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Does it Pay to Delay? Flesh Flies Show Adaptive Plasticity in Reproductive Timing

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Abstract

Life-history plasticity is widespread among organisms. However, an important question is whether this plasticity is adaptive, enhancing the organism's fitness. Most models for plasticity in life-history timing predict that once they have reached the minimal nutritional threshold animals under poor conditions will accelerate timing to development or reproduction. Adaptive delays in reproductive timing are not common, especially in short-lived species. Examples of adaptive reproductive delays exist in mammalian populations experiencing strong interspecific (e.g. predation) and intraspecific (e.g. infanticide) competition. But are there other environmental factors that may trigger an adaptive delay in reproductive timing? We show that the short-lived flesh fly *Sarcophaga crassipalpis* will delay reproductive timing under nutrient poor conditions, even though it has already met the minimal nutritional threshold for reproduction. We test if this delay strategy is consistent with an adaptive response allowing the scavenger time to locate more resources by providing additional protein pulses (early, mid and late) throughout the reproductive delay period. Flies receiving additional protein produced more eggs and larger eggs, demonstrating a benefit of the delay. In addition, by tracking the allocation of carbon from the pulses using stable isotopes, we show that flies receiving earlier pulses incorporated more carbon into eggs and somatic tissue than those provided a later pulse. These results indicate that the reproductive delay in *S. crassipalpis* is consistent with adaptive post-threshold plasticity, a nutritionally-linked reproductive strategy that has not been previously reported in an invertebrate species.

Keywords

resource allocation; phenotypic plasticity; stable isotopes; adaptation; evolutionary physiology

Introduction

Much of an organism's life-history strategy is centered on preparing for and executing a few key life-history events (e.g. reproduction) that require a substantial quantity of resources. The efficient acquisition and allocation of nutritional resources has a profound influence on these processes (Boggs 2009). However, because resources are often limited, organisms must adjust resource acquisition and allocation patterns to compensate for changing environmental conditions (Zera 2005; Boggs 2009). This plasticity can be adaptive if it enables individuals to increase their fitness within a particular environment (Reznick 1990; Wingfield 2005; Shine & Brown 2008). The ubiquitous nature of life-history plasticity

suggests that it is important, allowing individuals to buffer changes in their environment and thereby efficiently allocate resources to growth, storage and somatic maintenance (West-Eberhard 1989; Stearns 1992; Boggs 2009). Plasticity can be expressed on both the organismal (morphological and behavioral plasticity) and sub-organismal level (physiological plasticity) (West-Eberhard 1989; Hatle et al. 2004). While organismal plasticity is often more apparent than sub-organismal changes, both can have profound influences on life-history.

Reproduction is arguably the most important event in an organism's life-history, and as such it is a common correlate of fitness. For many organisms, the timing of reproduction and the magnitude of reproductive allotment are not static; they are flexible and highly tuned to environmental conditions (Bertness 1981; Moehrli & Juliano 1998; Shine & Brown 2008; Wheeler 1996). Reproductive plasticity can be manifest in the timing of reproductive development, the magnitude of reproductive allotment, or a combination of both. However, reproductive plasticity is not infinite, like any other trait there are limits to the extent of plasticity. The physiological control of life-history plasticity depends on numerous regulatory mechanisms (endocrine, genetic, etc.) each with their own feedback loops and constraints. These mechanisms can limit plasticity because they only allow for a certain number of physiological, behavioral, or anatomical states to occur simultaneously (Ricklefs & Wikelski 2002). Physiological limitations may restrict reproductive plasticity to relatively short windows of opportunity followed by fixed (or canalized) phases of development that are independent of environmental input (Hatle et al. 2000; 2001; 2004; Juliano et al. 2004; Twombly 1996). Determining whether reproductive plasticity is adaptive is important for ultimately understanding how selection may shape life-histories. Adaptive plasticity implies that an organism has the ability to compare its intrinsic condition with extrinsic environmental cues, and to respond to these signals by altering its physiology (Reznick 1990; Juliano et al. 2004). However, because not all examples of plasticity are adaptive, discerning adaptive responses from non-adaptive plasticity can be difficult and requires not only identifying plasticity, but also evaluating its influence on life-history traits (Reznick 1990).

