Evolution of larval foraging behaviour in *Drosophila* and its effects on growth and metabolic rates

LAURENCE D. MUELLER[1], DONNA G. FOLK[2], NGOC NGUYEN[1], PHUONG NGUYEN[1], PHI LAM[1], MICHAEL R. ROSE[1] and TIMOTHY BRADLEY[1]

[1]Department of Ecology and Evolutionary Biology, University of California, Irvine, Irvine, California, U.S.A. and [2]Department of Biology, College of William and Mary, Williamsburg, Virginia, U.S.A.

Abstract. The evolution of foraging in *Drosophila melanogaster* (Meigen) is studied using outbred populations that had been differentiated using laboratory selection. The foraging behaviour of *Drosophila* larvae is measured using the foraging path length of 72-h-old larvae. The foraging path length is the distance travelled by foraging larvae over 5 min. Populations of *Drosophila* selected for rapid development show significantly greater path lengths than their controls. Populations of *Drosophila* selected for resistance to ammonia and urea in their larval food have shorter path lengths than their controls. Individuals in the ammonia-resistant populations are smaller than those in the control populations, but the size-adjusted metabolic rates are not significantly different. A simple model is proposed suggesting that changes in larval foraging behaviour may be a means for *Drosophila* larvae to adapt to new environments that require additional maintenance energy. In the ammonia-selected populations, crucial tests of these ideas will have to be conducted in environments with ammonia.

Key words. Life-history evolution, trade-offs, feeding rates, experimental evolution, foraging path length.

Introduction

Life histories depend on the timing and duration of development before 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 (Stearns, 1992; Roff, 2000). Many life-history characters, such as development time and the number of offspring produced, are closely connected to fitness.

An important theme in the study of life-history evolution is the notion that the relative allocation of resources to development and reproduction will have fitness consequences that depend on the environment. One of the earliest problems in life-history research questioned why birds at higher latitudes tend to lay more eggs than birds close to the equator (Ricklefs, 2000). Although Moreau (1944) and Lack (1947) made important contributions to this problem, Cody (1966) framed the problem in terms of limited energy resources and trade-offs between competing demands for this energy. The importance of finite energy became part of formal life-history theory in the classic paper by Gadgil & Bossert (1970). They summarized the prevailing 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 & Bossert, 1970, p. 3).

Due to the important role trade-offs have in understanding life-history evolution, much of the research over the last 30 years has focused on collecting evidence for trade-offs (Stearns, 2000). There have also been conflicting ideas over what would constitute appropriate evidence of trade-offs (Reznick, 1985; Reznick *et al.*, 2000). Much life-history research has focused on the sign of genetic correlations

Correspondence: Dr L. D. Mueller, Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697, U.S.A. Tel.: +1 949 824 4744; fax: +1 949 824 2181; e-mail: ldmuelle@uci.edu

between traits of interest (Van Noordwijk & de Jong, 1986; Houle, 1991; de Jong & 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 longevity, but this is accompanied by declines in early fecundity (Rose, 1984; Luckinbill *et al.*, 1984). Similarly, 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 & Ayala, 1981; Mueller *et al.*, 1991).

The research referred to above reinforces the importance of trade-offs as important constraints on, and determinants of, the course of life-history evolution. The ability to understand trade-offs and their mechanisms will ultimately enhance an understanding of the diversity of life.

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

Ricklefs & Wikelski (2002) recognized the importance of physiological and behavioural mechanisms of life-history trade-offs. They suggested that '... studies should integrate behaviour and physiology within the environmental and demographic contexts of selection' (Ricklefs & Wikelski, 2002; p. 462). Changes in behaviour may be a mechanism to accomplish redistribution of energy. As an end-product of evolution, behaviour may reflect important energetic or fitness trade-offs. For example, Ghalambor & Martin (2001) show that predation risk affects foraging behaviour of birds in a way that is consistent with the expected effects on adult or juvenile mortality. Of course, showing that particular behaviours are consistent with theoretical predictions is not the same as showing that the behaviour actually evolved by natural selection subject to the presumed trade-offs.

