., 1993), and spermatophore size may be positively correlated with male size (Delisle and Hardy, 1997; Oberhauser, 1988; Thornhill and Alcock, 1983;

Wiklund and Kaitala, 1995). Females may thus exhibit choice based on male size as a proxy for spermatophore size when selecting between virgin males. Indeed, larger males of the moth *Rothschildia lebeau* Guerin-Meneville (Lepidoptera: Saturniidae: Saturniinae) had a higher probability of being observed mating in the field (Agosta, 2010). Males may increase their fitness by choosing females of higher quality (Clutton-Brock, 2007; Johnstone et al., 1996; Tang-Martinez and Ryder, 2005). Similarly, female size may be an indicator of potential fecundity (Honek, 1993; Lederhouse, 1981), and males in many species discriminate between females based on size (Alcock and Gwynne, 1987; Andersson, 1994; Frey et al., 1998; Rutowski, 1982a; Thornhill and Alcock, 1983).

Suboptimal larval diets often negatively affect pupal mass (Lee et al., 2004; Lee et al., 2002; Lindroth et al., 1991) (see also Chapters I, II). Pupal mass may be strongly correlated with female fecundity (Awmack and Leather, 2002; Honek, 1993). Adult body mass and wing length in many species decreases in individuals under larval food stress (Nylin and Gotthard, 1998). Therefore, larval diet may affect the ability of a butterfly to secure a mating due to effects on adult size. Adult diet also affects female (Bauerfeind and Fischer, 2005; Boggs and Ross, 1993; Geister et al., 2008; Mevi-Schutz and Erhardt, 2005; Romeis and Wackers, 2002) and male (Beck, 2007; Lederhouse et al., 1990; Lewis and Wedell, 2007) fitness, although adult size (wing length) is affected by larval diet alone.

Plant species typically pollinated by butterflies tend to have higher concentrations of amino acids in their floral nectars than those principally pollinated by bees, birds, etc. (Baker and Baker, 1973, 1973a; Baker and Baker, 1977; Baker and Baker, 1985; Baker and Baker, 1986, 1990); the latter have other dietary sources of nitrogen. Amino acids acquired during adult lepidopteran feeding may positively influence female fecundity (Dunlap-Pianka et al., 1977; Mevi-Schutz and Erhardt, 2005), as well as male longevity (Beck, 2007) and virility (Lederhouse et al., 1990). Larval diet affects adult size and therefore may affect the ability of individuals to secure matings. By contrast, adult diet does not affect butterfly size and it is unknown whether it affects the ability to secure matings.

The objective of this study was to determine if larval and adult nutrition affects *V. cardui* mate choice. A second objective of this study was to determine if *V. cardui* size is affected by larval nutrition. Theoretically, adults reared on protein-biased larval diet should be larger than those reared on carbohydrate-biased diet, as individuals reared on protein-rich diet exhibit greater pupal mass than those reared on carbohydrate-rich diet (Chapters II, III). If *V. cardui* selects between potential mates due to size, it is expected that adults will prefer to mate with individuals reared on protein-biased diet versus those reared on carbohydrate-biased diet, while adult diet is expected to not affect mating preference as size is only affected by larval nutrition.

MATERIALS AND METHODS

Insect culture

A colony of *Vanessa cardui* originating from > 300 original eggs purchased from Shady Oak Butterly Farm (Brooker, FL) was continuously maintained under controlled conditions in the lab. Larvae were fed a commercial *Manduca sexta* L. (Sphingidae) diet (BioServ Entomology Division, Frenchtown, NJ) and reared individually in clear plastic cups (3 cm H x 4 cm ID) under a 16:8 L/D, 24:24 ^{o}C D/N, 70% RH cycle in a Percival Scientific environmental chamber (Model: I-30BLL, Series 101, Perry, IA). Pupae were hung in a 37W x 36L x 48H cm communal eclosion cage. Adults were transferred to a 58W x 74L x 81H cm flight cage after emergence. Adults were given a constant food source of sucrose and honey water and an oviposition source of moistened cotton wicks. Eggs were removed from wicks daily, soaked in 2.5% bleach solution for 2 minutes, and placed within a 100 x 15 mm plastic Petri dish on moistened #1 Whatman® filter paper in the environmental chamber until hatching. Dishes containing eggs were held at the above conditions until hatching.