The majority of studies on reproductive plasticity in response to dietary input have been influenced by the model of amphibian metamorphosis proposed by Wilbur and Collins (1973), describing the development of frogs and salamanders in ephemeral ponds (Moehrli & Juliano 1998; Hatle et al. 2004; Juliano et al. 2004). The model predicts that individuals with access to high quality resources should delay metamorphosis after reaching the minimum nutritional threshold to accumulate more resources and grow larger. In contrast, individuals in ponds with poor resource quality should undergo metamorphosis as soon as they reach the minimal size threshold, allowing them to disperse to areas with greater resource abundance (Wilbur & Collins 1973). Clearly, resource availability will affect the time it takes to reach reproductive and developmental thresholds, so Wilbur and Collins (1973) predicted that amphibians will show adaptive plasticity only after the minimum threshold has been reached. While the Wilbur and Collins framework was developed in light of amphibian metamorphosis, the concept of post-threshold plasticity can be extended to other major life-history events, such as reproduction. An important question is how reproduction will follow the predictions of this model, for example will reproductive development in a poor environment result in faster post threshold reproductive development as predicted for metamorphosis?

Studies on reproductive plasticity in insects have not supported adaptive post-threshold plasticity as predicted by Wilbur & Collins (1973) (Boggs & Ross 1993; Moehrli & Juliano 1998; Fischer & Fiedler 2001; Good & Tatar 2001; Bauerfeind & Fischer 2005). In contrast, most empirical studies with insects have shown that after achieving the minimal nutritional

threshold for reproduction, allocation towards reproductive tissues is canalized (Juliano et al. 2004). Fixed allocation patterns suggest that insect reproductive plasticity is constrained by regulatory mechanisms that are activated once the minimal nutritional threshold has been met (Ricklefs & Wikelski 2002; Fronstin and Hatle 2008). While canalization may be common, it is not necessarily the rule. Hahn et al. (2008a) reported post-threshold plasticity in reproductive timing in the flesh fly, *Sarcophaga crassipalpis* Macquart. They found that adult female flesh flies that were switched to a protein-restricted diet after reaching their nutritional threshold for reproduction took longer to reproduce and had lower reproductive allotment (smaller and fewer eggs) than those fed a high protein diet (Hahn et al. 2008a). This plasticity differs from the Wilbur & Collins (1973) model in that resource poor individuals delay reproduction despite having already reached their minimum threshold, compared to resource poor anurans that undergo metamorphosis rapidly. Perhaps adaptive plasticity or the lack thereof is best viewed from an evolutionary perspective of each particular species under investigation. Unlike larval amphibians that are constrained to an ephemeral aquatic habitat until metamorphosis, adult flesh flies are highly mobile income breeders that must acquire sufficient protein and carbohydrate resources to provision their eggs (Wessels et al. 2010). In addition, flesh flies undergo energetically-demanding synchronous oocyte development and provision large clutches of eggs at a time, but they are short lived as adults, suggesting that early reproduction is important. Considering the spatially and temporally patchy nature of their food sources (carrion and nectar), Hahn et al. (2008a) proposed that the delay in flesh fly reproduction might be adaptive if it allows flies more time to acquire additional resources to maximize the quality of the first clutch.