Physiological examination of existing phenotypic polymorphisms supports the concept of energetic trade-offs. For example, the cricket *Gryllus firmus* consists of two morphs: a winged and a wingless morph. Crnokrak & Roff (2002) show that the winged morph has higher rates of respiration but reduced fecundity relative to wingless morphs. These energetic trade-offs might explain the maintenance of this wing polymorphism.

Steven Stearns 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; p. 484). The most direct and unambiguous way to implicate a mechanism as causal for life-history evolution would be to observe its effects in systems whose evolutionary history and phenotypic evolution are well known. Evolved laboratory systems are ideal for this purpose because their history is known exactly and the forces that have varied and thus shaped evolutionary change are under careful control (Rose *et al.*, 1996).

An important focal point in the study of life-history evolution is energy. Energy is viewed as a limiting resource that must be divided between the competing functions of growth, reproduction and maintenance, as illustrated by the comments of Gadgil & Bossert (1970), quoted above. This trichotomy is simplified in sexually immature stages, because reproduction is not an immediate drain on energy. In *Drosophila*, the immature larval stages expend substantial energy on growth and maintenance, whereas adults expend most of their energy on reproduction and maintenance. *Drosophila* larvae expend considerable energy when moving and foraging (Berrigan & Lighton, 1993), making the foraging behaviour of *Drosophila* larvae of interest to behavioural geneticists and evolutionary biologists. Two components of foraging behaviour have been studied extensively. Larval feeding rates are a simple measure of the rate of movement of the larva's cephalopharyngeal mouthparts. Associated with the movement of the larva's mouth is the movement of the larvae in two dimensions. The distance travelled by a foraging larva is called the foraging path length.

Both the larval feeding rate and the larval foraging path length respond to natural and artificial selection (Burnet *et al.*, 1977; Sokolowski *et al.*, 1983, 1997; Joshi & Mueller, 1988). A specific gene has been identified as affecting foraging path length directly (Osborne *et al.*, 1997). Usually, these two behaviours are studied separately. However, in a few instances, there is documentation of feeding rates increasing as the foraging path length increases (Sokolowski *et al.*, 1997). One goal of the present study is to measure the foraging path length in a collection of populations whose feeding rates have also been studied.

One possible consequence of altered foraging behaviour is a change in a larva's energy budget. This possibility is examined by measuring the metabolic rate of feeding larvae with significantly different foraging behaviours. A simple model of foraging behaviour evolution is then developed based on the notion that, in *Drosophila* larvae, important energetic trade-offs are mediated through changes in larval foraging behaviour.

Materials and methods

Populations

The populations used in this study were derived from laboratory evolved populations maintained at low larval densities and breeding populations of 1000–2000. There were five replicate control populations (AUC); five replicate ammonia-resistant experimental populations (AX) and five replicate urea-resistant experimental populations (UX). The AX larvae were raised in 8-dram vials with banana corn-syrup barley-malt food with 1 M ammonium chloride. Approximately 60 eggs were put in each vial and there were 40 vials per population. A plastic sleeve was placed inside each vial. Pupae settled on the sleeve, which was removed from the vials and placed in a large Plexiglass cage before

the emergence of adults. This allowed adults to emerge in a cage supplied with standard food. Thus, all the selection for ammonia tolerance was limited to the larval stage. It is important to note that the control vials also received plastic sleeves. Thus, any inadvertent selection on foraging behaviour that these sleeves may cause was felt with equal intensity on both the controls and selected populations. After approximately 1 week in the cage, eggs were collected and used to start the next generation. The UX populations were maintained in a similar fashion except the larval food contains 0.46 M urea. Controls were handled in exactly the same way, except there no ammonium chloride or urea was added to their food.

The populations selected for accelerated development are described elsewhere (Chippindale *et al.*, 1997), although their derivation and maintenance are summarized here. In 1989, five populations (CO) were derived from large laboratory populations (O) selected for postponed senescence. The CO populations spent their first 2 weeks in vials, reared at low larval densities of approximately 60 per 8-dram vial. Subsequently, the flies were transferred into Plexiglass cages and maintained until the eggs for the next generation were collected 2–3 weeks later. In 1992, the five accelerated development populations (ACO) were derived from the CO populations. Selection for accelerated development was imposed by selecting the first 20% of the flies first emerging from each population. These flies were placed in Plexiglass cages and allowed to lay eggs for 24 h, after which eggs were collected for the next generation. After approximately 125 generations, the ACO populations were maintained on a fixed 9-day generation cycle without further selection for decreased development time. A detailed phylogeny of all these populations is provided by Borash *et al.* (2000).