Methods for Rearing Experimental Larvae

Larvae were reared as defined under 'insect culture' until they reached the final (5th) instar. Final instar larvae were weighed and randomly assigned individually to clean clear plastic cups (3 cm H x 4 cm ID) and provided with one of three chemically defined diets that contained casein and sucrose as the only digestible sources of protein and carbohydrate, respectively. The experimental diets were a modification of one initially created for the larvae of *M. sexta* (Ahmad et al., 1989). Diets had nutrient ratios of 5 parts casein to 1 part sucrose (protein-biased), equal parts casein and sucrose (equal-ratio), and 1 part casein to 5 parts sucrose (carbohydrate-biased). Results from Chapter II demonstrated that diets of unequal nutrient ratios negatively affected larval fitness, while the equal-ratio diet was optimal for larval growth and development. All experimental diets contained a total combined content of 120g casein and sucrose per liter and were equivalent in total energy; protein and carbohydrate are approximately equivalent in energy released during metabolism (West et al., 1970). Diets also contained 20g agar, 850ml water, 14g Wesson's salt mixture, 4g cholesterol, 5g ascorbic acid, .25g inositol, 2g sorbic acid, 2g methylparabenzoate, 1g choline chloride, 10ml vitamin mixture, and 20ml formaldehyde per liter. Diet was changed every 3 days until pupation. Sex was determined by noting the sex mark located on the 9th abdominal sternite (Scott, 1986). Pupae were hung in communal eclosion chambers according to sex and larval diet upon which butterflies were reared.

Individuals constrained to carbohydrate-biased diet during the 5th instar take approximately 2 days longer to pupate than those constrained to equal-ratio or protein-biased diet (Chapter II); therefore, insects reared on carbohydrate-biased diet were reared from a larval cohort that hatched 2 days prior to those reared on equal-ratio or protein-biased diets. This assured that all adults used for mate trials would eclose on the same day.

Experimental Setup for Adults

Pupal eclosion chambers were placed in a controlled environmental rearing room under a 16:8 L/D, 29:29 ^{o}C D/N, 20% RH cycle. Supplementary lighting was on a 7:17 D/N cycle that provided additional lighting 2 hours after initial light to simulate a midday increase in light intensity. Upon eclosion, adults were put into communal cages that were separated according to adult diet to which they were assigned. Animals reared on equal-ratio diet were used as individuals making a choice among potential mates. Butterflies reared on equal ratio diet were provided water. This assured that butterflies making a choice among potential mates were from different nutritional backgrounds than the potential mates between which they were choosing. Therefore, any potential choice exhibited by animals cannot be attributed to them choosing a mate due to similar dietary history. Insects from carbohydrate-biased and protein-biased larval diets were randomly assigned to either an adult-diet treatment of sugar or sugar plus amino acids nectar (see ‘Nectar solutions’: Chapter III). This resulted in a 2x2 factorial assignment of feeding regimes that potential mates were provided: carbohydrate-biased larval diet given sugar nectar as adults, carbohydrate-biased larval diet given sugar and amino acids nectar as adults, protein-biased larval diet given sugar nectar as adults, and protein-biased larval diet given sugar and amino acids nectar as adults. Potential mates offered to selecting butterflies were marked with a numeric code on the left ventral hind wing to denote the combination of larval and adult nutritional treatments to which they were assigned while those individuals making the choice between mates were left unmarked. All animals were allowed to feed for three days prior to mate choice trials.

Mate Choice Observations

Butterflies reared on equal-ratio diet were transferred individually to clear water containers (22.86cm x 12.7cm x 12.7cm) filled 2 cm high with plaster of Paris to assure a level lower surface, and were provided with a choice between two adults from each of the four larval/adult diet treatments. This resulted in six mate choice combinations with which selecting individuals were presented (Table 5.1). Sexual activity of adults began approximately 13hrs after the beginning of photophase. Once animals initiated mating, the individual chosen to mate was noted, and all individuals were removed and transferred to a sealable plastic bag. The options of mates the butterfly was offered as well as the individual chosen as a mate were noted on the bag, and animals frozen. Adult size was later determined by measuring the length of the leading edge of the left forewing using digital calipers (Absolute Digimatic Series No. 500; Mitutoyo U.S.A.). Adults were observed until the end of photophase, and individuals that had not initiated mating by this time were excluded from analyses. Because *V. cardui* mate for several hours (*pers. obs.*), it is not likely that instances of coupling were missed during the experiment.

Statistical Analyses

Two-way ANOVA was used to determine the effects of larval diet and sex on wing length. Differences among treatment means were determined using post-hoc Tukey-Kramer multiple comparisons of least squares means. Butterfly mate choice decisions were analyzed using the Bradley-Terry model (Agresti, 2002) for paired comparisons using PROC LOGISTIC in SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA).

RESULTS

In total, 391 females and 428 males were observed in cages where mate pairings occurred. Overall, 155 females chose among 310 males and 118 males chose among 236 females, resulting in 273 observed pairings (Table 5.1).

Butterfly Wing Length

Butterfly forewing length was significantly affected by larval dietary nutrient ratio and sex, and there was a significant interaction between larval dietary nutrient ratio and sex (Table 5.2). Post-hoc Tukey-Kramer multiple comparisons of least squares means revealed that butterflies reared on carbohydrate-biased diet were significantly smaller than butterflies reared on equal-ratio or protein-biased diets (Figure 5.1A). Butterflies reared on carbohydrate-biased diet had wing lengths that were 5.67% shorter on average than those reared on protein-biased diet. Females were significantly larger than males, with wing lengths 4.7% longer on average than males (Figure 5.1B). Furthermore, Tukey-Kramer multiple comparisons within larval diet treatments revealed that females differed from males in size to a greater degree when reared on equal-ratio (6.04%) and protein-biased diets (5.46%) than when reared on carbohydrate-biased diet (2.46%) (Figure 5.1C).