Despite the importance of shifts in resource allocation to life-history plasticity, the flow of resources within an organism is one of the more difficult types of sub-organismal plasticity to measure directly (Zera & Harshman 2001). Evidence of this difficulty can be seen in the large gap between theoretical and empirical studies of resource allocation in life-history strategies (Rivero & Casas 1999; Giron & Casas 2003). Some the difficulty of tracking internal resource allocation has been mitigated by labeling diets with naturally-occurring stable isotopes (O'Brien et al. 2000). Stable isotopes have been used to follow resource allocation in multiple studies ranging from tracking the allocation patterns of larval and adult resources in moths (O'Brien et al. 2000; 2002) to understanding the flexibility of reproductive allocation in lizards (Warner et al. 2008). In this study, we evaluate whether delaying reproduction can be beneficial to *S. crassipalpis* by providing resource “pulses” at different times during the delay period to determine if the resource pulse affects reproductive allocation or timing (Fig. 1a). If the reproductive delay is beneficial and therefore consistent with an adaptive plastic response, we expect that resources acquired during the post-threshold delay period will result in faster development time, increased reproductive allocation, or a combination of both (Fig. 1a). We evaluate both the timing and allotment of resources using conventional organismal metrics (fecundity, egg size, etc.), but we go beyond most other studies by quantifying allocation of the pulsed resources to reproduction and soma using a diet-labeling approach with diets that have distinct stable carbon isotope profiles.

Materials and Methods

Animal Rearing and Experimental Design

Sarcophaga crassipalpis (flesh flies) used in this study were obtained from a laboratory colony maintained at the University of Florida according to procedures outlined in Hahn et al. (2008a) and Denlinger (1972). Newly-eclosed flies were hand sorted by sex into twelve screened cages (30 × 30 × 30 cm); each cage contained 150 females and 150 males. All cages were provided with water and granulated sugar *ad libitum* throughout the experiment. Of the twelve cages, two were provided liver from organic grass-fed cattle raised in Florida

(a high ^{13}C food source, abbreviated FL liver) *ad libitum* throughout the experiment, two were provided FL liver for the first four days after eclosion, and the remaining eight cages were provided FL liver for 2 days post-eclosion (Fig. 1b). Of the eight cages that were given FL liver for 2 days, two did not receive any additional resource pulses and the remaining six were given isotopically distinct resource pulses for an additional two days: on days 5 and 6, on days 7 and 8, or on days 9 and 10 (Fig. 1b). The isotopically distinct resource pulses consisted of switching the granulated sugar source from cane sugar (high ^{13}C) to beet sugar (low ^{13}C) and providing *ad libitum* access to beef liver from organic, grass-fed cattle raised on a farm in Dillon, MT (low ^{13}C , abbreviated MT liver).

Female flesh flies do not begin to provision eggs until the fourth day post-eclosion (Hahn et al. 2008b). Therefore, starting three days post-eclosion, seven females were sampled from each cage daily until all females contained fully-mature eggs. The two cages provided with FL liver for 2 days were sampled for 16 days total, longer than the other treatments because a portion of the females in this treatment did not fully provision eggs on any given sampling day. Females were frozen at $-20\text{ }^{\circ}\text{C}$ immediately following sampling and were later dissected. Females were thawed on ice and dissected in ultrapure water, the ovaries and any eggs in the uterus were removed. Oogenic development was characterized by dissecting the ovaries and observing the oocytes; the developing oocytes were staged using an eight point scale ranging from previtellogenic follicles (1) to mature chorionated eggs (8) as described in Hahn et al. (2008a). Fully-mature eggs were removed from the uterus and counted as a measure of fecundity. The length and width of four mature eggs from each female were measured to the nearest 0.1 mm using a microscope-mounted ocular micrometer, and the mean value for each female was used in further analyses. The eggs from mature females and the remaining carcass (which we refer to as the soma) were stored in separate 1.5 ml microcentrifuge tubes and frozen at $-80\text{ }^{\circ}\text{C}$ in preparation for stable isotope analysis.