These three sets of populations were chosen because a previous study has demonstrated differences in feeding rates (Borash *et al.*, 2000). This allowed for a rigorous test of whether foraging path length changes in a congruent fashion with feeding rates.

Foraging path length

Foraging path lengths were measured on rectangular Plexiglass sheets that had six circular wells (depth 0.5 mm, diameter 8.5 cm). Each of these wells was evenly filled with a yeast paste mixture (50 g yeast in 105 mL of water). For each population, 50, 72-h-old larvae were tested. A single larva was placed in each well and allowed 5 min to forage. Because the experiments were always carried out with paired populations (AX1 with AUC1, etc.), three of the wells on a given Plexiglass sheet were from the experimental populations and three from the control population. Thus, both experimental and control populations experienced the same Plexiglass sheets and were measured over exactly the same time intervals. After 5 min, the larva was removed and a Petri dish placed over the well. The path was traced onto a Petri dish. The path was later scanned and the

digital image used to measure the length of the path with ImageJ software (http://www.rsb.info.nih.gov/ij/). These experiments were run in blocks. On any day, one experimental and one control population were tested (e.g. ACO2 and CO2 or AX3, UX3, and AUC3).

Metabolic rates

Metabolic rates were measured in the five AX and five AUC populations. Larvae from these populations were raised under standard conditions that resembled the AUC environment. Eggs from the adult females emerging from these standardized conditions were collected on agar plates and allowed to hatch 24 h later. Larvae were then added to vials with standard banana-molasses food at approximately 50 larvae per vial. Adding larvae to vials rather than eggs ensured that all larvae within a vial began feeding and growing at essentially the same time, thus synchronizing their developmental stages. After 72 h, a liquid solution of the banana molasses food (e.g. without agar) was added to the vial and shaken vigorously. The solution was then drained through a strainer and 30 larvae were removed and placed in fresh 4-dram vials with 2 mL of food for the measurements of metabolic rate. This technique allowed larvae to be removed from their food faster than would be possible by dissecting the food by hand. The technique does not appear to result in severe damage (e.g. almost all larvae were recovered alive). The experimental and control populations were handled in exactly the same fashion. In some instances, less than 30 larvae were found and so the precise number used was recorded.

Measurements of the rates of release of CO_2 were made in a flow-through respirometry system using a LI-COR LI-6251 carbon dioxide analyser (LI-COR Biosciences, Lincoln, Nebraska). A data acquisition and analysis program, DATACAN V (Sable Systems, Las Vegas, Nevada), was used for acquisition and analysis of the data. Four samples of 30 larvae from each of two populations (one AX and one AUC) were run at a time. All measurements were carried out as paired comparisons of a selected and a control population. Four experimental vials containing 30 larvae and 3 mL of food, plus one vial containing only food, were attached to a multiplexer valving system (Sable Systems) that allowed computer controlled, automatic, timed sampling of chambers. The above system has been used to measure the metabolic rate of single adult fruit flies and, accordingly, the sensitivity was adequate for these measurements (Williams *et al.*, 1997, 1998; Williams & Bradley, 1998).

All measurements were performed at 25 °C under constant light. The noise in the system was below 0.05 p.p.m. Measurements were carried out in food vials that were cut down to a volume of 25 mL to reduce the time constant for respirometric analysis. The mass of the larvae was measured after the experiment and mass specific metabolic rates were calculated.

Analysis of variance (ANOVA) was used to aid in the interpretation of all experimental results. The implementation of ANOVA was performed using R (version 1.6.2 http://www. R-project.org). For the path length and metabolic rate experiments, groups of populations were tested on different days (e.g. AX1 and AUC1 on Monday, AX2 and AUC2 on Tuesday, etc.). Days were treated as a random block effect in the ANOVA. For the metabolic rate measurements, samples were divided into two sets of replicates within a block. Only one replicate set could be tested on the respirometer at a time. These replicates were treated as factors nested within blocks.