Butterfly Mate Choice

Pooled data allowing for a sex effect and including larval diet and adult diet as explanatory variables for choice showed that the effect of adult diet was significant (Wald Chi-sq = 6.8035; P = 0.0091) while the effects of larval diet and sex were not (Wald Chi-sq = 2.6090, 1.7144; P = 0.1063, 0.1908, respectively). Individuals reared on protein biased diet and fed sugar and amino acids nectar were most preferred as mates, followed by those reared on carbohydrate biased diet and fed sugar and amino acids nectar, those reared on protein biased diet and fed sugars, and finally those reared on carbohydrate biased diet and fed sugar nectar. Adults were significantly more likely to mate with individuals reared on protein-biased diet and fed sugar and amino acids nectar than those reared on either larval diet and fed sugar nectar alone (Table 5.3). Furthermore, adults were significantly more likely to mate with individuals reared on carbohydrate-biased diet and given sugar and amino acids nectar than those reared on carbohydrate-biased diet and given sugar nectar (Table 5.3).

DISCUSSION

Larval diet affected butterfly size as expected, with individuals reared on protein-rich diet significantly larger than those reared on carbohydrate-rich diet. Here, females were significantly larger than males which was unexpected as sexes did not differ in pupal mass (Chapters II, III). The fact that females differed from males in wing length to a greater degree when reared on equal-ratio or protein-biased diets than when reared on carbohydrate-biased diet indicates that the degree of sexual dimorphism may be affected by dietary quality. *V. cardui* differ significantly in pupal mass when fed *Glycine max* L. (soybean) foliage (O'Neill et al., 2008; Poston et al., 1977), but not when fed foliage grown under elevated CO_2, which increases the C:N ratio of foliage (O'Neill et al., 2008). This indicates that the degree of sexual dimorphism may be similarly affected by dietary nutrient ratios of natural diets as well (i.e. sexual dimorphism less apparent on diets lower in nitrogen content).

Results of this study are in contrast to expectations that larval diet, but not adult diet, affect mate choice in *V. cardui*. Since larval diet affected butterfly size as predicted but did not affect mate choice, results indicate that adults were not selective based on the size of potential mates offered in this study. Similarly, male size was not correlated with mating success for *Pieris napi* L. (Lepidoptera: Pieridae) in the field (Wiklund and Kaitala, 1995). Furthermore, the correlation between size and fecundity is often not strong, or can be absent, in female Lepidopterans (Leather, 1988). Butterfly size did not affect fecundity for *Pararge aegeria* L. (Nymphalidae) (Wiklund and Persson, 1983), *Lasiommata megera* L. (Nymphalidae) (Wiklund and Karlsson, 1984), *Speyeria mormonia* Edwards (Nymphalidae) (Boggs, 1986), *Danaus plexippus* L. (Nymphalidae) (Oberhauser, 1997; Svard and Wiklund, 1988), and *Pieris napi* L.

(Pieridae) (Wiklund and Kaitala, 1995). Therefore, female size may not be a good predictor of female quality (i.e potential fecundity) for males to use when selecting between potential mates. Alternatively, individuals reared on protein-biased and carbohydrate-biased diets may not have differed greatly enough in size to affect mate choice in this study.

Adult diet significantly affected the probability that an individual would be chosen as a mate. Adults were significantly more likely to mate with individuals fed sugar and amino acids nectar than with those fed sugar nectar alone. This is consistent with the hypothesis that nectar-derived amino acids enhance butterfly fitness. Mate choice was not affected by sex, indicating that both males and females exhibited mate choice. Both males and females may benefit by choosing mates of higher quality (Clutton-Brock, 2007; Johnstone et al., 1996; Tang-Martinez and Ryder, 2005; Thornhill and Alcock, 1983). Adults may prefer to mate with individuals with adult amino acid intake due to their greater potential fitness. Nectar containing sugars and amino acids increased female *Araschnia levana* L. (Lepidoptera: Nymphalidae) fecundity compared to females fed sugars only when larvae were reared on low quality diet (Mevi-Schutz and Erhardt, 2005). Male *Papilio glaucus* L. (Lepidoptera: Papilionidae) sired seven times more viable offspring when honey-water was supplemented with amino acids and electrolytes than when not (Lederhouse et al., 1990).