Stable Isotope Analysis

In this study, we used stable isotopes of carbon to track the incorporation of resources to reproductive and somatic tissue over time. Stable isotopes are naturally occurring isotopes of an element that have a different number of neutrons, and unlike radioactive isotopes, they do not decay. The most common isotopes used as dietary tracers are carbon (^{13}C) and nitrogen (^{15}N) (Hood-Nowotny & Knols 2007). Stable isotopes are substantially less abundant than their natural counterparts, but their concentration can vary naturally in biological systems. Stable isotope concentration is typically reported as a ratio of the less abundant isotope to the more abundant isotope compared to a standard, commonly known as delta notation (expressed in ‰) (Equation 1). In the case of carbon, animal and plant tissue is always more depleted in ^{13}C than the standard resulting in a negative number in delta notation; the less negative the number, the more ^{13}C the tissue contains.

$$\delta^{13}\text{C} = \left(\frac{(^{13}\text{C}/^{12}\text{C})_{\text{SAMPLE}}}{(^{13}\text{C}/^{12}\text{C})_{\text{STANDARD}} - 1} \right) \times 1000 \quad (1)$$

C_4 plants typically have more ^{13}C ($\delta^{13}\text{C} \sim -13\text{ }‰$) than C_3 plants ($\delta^{13}\text{C} \sim -27\text{ }‰$) (Cerling & Harris 1999). In this experiment, we take advantage of this naturally-occurring variation in isotope concentrations to create high and low ^{13}C labeled diets. We labeled the sugar source provided to the flies by using granulated sucrose from sugar cane (C_4 plant, $\delta^{13}\text{C} = -11.26\text{ }‰$) and sugar beet (C_3 plant, $\delta^{13}\text{C} = -24.76\text{ }‰$). In addition, we provided flies with beef liver as a protein source; the liver used was from USDA organic grass-fed cattle raised in either Florida or Montana. In North America, the proportion of C_3 and C_4 grasses varies with latitude (MacFadden et al. 1999). C_3 grasses are more common at higher latitudes and

C₄ grasses are more common in equatorial latitudes (Terri & Stowe 1976). Therefore, the tissues of naturally-grazing cattle raised at lower latitudes will contain more ¹³C (FL liver = $\delta^{13}\text{C} - 17.3\text{‰}$, latitude 29.17°N) than those raised at higher latitudes (MT liver = $\delta^{13}\text{C} - 25.7\text{‰}$, latitude 45.22°N). By providing flies with either cane sugar and FL liver (High ¹³C) or beet sugar and MT liver (Low ¹³C) we have created both high and low ¹³C diets.

All flesh flies in this experiment were raised on the high ¹³C diet as larvae and were initially provided with the high diet as adults. The treatments that received a resource pulse during the reproductive delay were switched to the low ¹³C diet during the pulse period (Fig. 1b). This separation allows us to track and quantify the incorporation of carbon from the resource pulses. Prior to isotope analysis, insect tissues were frozen at -80 °C, then lyophilized. Next, the dried tissues were homogenized in a vibratory bead homogenizer using zinc-coated steel pellets. Between 650–750 μg of homogenized tissue from each individual was placed into a Costech 5 × 9 mm pressed tin capsules for stable isotope analysis at the University of Florida Stable Isotope Geochemistry Lab. Samples were first combusted in a Carlo Erba NA 1500 CNS elemental analyzer. The purified N₂ and CO₂ gas from the elemental analyzer was carried to a ConFlo II interface and then into a Finnigan-MAT 252 isotope ratio mass spectrometer. L-glutamic acid (NIST USGS40) was used as a standard, results were reported versus VPDB for $\delta^{13}\text{C}$.

Stable Isotope Incorporation – Mixing Model

Accounting for the discrimination of the tracer as it is metabolized by the consumer is important. Discrimination results in differences in the composition of the consumer's tissue and the diet (Martinez del Rio and Wolf 2005). The easiest way to account for discrimination is to rear individuals solely on both diets and measure the difference between the dietary ¹³C concentration and the ¹³C composition of the tissue of interest (Table 1). To quantify discrimination, we raised a cohort of *S. crassipalpis* solely on our high ¹³C diet, and another cohort solely on our low ¹³C diet, and sampled four females when they had fully-mature eggs present in their uterus. The eggs were dissected and the egg and somatic tissue were prepared separately for stable isotope analysis (Table 1).