Results

Foraging path lengths

The average path length for each ACO population was greater than its control CO population (Fig. 1). These differences were highly significant as revealed by ANOVA (Table 1). The measurements of path length could vary substantially from one block to the next (compare replicates 4 and 5 to 1, 2 and 3 in Fig. 1). These effects emphasize the necessity of the block design in the ANOVA. There were also large differences in foraging path length due to selection for ammonia and urea resistance (Fig. 2), which were statistically significant (Table 2). Tukey's simultaneous confidence intervals showed that the mean foraging path length of the AUC population was significantly greater than the AX and UX population and the mean path length in the AX population was greater than the UX population.
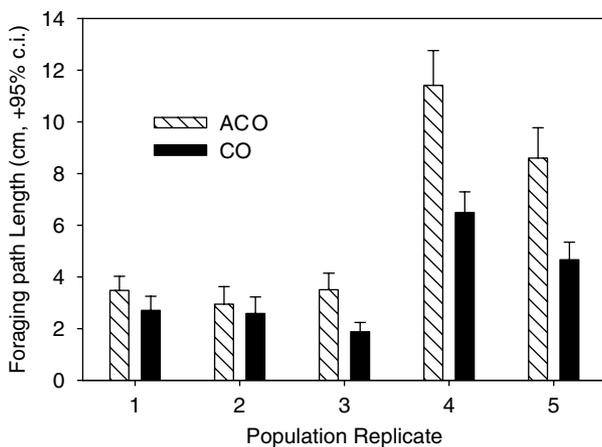
Metabolic rates

The AUC larvae tended to be larger than the AX larvae (Fig. 3, top panel) although this difference was not statistically significant ($P = 0.068$) (Table 3). To provide a meaningful basis to compare metabolic rates, the production of CO_2 was analysed on a unit weight basis. These results show that, although the AUC larvae appeared to produce more CO_2 (Fig. 3, lower panel), the difference was not statistically significant (Table 4).

Discussion

The results from the present study support the idea that larval feeding rates and foraging path length evolve in a correlated fashion. In every population examined, if feeding rates increase then their foraging path length also increases. In the present study, the ammonia-resistant lines and the urea-resistant lines show significantly shorter foraging path lengths than their controls. Previous work demonstrated that adaptation to urea and ammonia also resulted in decreased larval feeding rates (Borash *et al.*, 2000). Similarly, the populations selected for rapid development time show increased foraging path lengths relative to their controls. The accelerated development lines also have elevated feeding rates relative to their controls (Borash *et al.*, 2000).

Life-history evolution in Drosophila may depend on larval foraging behaviour

Drosophila larvae complete three instars before their metamorphosis in the pupal stage. Except for several hours before pupation, the larvae feed continuously. Larval feeding involves stereotypical behaviour that consists of thrusting their mouth hooks forward and then retracting them. This single motion may be repeated 100–200 times per minute. This behaviour is associated with food consumption, passage of food through the animal's alimentary tract and locomotion. This behaviour can be quantified in two different ways: (i) the larval feeding rate is a count of the number of mouth thrusts made in a fixed period of time, normally 1 min and (ii) the foraging path length is the distance travelled by a foraging larva on a flat surface in a fixed period of time, normally 5 min.

Of course, any study of larval life histories will include viability and development time as fitness components, but not reproduction because larvae are prereproductive. However, it is important to remember that larval growth affects adult size, which in turn determines fertility in both males and females (Partridge *et al.*, 1987; Wilkinson, 1987; Mueller & Joshi, 2000; ch. 6). Below, evidence is reviewed suggesting that larval feeding rate and foraging path length are important aspects of life-history evolution in *Drosophila* and may be a primary pathway for energy diversion.

Fig. 1. The foraging path length in the five accelerated development populations (ACO) and their controls (CO).