Animals fed protein-rich larval diet and provided sugar and amino acids nectar as adults were the most likely to be chosen as mates by selecting individuals, while those fed carbohydrate-rich larval diet and given sugar nectar as adults were the least likely to be chosen. This follows theoretical predictions that animals should select between potential mates based on mate quality (Clutton-Brock, 2007; Johnstone et al., 1996; Tang-Martinez and Ryder, 2005; Thornhill and Alcock, 1983), and that animals may benefit from, and thus choose to mate with, individuals with greater nitrogen reserves either from larval or adult feeding. Nitrogen is a limiting nutrient for folivores (Tabashnik and Slansky, 1987) and is especially limiting in the adult diet of nectarivorous Lepidoptera (Rutowski, 1982b). Males of many butterfly species elicit puddling behaviors in which they feed on mud, excrement, or carrion, and it has been suggested that nitrogenous sources may also be sought in addition to sodium (Molleman, 2010). Proteins can elicit adult feeding responses (Boggs and Dau, 2004), and labeled amino acids from mud have been shown to be incorporated into the eggs of females that mated with puddling males (Arms et al., 1974). Butterflies may prefer nectars containing amino acids (Alm et al., 1990; Erhardt and Rusterholz, 1998; Goverde et al., 2008; Hawn and Lanza, 2004; Mevi-Schutz and Erhardt, 2002, 2003, 2004; Mevi-Schutz et al., 2003), and in most cases only female butterflies display this preference (Alm et al., 1990; Erhardt and Rusterholz, 1998; Goverde et al., 2008; Mevi-Schutz and Erhardt, 2002, 2003, 2004; Mevi-Schutz et al., 2003). The current study indicates that butterflies can select between potential mates due to adult diet and may be a further explanation for the prevalence of puddling in male lepidopterans and amino acid preference in female nectar feeding.

It is often difficult to determine if observed mate pairing is due to mate choice or due to some form of mating competition (i.e. due to intersexual choice (epigamic selection) or to intrasexual competition) (Halliday, 1983; Havens et al., 2011; Thornhill and Alcock, 1983). The observed mating preferences by *V. cardui* in this study may indicate that insects were exhibiting mate choice between potential mates or alternatively may indicate that individuals fed different adult diets differed in intrasexual competitive ability or sexual responsiveness. Females may respond to males that court them more vigorously (Rutowski, 1982b). Females of the black field cricket *Teleogryllus commodus* were more sexually responsive to male calls when females were reared on a high protein diet versus a low protein diet (Hunt et al., 2005). Future studies should examine whether *V. cardui* offered a choice between potential mates are exhibiting mate choice, or if potential mates differ in competitive ability or sexual responsiveness to further elucidate how adult diet affects mate choice. Furthermore, future studies may examine whether *V. cardui* exhibit mate choice when potential mates are reared on a leaf diet of low or high nitrogen content and either given sugars or sugars plus amino acids as adults to ascertain if mating preferences observed in this study occur under more natural conditions.

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TABLES AND FIGURES

Table 5.1 Mate choice combinations with which butterflies were presented. Butterflies presented choices of potential mates were reared on equal-ratio diet and given water as adults.

Choosing Sex	Mate Choice Combination	Choice 1	Choice 2	n	Choice 1 Fequency	Choice 2 Frequency
Male	1	CS	CSA	21	7	14
Male	2	CS	PS	19	8	11
Male	3	CS	PSA	19	4	15
Male	4	CSA	PS	18	8	10
Male	5	CSA	PSA	18	6	12
Male	6	PS	PSA	23	13	10
Female	1	CS	CSA	25	10	15
Female	2	CS	PS	26	14	12
Female	3	CS	PSA	25	11	14
Female	4	CSA	PS	27	17	10
Female	5	CSA	PSA	25	11	14
Female	6	PS	PSA	27	10	17

Table 5.2 Effects of larval dietary nutrient ratio and sex on *V. cardui* wing length.

Dependent Variable	**df**	**Mean Square**	***F***	***P***
Wing Length				
Larval Dietary Nutrient Ratio	2	250.3899	212.65	<0.0001
Sex	1	357.5067	303.62	<0.0001
Interaction	2	22.0263	18.71	<0.0001
Error	753	1.1775		

Table 5.3 Pairwise comparisons of mate preference.

Potential Mate Pairing	**df**	**Wald Chi-sq**	***P***	**Sequential Bonferroni *P***
CS vs CSA	1	6.8035	0.0091	0.0455
CS vs PS	1	2.6090	0.1063	0.2126
CS vs PSA	1	8.5777	0.0034	0.0204
CSA vs PS	1	0.4542	0.5004	0.5004
CSA vs PSA	1	2.6090	0.1063	0.3189
PS vs PSA	1	6.8035	0.0091	0.0364