We quantified the incorporation of carbon from the resource pulses into the eggs and somatic tissue using a two-source linear mixing model (Equation 2):

$$\delta^{13}\text{C}_{\text{Tissue}} = [(p) \times (\delta^{13}\text{C}_{\text{HIGH DIET}} + d_{\text{HD}})] + [(1-p) \times (\delta^{13}\text{C}_{\text{LOW DIET PULSE}} + d_{\text{LDP}})] \quad (2)$$

where $\delta^{13}\text{C}_{\text{Tissue}}$ is the consumer tissue of interest, and p represents the proportion of the tissue derived from carbon from the high diet. The term $\delta^{13}\text{C}_{\text{High Diet}}$ is the isotopic composition of the high diet and d_{HD} represents the discrimination of the high diet by the consumer, $\delta^{13}\text{C}_{\text{Low Diet Pulse}}$ refers to the isotopic composition of the low diet and d_{LDP} represents discrimination of the low diet pulse. All statistical analyses were performed using JMP version 7.0.2 (SAS Institute, Cary, North Carolina, USA) and mixing models were calculated using Microsoft Excel (Microsoft Corporation, Redmond, Washington, USA).

Results

Reproductive Timing and Allocation

The non-pulsed treatments showed a reproductive delay similar to that observed in Hahn et al. (2008a) (Fig. 2a). Receiving a resource pulse substantially decreased the time required to fully provision eggs in all pulsed treatments. Egg development time in individuals given the earliest pulse on days 5 & 6 post-eclosion was not different from the 4 day non-pulsed

treatment, although both took slightly longer to provision eggs than the *ad libitum* treatment (Fig. 2b). Development time in the 7 & 8 and 9 & 10 pulse treatments was slower than the *ad libitum* and 4-day, non-pulsed treatments. In addition, egg development time did not differ between individuals given a pulse on days 7 & 8 and individuals given a later pulse on days 9 & 10 (Fig. 2b). Most important, development time in the 7 & 8 and 9 & 10 day pulse treatments was faster than the 2-day, non-pulsed treatment, suggesting that pulsed flies were able to beneficially use the pulsed resources to decrease the time required to provision eggs (Fig. 2b). The majority of females fed liver for 2 days were able to develop fully mature eggs by 16 days, however, a small portion (36%) had not completely provisioned their oocytes (Fig. 2a) (Table 2).

Resource availability and pulse timing had a substantial effect on reproductive allotment in *S. crassipalpis*. There was no difference in fecundity between the *ad libitum* and 4 day non-pulsed treatments, but the 2-day treatment had fewer eggs (Fig. 3a). Individuals given an early pulse (days 5 & 6) produced more eggs than individuals receiving the intermediate pulse (days 7 & 8) which, in turn, produced more eggs than females receiving the late pulse (days 9 & 10) (Fig. 3a), indicating that resources acquired earlier during the delay period had a greater effect on fecundity than those acquired later. Feeding treatment similarly affected egg size (Fig. 3b). In the pulsed treatments, individuals provided with the earliest pulse (days 5 & 6) had larger eggs than individuals that received the two later pulses (days 7 & 8, and 9 & 10) (Fig. 3b).

Female flies were capable of incorporating resources from pulses during the delay period into eggs, and the timing of the resource pulse affected the magnitude of resource allocation. Females provided with the earlier resource pulses (days 5 & 6, and 7 & 8) incorporated more carbon from the pulse into their eggs than those provided a later pulse (days 9 & 10) (Fig. 4a). Similarly, females provided the earliest two resource pulses (days 5 & 6, and 7 & 8) were able to incorporate more carbon from the pulse into their somatic tissue than those provided with the later pulse (days 9 & 10) (Fig. 4b). Further, for all three pulsed treatments, the percentage of carbon allocated to reproductive tissue from the pulse was greater than the percentage of somatic tissue from the pulse.