Table 1. The analysis of variance of path lengths in the accelerated development and control populations.

	d.f.	Sum of squares	Mean square	F	P (> F)
Population	1	674.1	674.1	79.0	$< 2.2 \times 10^{-16}$
Block	4	3215.7	803.9	94.2	$< 2.2 \times 10^{-16}$
Residuals	494	4214.0	8.5		
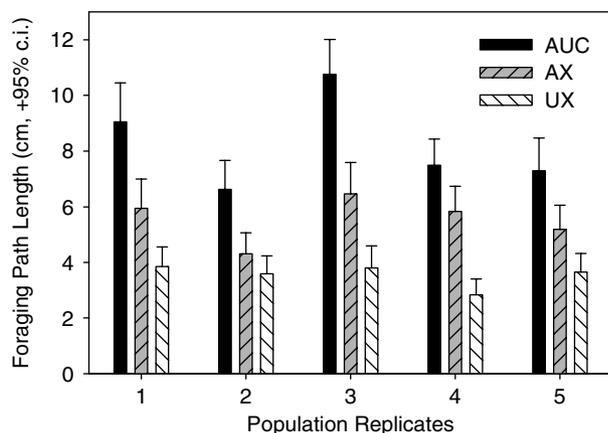
Fig. 2. The foraging path length of the ammonia-resistant populations (AX), urea-resistant populations (UX), and their controls (AUC).

Feeding rates respond to many different types of natural selection. In the laboratory, *Drosophila* populations' feeding rates evolve in response to larval crowding (Joshi & 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; Prasad et al., 2001). Populations that are resistant to parasitoids, ammonia and urea show lower feeding rates. Populations adapted to crowding show elevated feeding rates. Selection for development time has not always produced a consistent response in feeding rate, but it appears that the response to this type of selection also reduces feeding rates (Prasad et al., 2001). The wide range of environmental conditions that affect foraging behaviour suggests that it is plausible to assume that these behaviours also evolve in natural populations subject to variation in these conditions.

Larvae that feed faster are better competitors for limited food. This has been demonstrated in populations artificially selected for high and low feeding rates (Burnet et al., 1977), and for populations that have evolved different feeding rates in response to density (Joshi & Mueller, 1988) and parasitoids (Fellowes et al., 1998).

Larvae that feed fast are less efficient. Fast-feeding larvae require more food than slow feeders to successfully pupate (Mueller, 1990; Joshi & Mueller, 1996). As a corollary, it is reasonable to assume that those larvae feeding more slowly may be more efficient. This relationship is a crucial component of the hypotheses underlying the explanation of foraging behaviour evolution in the populations described in above.

Increased immune response is associated with reduced feeding rates. This has been observed in the parasitoid-resistant lines where the immune response is thought to contribute directly to resistance (Kraaijeveld et al., 2001).

Changes in feeding rates are often accompanied by changes in larval foraging path length. For several experimental systems of *Drosophila*, increases in feeding rates have been accompanied by increases in foraging path length. The *r*- and *K*-populations and the UU and CU-populations are two different sets of populations selected at extreme densities. The high density treatments show both increased feeding rates and increased foraging path length (Joshi & Mueller, 1988; Santos et al., 1997; Sokolowski et al., 1997). Populations selected for accelerated development, ammonia resistance and urea resistance also show a positive correlation between high feeding rates and long path lengths (Borash et al., 2000; Prasad et al., 2001; this study).

Studies of the energetics of larval movement in *Drosophila* demonstrate that this cost of transport is one of the highest for terrestrial locomotion (Berrigan & Lighton, 1993; Berrigan & Pepin, 1995). Taken together, these observations suggest that, when evolution requires energy to be reallocated to new functions in *Drosophila* larvae, this energy will be made available by reducing

Table 2. The analysis of variance of path lengths in the ammonia-resistant populations, urea-resistant populations and and their controls.

	d.f.	Sum of squares	Mean square	F	P (> F)
Population	2	2780.2	1390.1	120.3	$< 2.2 \times 10^{-16}$
Block	4	444.2	111.0	9.6	1.4×10^{-7}
Residuals	743	8584.2	11.6		
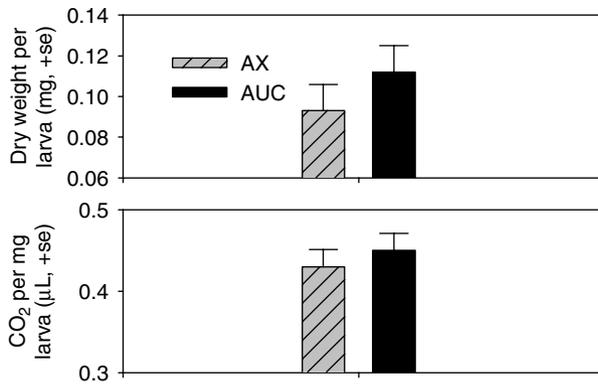
Fig. 3. Average physiological measurements on 72-h larvae in the ammonia-resistant populations (AX) and their controls (AUC). The top panel shows the average dry weight per larva, and the bottom panel shows the amount of CO_2 produced per mg of larva.

foraging activity. These ideas are developed in more detail below with respect to populations of *D. melanogaster* that have evolved resistance to ammonia in their larval food.