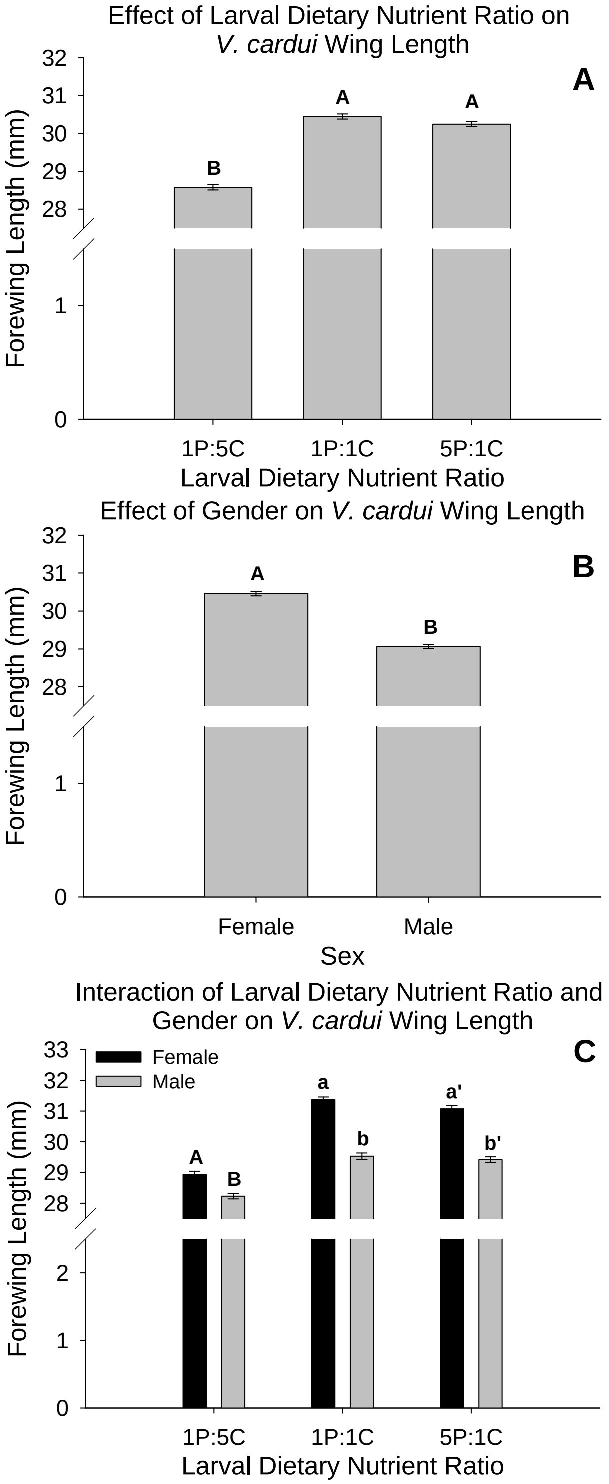

Figure 5.1 Main effects of larval dietary nutrient ratio (A), gender (B), and interaction (C) on *V. cardui* wing length. Values are least squares means +/- 1 S.E. Different letters represent significant differences between means.

CHAPTER VI

CONCLUSIONS

The broad objective of this dissertation research was to determine if the generalist feeding Lepidopteran *Vanessa cardui* L. (Nymphalidae) displays dietary self-selection during the 5th instar, what the rules of compromise are when larvae are constrained on suboptimal diet, and whether adults respond to variations in larval nutrition by altering adult foraging. Experiments further examined the importance of larval and adult nutrition on fitness parameters and whether larval and adult nutritional histories affect mate choice.

It is well established that animals and insects regulate nutrient intake to optimize performance and growth (Raubenheimer and Simpson, 1997; Waldbauer and Friedman, 1991), while a suboptimal diet can adversely affect caterpillar growth, development, survivorship, and fecundity (Awmack and Leather, 2002). Most research into nutritional quality and self-selection behaviors has focused on the ratio of protein to digestible carbohydrate (P:C) available to insects. This ratio is a chief aspect of dietary quality that significantly impacts performance. I applied the geometric framework (Raubenheimer and Simpson, 1997, 1999; Simpson and Raubenheimer, 1993, 1995) to determine the 5th instar larval feeding behaviors of *V. cardui* in Chapter II. In choice-trials, where larvae were given two food blocks consisting of different ratios of protein to carbohydrate, *V. cardui* independently regulated intake of protein and carbohydrate to a ratio approximating 1P:1.09C. Generalist caterpillar species studied to date are characterized by selection of a protein-biased diet, such as *H. virescens* (4P:1C), *S. littoralis* (1.33P:1C), and *S. exigua* (1.1P:1C) (Lee et al., 2002; Merkx-Jacques et al., 2008; Simpson et al., 2002; Telang et al., 2001; Waldbauer et al., 1984). Oligo- and monophagous species, by comparison, such as *M. sexta*, *H. subflexa*, and *S. exempta*, select diets with either equal or slightly carbohydrate-biased ratios, and it has been suggested that these specialists evolved selection behaviors that match the nutrient content of their host plants (Lee et al., 2006; Lee et al., 2004b; Thompson and Redak, 2005). Therefore, although *V. cardui* are extremely polyphagous, the ratio of protein to carbohydrate selected was more akin to that selected for by more specialist herbivores.