Discussion

We expected that if the observed reproductive delay in flesh flies was adaptive, flies receiving additional resource pulses during the delay period should benefit from those resources by allocating them to reproduction. Flies that received additional resources during the delay period had more eggs, larger eggs, and reproduced faster than individuals that did not receive additional resources. The later the resource pulse was provided, the fewer eggs females provisioned. Therefore, resources acquired late during the delay period did not enhance reproduction as much as those acquired earlier, suggesting that flesh flies cannot delay reproduction indefinitely and that resources acquired earlier in the delay period have more value for the current reproductive bout than those acquired later. The observed benefits of resources acquired during the delay period are consistent with adaptive post-threshold reproductive plasticity (diamond symbols in Fig. 1a, Figs. 3a & b).

We further support the organismal metrics of reproductive output by quantifying allocation of the pulsed nutrients towards reproduction and soma using stable carbon isotopes. Flesh flies allocated carbon from all of the resource pulses towards reproduction, but the timing of the pulse affected both the magnitude of allocation and reproductive timing. Therefore, the environmental variable that initiated the plasticity (e.g., more food) was itself involved in the adaptive physiological change (allocation of nutrients from that food to the eggs) in response to that plasticity. In general, flies receiving early pulses were able to use the

nutrients from this food to a greater degree than flies receiving late pulses. Individuals that received the early and intermediate resource pulses allocated approximately 15% more carbon from the pulse to eggs than those that received the latest pulse (Fig. 4a). Similarly, females receiving a late pulse had somatic tissues that contained less carbon from the pulse than did females receiving an early pulse. This is despite the fact that all pulse groups required a similar time period from receiving the pulse until they fully provisioned their eggs. For example, the earliest pulse group received additional protein on day 5 and completed egg provisioning on day 9, while the latest pulse group received additional protein on day 9 and completed provisioning on day 12. Hence, there is a clear link between the environmental change (food availability) and the physiology of the flies (allocation of the meal to eggs and soma), and this physiological ability to incorporate nutrients changes with age.

A critical question underlying the presence of the reproductive delay in *S. crassipalpis* is the biological significance of this strategy. *Sarcophaga crassipalpis* females are capable of producing multiple clutches of offspring as adults. In the laboratory, they regularly lay 1–2 clutches of eggs, but conditions in the field are likely to be more stressful and opportunities for multiple clutches are probably more limited in nature than in the lab. Therefore, an obvious question is why females would delay reproduction to maximize the first clutch, when they could presumably lay a rapid, but suboptimal, first clutch, and focus on acquiring higher quality resources for the second clutch. The delay strategy suggests that there are substantial benefits associated with optimizing allotment in the first clutch of eggs. Presumably, the benefits are substantial enough to allocate additional time towards, even at the cost of future reproduction. Evolutionary theory predicts that early reproduction is more likely to be successful, and therefore more valuable, than later reproduction because the chance of mortality increases with age (Magnhagen 1990, 1991; Stearns 1992). Results from a parallel laboratory study suggest that flesh flies experience relatively high mortality after the first clutch (under optimal laboratory conditions, a clutch is ready to lay in 10 days). When reared under a sex ratio similar to this study (1:1 female: male initial ratio) female mortality is approximately 56% by day 20 and 89% by day 30 when fed continuous liver (Bastea L.I., Brix K.V., Hatle J.D., unpublished data). Therefore, even under optimal conditions there is a high probability of mortality prior to laying the second clutch. Because resources are patchy and clutch laying represents such a large investment of resources, the ability to produce a high-quality first clutch may be particularly important in field populations.

