INTRODUCTION

Why are trade-offs important?

Life-histories refer to the timing and duration of development prior to reproduction and the levels and duration of reproductive events after sexual maturity. Evolutionary biologists have long been interested in understanding the forces that mold life-histories because of their great variability in nature. Many life-history characters like development time, and numbers of offspring produced, are closely connected to fitness. Yet there is not a single set of life-history traits that always has greatest fitness in all environments.

An important theme in most explanations of life-history evolution is the notion that the relative allocation of resources to different aspects of development and reproduction will have fitness consequences that depend on aspects of the environment. Certainly one of the earliest problems in this field was why birds at higher latitudes tended to lay more eggs than birds close to the equator (Ricklefs, 2000). While Moreau (1944) and Lack (1947) made important contributions to this problem, Cody (1966) framed his explanation simply, in terms of limited energy resources and trade-offs between the competing demands for this energy. The importance of finite energy became part of formal theory in the classic paper by Gadgil and Bossert (1970). They summarize the point of view, “An organism’s life history may be looked upon as resultant of three biological processes, namely, maintenance, growth, and reproduction. Any organism has limited resources of time and energy at its disposal. The three component processes of the life history compete for these limited resources.” (Gadgil and Bossert, 1970, pg. 3).

Much time and effort over the last 30 years has been spent on collecting evidence of trade-offs (Stearns, 2000). There have also been conflicting ideas over what would constitute evidence of trade-offs (Reznick, 1985; Reznick et al., 2000). Much of the research focused on the sign of genetic correlations between traits of interest (Van Noordwijk and de Jong, 1986; Houle, 1991; de Jong and Van Noordwijk, 1992; Roff, 2000). In other studies changes in traits in direct response to natural selection were observed that confirmed the idea of trade-offs. In populations of Drosophila selection for late life survival and reproduction, increases in longevity were accompanied by declines in early fecundity (Rose, 1984). Likewise, populations of Drosophila that have evolved at high population density show increased rates of population growth at high densities but reduced rates of growth at low densities relative to populations maintained at low density (Mueller and Ayala, 1981; Mueller et al., 1991).

The body of research referred to in these last few paragraphs has reinforced the importance of trade-offs as important constraints and determinants of the course of life history evolution. Our ability to understand trade-offs and their mechanisms will ultimately enhance our understanding of the diversity of life.

What do we know about the mechanisms of life-history trade-offs?

Ricklefs and Wikelski (2002) recognize the importance of physiological and behavioral mechanisms of life-history trade-offs. They suggest that “… studies should integrate behavior and physiology within the environmental and demographic contexts of selection. (Ricklefs and Wikelski, 2002, pg. 462). Behavior may be a mechanism by which important trade-offs become balanced. As the end product of evolution behavior may result in decisions that reflect important energetic or fitness trade-offs. For instance Ghalambor and Martin (2001) show that predation risk affects foraging behavior of birds consistent with the expected effects on adult or juvenile mortality. Of course showing certain behaviors are consistent with theoretical predictions is not the same as showing the behavior evolved by
natural selection subject to the requisite trade-offs.

Physiological examinations of existing phenotypic polymorphisms have been made to infer energetic trade-offs. For instance the cricket *Gryllus firmus* consists of two morphs: a winged and a wingless morph. Crnokrak and Roff (2002) show that the winged morph has elevated respiration rates but reduced fecundity relative to wingless morphs.

Immune system function may also be an important focal point of life-history trade-offs (Lochmiller and Deerenberg, 2000; Norris and Evans, 2000; Ricklefs and Wikelski, 2002). At present much of the evidence for the relationship between immune system function, energy, and fitness is indirect. However, this is a potentially attractive area for additional research.

Steven Stearns, who has made many important contributions to life-history theory and research, has stated recently that "We have a lot of evidence that trade-offs exist; we have very little understanding of the mechanisms that cause them." (Stearns, 2000, pg. 484). The most direct and unambiguous way to implicate a mechanism as casual for life-history evolution would be to observe its effects in systems whose evolutionary history and mechanisms were well known. Of course laboratory systems are ideal for this purpose since their history is known exactly and the forces that have varied and thus been the cause of evolutionary change have been under careful control. We next review evidence that larval feeding rates in *Drosophila melanogaster* are likely to be a key phenotype to understanding important trade-offs that occur during the evolution of many life-history characters in this species.

**Life-history evolution in Drosophila may rely on larval feeding rates for important energy trade-offs.**

*Drosophila* larvae complete three instars prior to their metamorphosis in the pupal stage. Except for several hours prior to pupation the larvae are feeding continuously. Larval feeding involves stereotypical behavior consisting of thrusting their mouth hooks forward and then retracting them. This single motion may be repeated 100-200 times per minute. This behavior is associated with food consumption and the passage of food through the animal’s alimentary tract. Below we review evidence that suggests this behavior is an important aspect of life-history evolution in *Drosophila* and may be a primary pathway for energy diversion. In this discussion the life-history we primarily focus on is larval viability and development time. However, it is important to remember that larval growth affects adult size that determines fertility in both males and females (Partridge et al., 1987; Wilkinson, 1987; Mueller and Joshi, 2000, chpt. 6).