When constrained to a single food source, an animal is unable to independently regulate protein and carbohydrate consumption and has to feed in a way to compromise between overingesting the nutrient in excess and underingesting the nutrient in deficit within the diet and assure its minimum nutritional requirements are met. The feeding behavior exhibited by the animal is termed the "rule of compromise", and reflects the costs and benefits of over and under ingesting specific nutrients (Raubenheimer and Simpson 1997, 1999). Which rule the animal follows when faced with nutrient imbalances is a qualitative indicator of dietary breadth (Lee et al., 2003; Raubenheimer and Simpson, 1999, 2003). The rule of compromise exhibited by fifth instar *V. cardui* is consistent with that expected for a generalist herbivore (Behmer, 2009). Larvae constrained to single dietary blocks consumed less on increasingly protein-biased diets and more on increasingly carbohydrate-biased diets. Compensatory feeding on carbohydrate-biased diets by *V. cardui* is similar to that found for many generalist caterpillar species including *S. littoralis* that counteract protein limitations by increased consumption (Lee et al., 2002; Lindroth et al., 1991; Telang et al., 2001; Thompson et al., 2003), but is unlike the feeding strategy found for other generalist caterpillars, including *H. virescens* and *S. exigua*, in which larvae reduced nutrient intake on carbohydrate-biased diets (Lee et al., 2006; Lee et al., 2002; Merkx-Jacques et al., 2008).

Dietary nutrient ratio affected larval performance, with pupal mass decreasing and stadium duration increasing as nutrient ratio shifted away from an equal-ratio of protein:carbohydrate, but to a greater extent when shifted toward carbohydrate than when shifted toward protein. High survivorship on all dietary nutrient ratios indicated that 5th instar *V. cardui* can grow well on diets that are highly variable in their ratios of macronutrients. Other Lepidopteran species (e.g. *S. eridania*, *S. littoralis*, *S. exempta*, and *M. sexta*) can acquire lipids through the deamination of excess amino acids from dietary protein and subjection of their carbon skeletons to gluconeogenesis (Lee et al., 2004a; Lee et al., 2002; Lee et al., 2003; Thompson et al., 2003). More research is needed to determine if *V. cardui* are able to use excess dietary protein in the same fashion.

Larval nutritional resources can vary greatly within and between plant species (Bernays and Chapman, 1994; Schoonhoven et al., 2006; Schowalter, 2006). Adult nutritional resources, (eg. the carbohydrate and amino acid content of nectar) also vary within and among plant species (Baker and Baker, 1983; Corbet and Delfosse, 1984; Gardener and Gillman, 2001b; Gottsberger et al., 1990; Herrera et al., 2006; Langenberger and Davis, 2002; Lanza et al., 1995; Nicolson and Nepi, 2005; Percival, 1961; Petanidou et al., 1996; Plowright, 1981). Several studies have demonstrated that butterflies prefer nectars containing amino acids (Alm et al., 1990; Erhardt and Rusterholz, 1998; Goverde et al., 2008; Hawn and Lanza, 2004; Mevi-Schutz and Erhardt, 2002, 2003b, 2004; Mevi-Schutz et al., 2003). In most cases, only female butterflies display preference for the presence of amino acids (Alm et al., 1990; Erhardt and Rusterholz, 1998; Goverde et al., 2008; Mevi-Schutz and Erhardt, 2002, 2003b, 2004; Mevi-Schutz et al., 2003). These preferences for nectar derived amino acids may differ due to larval nutrition, mating history, and nutritional demands brought on by oogenesis (Mevi-Schutz and Erhardt, 2003b, 2004; Mevi-Schutz et al., 2003).

I found that larval dietary nutrient ratio affected initial nectar preference between sugar and sugar plus amino acids nectar by female *V. cardui*, but not by males. This is consistent with previous findings that have indicated that females, but not males, select for nectars containing amino acids (Alm et al., 1990; Erhardt and Rusterholz, 1998; Goverde et al., 2008; Mevi-Schutz and Erhardt, 2002, 2003b, 2004; Mevi-Schutz et al., 2003). Only female butterflies reared on carbohydrate-biased diet were significantly more likely to probe towards sugar aplusnd amino acids solution than towards sugar solution alone when they were held in tarsal contact with both. Those reared on equal-ratio or protein-biased diets did not exhibit nectar preference. This is consistent with previous results that showed female *Araschnia levana* reared on low quality leaves (i.e. low in nitrogen content) prefer sugar plus amino acids solution while females reared on high quality leaves display no preference between nectar containing or devoid of amino acids (Mevi-Schutz and Erhardt, 2003b).

Altered feeding responses by *V. cardui* appear to be limited to initial probing. Butterflies from different larval diets did not display any consistent preference for or avoidance of sugar and amino acids solution between two replicated consumption trials. This result suggests that *V. cardui* do not exhibit overall preferences between sugar and sugar and amino acids nectars when foraging. This is in contrast to what has been found for *A. levana*, which exhibited increased preference for amino acid rich nectar when reared on leaves low in nitrogen content relative to those reared on leaves high in nitrogen content (Mevi-Schutz and Erhardt, 2003b) and *C. pamphilus*, which exhibited a significant decrease in preference for amino acid rich nectar when reared on fertilized plants as opposed to butterflies reared on unfertilized plants (Mevi-Schutz et al., 2003). However, butterflies in this study may have been unable to associate the preferred nectar with its source due to flowers of both solutions being presented identically. Future research should use different visual stimuli to represent each solution to determine if butterflies show foraging preferences between solutions if visual aids help in butterfly learning.