Due to the cryptic nature of resource allocation, the role of nutrition in shaping reproductive allocation strategies is not well understood. Moehrli and Juliano (1998) manipulated the quantity and timing of food availability to the grasshopper *Romalea microptera* (=guttata). Diets were switched from high to low quantity and vice versa. *Romalea microptera* was able to detect food quantity for a short window of time after adult molt and respond to that input by altering the timing and magnitude of reproductive allocation accordingly (Moehrli and Juliano 1998). However, if food quantity was switched after the detection window, no changes in age at oviposition were observed (Moehrli and Juliano 1998). In the case of *R. microptera*, it appears that reproduction shows plasticity in response to nutritional conditions immediately after adult molt. However, unlike flesh flies, there appears to be a time threshold, after which reproductive allocation is fixed and cannot be “rescued” by switching to high quantity resources. Similarly, the cockroach *Nauphoeta cinerea* gradually matures oocytes following adult eclosion (Moore & Sharma 2005). However, in this species mating induces an increase in juvenile hormone synthesis that results in rapid oocyte development (Moore & Moore 2001; Moore & Sharma 2005). There is a direct fitness cost to delaying mating, wherein females that delay mating past peak receptiveness have reduced fecundity and take longer to lay their first clutch (Moore & Moore 2001). Cockroaches that are starved

following adult eclosion have lower oocyte volume and greater oocyte apoptosis (Barrett et al. 2008). Barrett and colleagues (2008) hypothesized that the oocytes are being resorbed to allocate additional resources towards somatic functions. Not surprisingly, reproductive allotment is decreased when nutritional resources are scarce. However, non-vitellogenic oocytes were also resorbed, suggesting that starvation may also negatively impact future reproduction (Barrett et al. 2008).

The mechanistic basis of plasticity and canalization has long been a subject of debate. One leading hypothesis suggests that the transition from plastic to canalized phases of development is due to attaining a nutritional threshold, and then releasing an endocrine signal that commits the animal to the major life-history transition (Bradshaw & Johnson 1995; Hatle et al. 2004). This can create constraints in life-history development, such as canalized phases of development. Canalization during changing environmental conditions may be the result of physiological lags associated with the endocrine cascades that initiate life-history transitions (Nijhout 1994; Ricklefs & Wikelski 2002). These constraints on the physiological progression of life-history transitions may explain why there are limits on the number of life-history options available at any one time for most organisms. In contrast to this typical pattern of attaining a threshold and then becoming fixed, *S. crassipalpis* reaches a threshold that allows completion of reproduction but retains plasticity after attaining the threshold.

To our knowledge, no other examples of nutritionally-induced adaptive reproductive delays have been documented in the literature. Perhaps this is due to the difficulty of identifying adaptive plasticity and establishing a cause for the plasticity. Simply finding a correlation between poor resources and delayed development or reproductive timing does not indicate an adaptive response. Adaptive reproductive delays have been noted in several mammalian systems (Wolff 1997; Agrell et al. 1998), but most of these are associated with either interspecific (Ylönen 1989; Norrdahl & Korpimäki 1995) or intraspecific competition (Huck 1982; Packer & Pusey 1983; Digby 1995; Woodroffe & MacDonald 1995). For example, in a laboratory setting, the presence of a predatory weasel in close proximity to breeding voles caused complete suppression of reproduction despite adequate nutrition, whereas voles not exposed to the predator reproduced normally (Ylönen 1989). The majority of studies finding an adaptive delay in reproduction due to strong intraspecific competition involved infanticidal species (Agrell et al. 1998). When new male lions “take over” a pride, they often kill existing cubs and the offspring of females that were pregnant at the time of take over (Packer & Pusey 1983). Females that experienced a take over mated more frequently, but took nearly twice as long to reproduce (244 days) as those that continued to mate with the same males (134 days). Packer and Pusey (1983) hypothesized that the adaptive significance of this delay was to incite competition between males, increasing competition and ensuring that the new coalition of males was the most fit. While it is easy to understand the adaptive significance of a delay strategy in these examples, the physiological effects of nutritional stress are often more cryptic and require creative approaches to elucidate them.