Ammonia detoxification as a model

As an example of how this evolution might proceed, the populations selected for ammonia resistance will be considered in some detail. Ammonia may be converted to glutamate via the reversible reaction:

$$NH4^+ + H^+ + NADH + \alpha - ketoglutarate \\ \leftrightarrow glutamate + NAD + H_2O.$$

The next important question is does this detoxification represent a significant energy drain? The energy in NADH is equivalent to approximately 2.25 ATP molecules. From Santos *et al.* (1997), we can determine that the slow feeding UU larvae gain 0.027 mg of wet weight per hour between the ages of 66 and 72 h. If we assume the AX larvae gain a similar amount of weight at this age, then the AX larvae

would have to consume at least 0.027 mg of their 1 M ammonia food. Consequently, they might have to process up to 2.7×10^{-8} M of NH_4^+ per hour. This would require an equivalent of 6.08×10^{-8} M of ATP. Our results from this study show CO_2 production of 1–2 µL per hour per larva. Assuming that this is all due to the aerobic metabolism of glucose, then this respiration rate is equivalent to the production of 2.36–4.72×10^{-7} M of ATP per hour. Thus, the detoxification of ammonia could cost 13–25% of a larva's total energy budget. Although these numbers are very approximate, they show that ammonia detoxification could be a substantial energy drain for feeding larvae.

Energy allocation in feeding larvae

It can be assumed that energy intake of feeding *Drosophila* larvae is related to their feeding rates in two ways. First, the total amount of food consumed will increase as larval feeding rates increase (Fig. 4a). Second, the amount of energy expended by foraging will also increase with increasing energy intake. This leads to the prediction that there will be some intermediate feeding rate, f_g, which will maximize the gross energy intake (Fig. 4a). Figure 4(b) shows explicitly how gross energy intake changes with feeding rate. The unimodal shape of this curve follows from the assumption that, at exceedingly high feeding rates, the incremental gains in food consumed are small compared with the cost of feeding.

It is not unreasonable to assume that, as larvae feed faster, their ability to extract all the usable energy declines. Previous experimental work has shown that, indeed, fast feeding larvae require more energy to complete development than do slower feeding controls (Mueller, 1990; Joshi & Mueller, 1996). This assumption is depicted in Figure 4(b) as the curve labelled net food energy after digestion, which is generally smaller than the gross energy curve and increasingly so at higher feeding rates. The maximum net energy intake is then represented by f_n. In the control populations, this would be the feeding rate that

Table 3. The analysis of variance of dry larval weight in the ammonia-resistant populations and their controls.

	d.f.	Sum of squares	Mean square	F	P (> F)
Population	1	0.004304	0.004304	3.62	0.068
Block	4	0.014354	0.003588	3.02	0.035
Replicate within block	5	0.003562	0.000712	0.60	0.70
Residuals	28	0.033310	0.001190		

Table 4. The analysis of variance of CO_2 production per mg of larva in the ammonia-resistant populations and their controls.