Lepidopteran fitness studies are often limited to the juvenile stages and do not consider effects of larval nutrition on adult fecundity and longevity (Karowe and Martin, 1989; Lee and

Roh, 2010; Lindroth et al., 1991; Morehouse and Rutowski, 2010; Wheeler and Halpern, 1999). However, a combination of larval and adult-derived nutrients and energy are used for reproduction by species that consume resources as adults (Boggs, 1981a). Adult carbohydrate intake significantly affects butterfly fitness parameters (Bauerfeind and Fischer, 2005; Boggs and Ross, 1993; Fischer et al., 2004; Hill, 1989; Hill and Pierce, 1989; Murphy et al., 1983; O'Brien et al., 2004). Furthermore, females can compensate for poor larval nutrition through adult consumption of amino acid rich nectar (Mevi-Schutz and Erhardt, 2005).

Adult nutrition played a greater role in realized fitness than larval nutrition for *V. cardui*, but the effects of adult diet were mediated by larval dietary history. Butterflies with access to sugars as adults, whether alone or in combination with amino acids, lived significantly longer than those without access to carbohydrates and were able to maintain egg production for a longer period of time. This result is congruent with current knowledge of butterfly biology; access to sugars often significantly increases butterfly fitness (Bauerfeind and Fischer, 2005; Boggs and Ross, 1993; Ferkau and Fischer, 2006; Karlsson and Wickman, 1990; Molleman et al., 2008; Romeis and Wackers, 2002). Animals reared on diet of equal protein to carbohydrate ratio performed best when provided only sugars as adults. Nectar-derived amino acids did not appear to aid female *V. cardui* fitness, and, interestingly, butterflies provided sugar and amino acids had reduced fecundity in comparison to adults given sugar alone when larvae were reared on equal-ratio diet. These are the first results to indicate that nectar-derived amino acids may have negative consequences under certain circumstances, such as when larvae are reared on diets approximating their nutrient targets.

Animals should select between possible mates if potential mates differ in quality (i.e. potential fecundity of females, resources offered by males) (Clutton-Brock, 2007; Johnstone et al., 1996; Tang-Martinez and Ryder, 2005; Thornhill and Alcock, 1983). Female size may be strongly correlated with fecundity (Awmack and Leather, 2002; Honek, 1993) and butterflies may select potential mates based on size (Agosta, 2010; Frey et al., 1998; Rutowski, 1982a; Thornhill and Alcock, 1983), which is affected by larval nutrition (Lee et al., 2004a; Lee et al., 2002; Lindroth et al., 1991) (see also Chapters II, III). The quality of adult nutrition also affects butterfly fitness (Bauerfeind and Fischer, 2005; Beck, 2007; Boggs and Ross, 1993; Dunlap-Pianka et al., 1977; Geister et al., 2008; Lederhouse et al., 1990; Lewis and Wedell, 2007; Mevi-Schutz and Erhardt, 2005; Romeis and Wackers, 2002), although butterfly size (wing length) is fixed at eclosion. I therefore predicted that *V. cardui* would select between potential mates based on larval nutrition but not adult nutrition and tested this prediction in Chapter V.

Adults were offered two potential mates that differed in both larval and adult nutrition. Animals did not become sexually active until 13 hours after the beginning of the photophase, corresponding to observations that *Vanessa* males do not take up perches until shortly before sunset (Opler and Krizek, 1984). Larval nutrition did not affect mate choice, although it affected the size of potential mates offered to selecting individuals. This result indicates that *V. cardui* do not discriminate between potential mates due to the range of sizes availably in this study. Similarly, male size was not correlated with mating success for *Pieris napi* in the field, indicating that females were not selective in this regard (Wiklund and Kaitala, 1995). Furthermore, the correlation between size and fecundity is often not strong or absent in female Lepidopterans (Leather, 1988). Therefore size may not be a good predictor of female quality (i.e potential fecundity) for males to use when selecting between potential mates.

Adult nutrition significantly affected the likelihood that a potential mate would be successful at securing a mating. Overall, potential mates that were given sugar and amino acid nectar were more likely to be chosen by selecting butterflies than those given sugar alone. This is consistent with the hypothesis that nectar-derived amino acids enhance butterfly fitness.