Adaptive life-history plasticity remains largely unknown from a biochemical perspective, and only a few model systems have demonstrated such mechanisms. One example is the wing-polymorphic cricket, *Gryllus firmus* (Scudder), that has distinct morphs for dispersal and reproduction (Zera & Harshman 2001). The two morphs differ in their metabolic allocation strategies, wherein the dispersal morph allocates resources to flight muscle maintenance and the reproductive morph allocates the majority of resources to ovarian development in early adulthood (Zera et al. 1994; Zera & Zhao 2006). Zhao and Zera (2002) linked this trade-off between somatic maintenance and reproduction to a physiological difference in lipid biosynthesis. Another clear example of physiological mechanisms and adaptive plasticity of metamorphosis was found in the western spadefoot toad, *Scaphiopus*

hammondii (Denver 1997). Denver (1997) linked the accelerated metamorphosis observed by Wilber and Collins (1973) to the elevation of the stress-induced corticotropin-releasing hormone that controls a suite of hormonal regulators of metamorphosis (Denver 1997). These systems represent valuable tools for characterizing phenotypic plasticity at the sub-organismal level, providing a foundation for studying mechanisms underlying the evolution of phenotypic plasticity.

Our findings demonstrate a clear link between the timing of resource availability and a physiological shift in resource allocation patterns that ultimately increases reproductive allotment and decreases the time required to reproduce. Our results go beyond most other studies of reproductive allocation by showing that flesh flies are able to incorporate additional nutrients acquired during the reproductive delay period and that there are benefits associated with receiving these resources. These findings are consistent with adaptive post-threshold plasticity in reproductive timing and provide the foundation for investigating the mechanisms underlying plasticity and canalization in resource allocation.

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Fig. 1.

(a) Predictive model for adaptive reproductive plasticity in *S. crassipalpis*. Arrows on the X-axis indicate three resource pulses at different times during oogenesis. (b) Experimental design outlining the timing of resource pulses provided during the reproductive delay period.

Fig. 2.

Egg development over time across feeding treatments, (a) shows non-pulsed control treatments. The *ad libitum* and 4-day feeding treatments do not delay reproduction, while the 2-day feeding regime shows a delay in reproduction. (b) Pulsed feeding treatments (black symbols) are overlaying the non-pulsed controls (grey symbols) showing the reduced reproductive timing when additional resource pulses are provided. Symbols represent means and bars represent standard error. Letters denote mean separation in a general linear model using Tukey's honestly significant difference.

Fig. 3.

(a) The relationship between reproductive timing (number of days until 50% of the population reached full reproduction) and fecundity and (b) the relationship between timing and egg size (length x width). Both show the benefits of acquiring resources during the reproductive delay period, where individuals receiving earlier pulses have more eggs (a) and larger eggs (b) than those receiving later treatments. The symbols represent means and error bars represent standard error. Letters indicate separation of the mean egg size using a Student's t-test.

Fig. 4.

(a) The amount of egg carbon (%) from pulsed resources provided during the reproductive delay and (b) the amount of somatic carbon (%) from pulsed resources. Both show greater allocation associated with early pulses although overall more resources were directed towards reproductive tissue. Bars represent means and error bars show standard error. Letters denote separation of the means using Tukey's honestly significant difference.

Table 1

Quantification of ^{13}C discrimination of egg and somatic tissue from female *S. crassipalpis* raised on high and low ^{13}C artificial diets.

Diet	Tissue	Average $\delta^{13}\text{C} \pm \text{SE}$	n
High	Soma	-16.36 ± 0.13	4
Low	Soma	-24.90 ± 0.09	3
High	Eggs	-14.76 ± 0.18	4
Low	Eggs	-24.75 ± 0.25	4

Table 2

Multivariable general linear model for the effects of treatment and timing on egg development.

Egg Development	d.f.	F	p
Whole Model	17	86.65	< 0.0001
Treatment	5	60.50	< 0.0001
Age: Days Post-Eclosion	1	165.15	< 0.0001
Interaction: Trt x Age	5	22.87	< 0.0001
Cage Effects	6	8.21	< 0.0001
Error	901		
Total	918		