	d.f.	Sum of squares	Mean square	F	P (> F)
Population	1	0.004287	0.004287	0.38	0.54
Block	4	0.036012	0.009003	0.80	0.54
Replicate within block	5	0.084715	0.016943	1.51	0.22
Residuals	28	0.315048	0.011252		
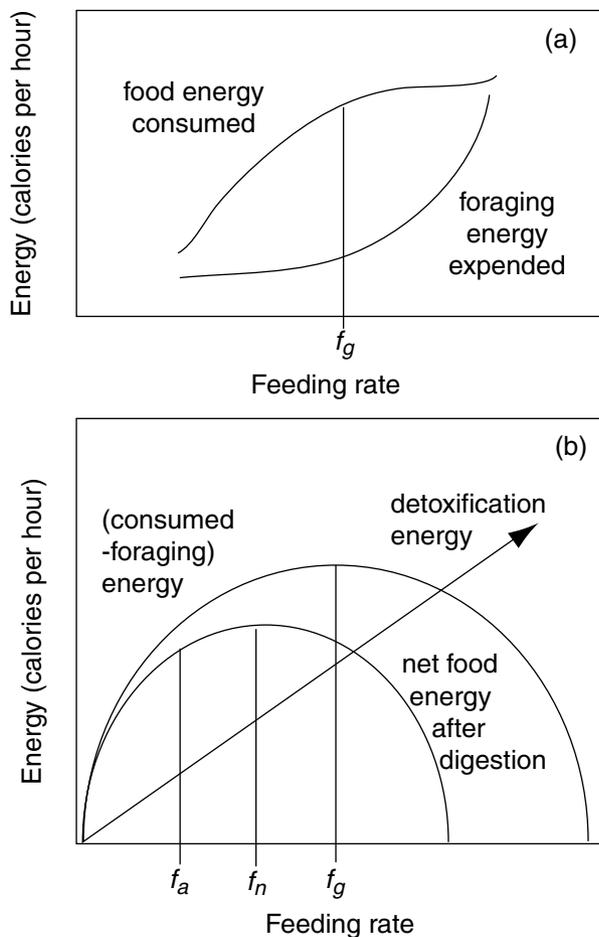
Fig. 4. The relationship between larval feeding rates and energy acquisition. As feeding rates increase, there will be a general increase in the amount of food consumed, although gain is expected to slow at high feeding rates (a). High feeding rates also expend more energy resulting in a feeding rate, f_g, which maximizes the gross amount of energy intake (a). (b) The difference between the food energy consumed and the energy used for foraging is shown as a function of feeding rates. Below that curve is a second curve showing the net energy available to the larva after digestion (see text for additional discussion). Environments with high levels of ammonia require energy to detoxify the ingested ammonia. This energy is shown as a straight line. The difference between the net energy curve and the detoxification line represents energy available after detoxification, which is maximized at f_a.

would maximize energy intake of a feeding larva. However, evolution would probably favour feeding rates somewhat higher than this value because that would result in higher competitive ability. The precise amount by which the feeding rate was above f_n, depends on the density of the population.

If larvae are presented with a stress (e.g. ammonia), then we assume that detoxification will require energy and that this required energy will increase as more ammonia is consumed (i.e. feeding rates increase). This energy requirement is shown in Figure 4(b) as a straight line. This new requirement shifts the feeding rate at which energy consumption is maximized to a lower value, f_a. In addition to this effect, it can also be predicted that the detoxification of ammonia would be more important, in a fitness sense, than competition. This change in the selection environment would also lead to reductions in the feeding rates of larvae living in environments with high levels of ammonia.

Adaptation to urea should affect feeding rates in a similar way to that hypothesized for ammonia effects on feeding rates. The impact of parasites will not increase with increasing feeding rates. However, the net intake of energy can be increased by decreasing feeding rates below the competitive optimum. Thus, it is predicted that adaptation to parasites will also result in decreasing feeding rates as a means of making more energy available for fighting parasites.

The effects of selection on development time are probably more complicated. Previous work on the ACO and CO populations has shown increased feeding rates in the accelerated development populations (Borash *et al.*, 2000). Prasad *et al.* (2001) have documented reduced feeding rates in their accelerated lines. The difference in these two studies may be related to whether the populations are experiencing strong selection for decreased development time, as in the study by Prasad *et al.* (2001), or have been maintained for a long time at reduced development time, such as the ACO populations. More work is needed to determine the consequences of selection for rapid development on feeding rates.

The foraging behaviour of *Drosophila* larvae appears to be an especially attractive system for studying energetic trade-offs. Because larval feeding rates are known to change as *Drosophila* larvae adapt to a range of environments, energy reallocation appears to be a unifying theme that can be used to explain the present observations. The ability to make detailed measurements of energy consumption, metabolic rate and growth rate with *Drosophila* larvae should permit the development of a detailed understanding of the mechanisms of larval life-history evolution in this model system.

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