Furthermore, animals fed protein-rich larval diet and provided sugar and amino acid nectar as adults were the most likely to be chosen as mates by selecting individuals, while those fed carbohydrate-rich larval diet and given sugar nectar as adults were the least likely to be chosen. This follows theoretical predictions that animals should select between potential mates based on mate quality (Clutton-Brock, 2007; Johnstone et al., 1996; Tang-Martinez and Ryder, 2005; Thornhill and Alcock, 1983), and that animals may benefit from, and thus choose to mate with, individuals with greater nitrogen reserves either from larval or adult feeding. Nitrogen is a limiting nutrient for folivores (Tabashnik and Slansky, 1987) and is especially limiting in the adult diet of nectarivorous Lepidoptera (Rutowski, 1982b). The current study indicates that butterflies can select between potential mates due to adult diet and may be a further explanation for the prevalence of puddling in male Lepidopterans and amino acid preference in female nectar feeding.

The apparent mate choice observed in Chapter V may have resulted from mate choice or due to potential mates differing in intrasexual competitive ability or sexual responsiveness. Nonetheless, results reveal that adult diet affects the probability that an individual will successfully secure a mating. Furthermore, they are the first to demonstrate that adult diet affects butterfly mating success and reveal another avenue in which amino acids acquired through adult foraging may enhance butterfly fitness.

This dissertation research revealed that 5th instar *V. cardui* larvae independently regulate dietary consumption of protein and carbohydrate, and alter consumption when constrained to suboptimal diets in the way as expected of a generalist herbivore. Initial preference for nectars containing amino acids may be altered in response to changes in larval nutrition for females, but not males. Adult consumption of carbohydrates is shown to be the most important factor increasing egg production, while consumption of nectar containing amino acids in addition to sugars may decrease egg production by butterflies reared on diets of equal-ratio protein:carbohydrate. Larval nutrition does not affect mate choice, but adult diet of animals affects the likelihood that they will successfully secure a mate. Overall, these studies indicate that nutrient regulation by *V. cardui* is likely constrained to larval feeding, while the most important nutrient acquired by adults for reproduction are the carbohydrates that nectar provides. However, while the intake of amino acids by adults may not affect fecundity or longevity, consumption of nectar containing amino acids in addition to sugar increase the probability that an individual will secure a mate. Therefore, consumption of amino acid rich nectar may nonetheless positively affect *V. cardui* fitness.

Future research should address the postingestive regulation of nutrients by *V. cardui* larvae. Furthermore, it is interesting to note that total fecundity did not differ between butterflies reared on equal-ratio and protein-biased diets (Chapter IV), but results from Chapter III indicate that butterflies reared on protein-biased diet consume greater quantities of nectar. Given that total fecundity and longevity may be affected by total nectar consumption (Boggs and Ross, 1993; Hainsworth et al., 1991; Mevi-Schutz and Erhardt, 2003a, 2005), any future study on butterfly fitness should also measure total consumption by adults for use as a covariate in analyses in order to determine the effects of diet while accounting for differences in total nectar consumption.

Amino acids acquired through adult feeding may enhance butterfly fitness through increasing or maintaining flight muscle, thereby increasing the capability for greater dispersal, mate courtship, territory defense, or predator avoidance. Proline may specifically aid butterfly flight capabilities through its presence in floral nectar (Carter et al., 2006). Proline is often found at high levels floral nectar (Gardener and Gillman, 2001a) including *L. camara* floral nectar (Alm et al., 1990), and is the most prominent amino acid in pollen (Zhang et al., 1982). Proline is oxidized in insect flight muscle as a source of energy predominantly during the first 30 seconds of flight by the oxidative proline degradation pathway because it is rapidly metabolized and results in the production of high levels of ATP (Balboni, 1978; Brosemer and Veerabhadrappa, 1965; Crabtree and Newsholme, 1970; Njagi et al., 1992). The initial steps of glucose metabolism require the consumption of ATP, although glucose is a superior fuel to proline for prolonged flight. Other amino acids cannot be metabolized as rapidly as proline and do not release as much ATP without complete metabolism. Enhanced flight capabilities may markedly affect butterfly fitness through increasing survival (predator avoidance) or ability to secure mates (courtship, territory defense). However, studies on the effects of adult dietary nutrients on butterfly fitness do not measure flight capability but instead measure other fitness parameters (i.e. fecundity, longevity, egg size, egg composition, hatching success, etc.). Consequently, future studies on potential effect of nectar-derived amino acids on butterfly fitness should include measurements of flight capability.

Results from Chapter V revealed that potential mates given sugar and amino acid nectar were more likely to be chosen by selecting butterflies than those given sugar alone, but did not determine if this was due to mate choice by selecting individuals or due to differences in intrasexual competitive ability or sexual responsiveness of potential mates. More research needs to be done to determine: 1) if adult diet is affecting mate choice or alternatively the competitive ability or sexual responsiveness of potential mates through more detailed observations of adult mating behaviors, 2) how nectar-derived amino acids are altering mate choice or competitive ability, and 3) if exhibiting mate choice indeed confers fitness benefits to the selective individual (if mating preferences observed are due to mate choice). Furthermore, future research may determine if adult dietary history affects mate choice in other butterfly species, or affects *V.*

cardui mate choice under more natural conditions (i.e. when larvae are reared on leaves of low vs. high nitrogen content).

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