1. **Feeding rates respond to many different types of natural selection.** In laboratory *Drosophila* populations feeding rates evolve in response to larval crowding (Joshi and Mueller, 1988; Borash et al., 1998), parasitoids (Fellowes et al., 1999), urea laced larval food (Borash et al., 2000), ammonia laced larval food (Borash et al., 2000), and development time selection (Borash et al., 2000). Populations resistant to parasitoids, ammonia, and urea show lowered feeding rates. Populations adapted to crowding, and rapid development show elevated feeding rates.

2. **Larvae that feed faster are better competitors for limited food** (Burnet et al., 1977; Joshi and Mueller, 1988; Fellowes et al., 1998).

3. **Larvae that feed fast are less efficient.** That is they require more food than slow feeders to successfully pupate (Mueller, 1990; Joshi and Mueller, 1996).

4. **Increased immune response is associated with reduced feeding rates** (Kraaijeveld et al., 2001).

The energetics of movement in *Drosophila*, which is closely tied to feeding behavior, has shown that the cost of transport is one of the highest for terrestrial locomotion (Berrigan and Lighton, 1993; Berrigan and Pepin,
1995). Taken together these observations suggest that evolution in Drosophila larvae that requires energy to be reallocated to new functions will acquire this energy through reductions in feeding activity. We develop these ideas in more detail next for populations of D. melanogaster that have evolved resistance to ammonia in their larval food.

**PRELIMINARY RESULTS**

**Populations**

The populations used for studying the evolution of resistance to ammonia have been derived from laboratory evolved populations that had been maintained at low larval densities and breeding populations of 1000-2000. There are five replicate control populations, called AUC, and five replicate experimental populations, called AX. The AX larvae are raised in 8-dram vials with banana-corn syrup-barley malt food with 1 M ammonium chloride. About 60 eggs are put in each vial and there are 40 vials per population. Each vial has a plastic sleeve placed inside. Pupae settle on the sleeve, which is removed from the vials and placed in a large plexiglass cage prior to emergence of adults. This allows adult to emerge in cage and to feed immediately on standard food. After about one week in the cage eggs are collected and used to start the next generation. Controls are handled exactly the same except there is no ammonium chloride added to their food.

**Selection response**

Borash et al. (2000) show that there are large differences between the AX and AUC populations in egg-to-adult survival in ammonia laced food. Additionally the AX larvae also survive better in food laced with urea than do controls. Previously we noted that the AX populations feed at a slower rate than do the AUC populations. Recently, we have measured a related larval behavior called foraging path length (Sokolowski). To measure this behavior 72 hr old larvae are placed on a flat plexiglass surface that has been coated with a yeast paste. The larvae are allowed to forage in the yeast paste for five minutes and the path the leave is traced onto a petri dish. The path is then scanned into a computer and the length of the digital path is estimated. The path length of all five AX populations was less than its paired control (figure 1). All the tested larvae in figure 1 were raised under standard conditions and tested in food without added ammonia or urea. Thus, the differences in foraging behavior reflected in figure 1 are genetically based.

![Figure 1](image-url) The foraging path length of the five replicate ammonia (AX), urea selected (UX) and control (AUC) populations. The bars are 95% confidence intervals. The path lengths of the AX populations are significantly shorter than the control ($p<0.05$). Populations of the same replicate number were tested on the same day. Day-to-day variation causes differences in path lengths and thus path lengths of different replicates cannot be compared.

From the discussion so far there are two obvious candidate loci that may contribute to ammonia resistance in Drosophila. The first is GDH and the second is for. Ammonia is generated from the catabolism of proteins and amino acids. The final step in this pathway is the deamination of glutamate to produce $\alpha$-ketoglutarate and ammonia and is catalyzed by glutamate dehydrogenase (GDH). The for locus is primarily responsible for foraging path length in Drosophila. The for gene has recently been identified as a cyclic guanosine
monophosphate-dependent protein kinase (Osborne et al., 1997). In collaboration with Dr. Kevin White at Yale University we have tested larvae from these populations for levels of gene expression using RNA arrays. Our preliminary test of the $AX$ and $AUC$ populations suggest that $GDH$ is overexpressed in the $AX$ populations and $for$ is underexpressed (table 1). These results are consistent across the four independent experimental and control populations.

**Table 1.** The ratio of expression level in the $AX$ population divided by the $AUC$ populations. Results for only four population pairs were obtained.

<table>
<thead>
<tr>
<th>Population</th>
<th>Expression Ratio</th>
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<tbody>
<tr>
<td>$AX_1/AUC_1$</td>
<td>GDH 1.24  for 0.79</td>
</tr>
<tr>
<td>$AX_3/AUC_3$</td>
<td>GDH 1.35  for 0.66</td>
</tr>
<tr>
<td>$AX_4/AUC_4$</td>
<td>GDH 1.54  for 0.54</td>
</tr>
<tr>
<td>$AX_5/AUC_5$</td>
<td>GDH 1.43  for 0.89</td>
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**PROPOSED RESEARCH**

**Central Hypothesis**

We propose that adaptation to larval stress will require energy and that this energy is most often obtained by reducing the energy spent on feeding. This type of trade-off is consistent with the energetic trade-off ideas of Cody (1966) and Gadgil and Bossert (1970). We have reason to believe that in *Drosophila* this particular mechanism is quite common, and utilized by *Drosophila* for adaptation to many different environments. However, since many organisms spend large amounts of energy feeding prior to sexual maturity the mechanisms utilized by *Drosophila* may be common to many other organisms.