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The confusing transition into adulthood: time allocation to the life-history transition of aquatic larvae

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ABSTRACT

Hypothesis: Organisms allocate their time towards reproduction (i.e. mature as soon as size threshold is reached) under extremely deprived conditions, and shift allocation towards growth should a nutrient influx occur.

Organism: Larvae from a laboratory colony of the mosquito *Anopheles gambiae* (Diptera: Culicidae).

Methods: We divided individuals into three treatment groups: well-fed, starved, and 'rescued' (switching from low- to high-food diets). We recorded their age and size at emergence.

Results: Only a few small mosquitoes emerge under low-food conditions, and when 'rescued' they only partially catch up in size to the constantly well-fed individuals.

Keywords: age–size conflict, *Anopheles*, life history, metamorphosis, plasticity, metabolic energy.

INTRODUCTION

The growth and development of organisms are often constrained by the availability of limited resources, such as time and energy. For organisms with complex ontogeny, optimizing age and size at each life-history transition under such constraints is crucial to their final reproductive success. This optimization involves a trade-off between growth and mortality: time and energy can be spent attaining a large size at the cost of delaying, and increasing risk of dying before, reproduction. Larger individuals tend to be fitter, in that compared with smaller individuals they are more competitive, have higher survival rates and fecundity. However, individuals that take longer to develop in order to grow to a large size have delayed reproduction and higher cumulative mortality risks.

Optimal timing of life-history transition depends upon (1) the relative fitness benefits of being large at the start of maturity, (2) potential for continued growth following the transition, and (3) mortality risks associated with each life stage (Kozlowski, 1992; Roff, 1992; Rudolf and Rödel, 2007). Across gradients of environmentally determined growth potential

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(typically resource availability), age and size at maturity often follow an L-shaped relationship (e.g. Plaistow *et al.*, 2004): individuals reared in salubrious environments mature early and large, whereas individuals that have experienced low-resource environments mature small and late. This negative, non-linear age–size pattern has been described by Day and Rowe (2002), using a formal mathematical model, as a consequence of a minimum size or energy threshold required for the life-history transition under the growth–mortality framework described above.

Trade-offs and plasticity in age and size at the transition to maturity have classically been studied in mosquitoes and other insects via mild food dilution titrations over time (e.g. Bradshaw and Johnson, 1995). By contrast, a scenario of severe food shortage coupled with random energy pulses is rarely taken into consideration. In this study, we used a simple starvation-rescue experimental design to investigate how the growth mortality trade-off might play out when organisms under extremely deprived conditions receive an abrupt, large influx of nutrients. Phelan (2012) used Day and Rowe's (2002) model to predict optimal development when the environment switches from low to high resources, and the model indicated that these organisms should be able to 'catch up' in size to those that have not experienced any food shortage. Thus, we hypothesize that in low-energy environments, organisms suffer a low survival rate, and mature small and late; however, should a nutrient influx occur, they will mature at the same size as those that are not constrained (Bradshaw and Johnson, 1995).

Our study organism is the larva of the African malaria mosquito, *Anopheles gambiae* (Diptera: Culicidae). These larvae often dwell in ephemeral and extremely nutrient-deprived habitats (Gimnig *et al.*, 2001) with a mortality rate of up to 93% (Service, 1977). Adult body size of females is highly correlated with fecundity (Lyimo and Takken, 1993), and since adults cannot grow, their larval growth is crucial to their fecundity and fitness. They are also iteroparous and overlapping generations are common, hence there are great fitness costs to delaying reproduction. The aforementioned L-shaped age–size relationship is common in mosquito systems, where it has been described without reference to ultimate causes using the pupation window model (Carpenter, 1984; Walker *et al.*, 1997).

MATERIALS AND METHODS

The *A. gambiae* colony used in this study originated from Tanzania (Njagi) in 1997, and was maintained according to the protocol described by Zappia and Roitberg (2012). Our experimental design consisted of three treatments: high food (HH), low food (LL), and a 'rescue' group (LH), which began on the low-food level and then switched to the high-food diet on day 10. This time frame was chosen because in pilot studies, it was observed that all HH larvae that were alive had emerged by this day whereas LL larvae had not, ensuring a strong comparison between the treatment groups. The two constant-food treatments can be considered controls for the treatment with the diet switch. The high- and low-food diets were 14 mg and 0.1 mg per day respectively of ground fish flake food (Nutrafin Basix – Hagen, Taiwan).

Our experimental apparatus consisted of four water baths, kept at a constant temperature of 30°C and 12:12 h (light/dark) photoperiod. Three custom-made plastic racks were floated in each water bath, and eight drosophila vials (depth 9 cm, diameter 2.5 cm) were secured in slots on each rack. At the beginning of each experimental run, freshly hatched larvae were haphazardly picked from a single batch and were transferred singly to vials filled with distilled water to a depth of 3 cm. All the replicates in a rack were of the same treatment and all of the treatments were represented in each bath. Each experimental run lasted a total of 20 days. Every 24 hours, the experimenter visually assessed the larval stage of individuals by head capsule size (Timmermann and Briegel, 1998), and ensured the water and food levels in each vial were constant. The position of the racks within each bath was rotated daily to eliminate positional bias. A total of four experimental runs were carried out, generating 384 replicates. When larvae reached pupation, food was no longer provided and netting was put in place to keep adults in the vial following metamorphosis. Emerged adults were killed, dried for at least 72 hours, and dissected for wing length measurements.

R was used for all statistical tests and graphs (R Development Core Team, 2012). Four individuals assigned to be LH were considered LL as they pupated prior to the experimental switch of food diet. Effects of treatment on the survivorship (i.e. percentage of each treatment population that survived) to emergence were analysed using χ^2 tests. A Kruskal-Wallis test was used to analyze whether treatment had an effect on the time larvae took to complete their development and emerge, as well as for an effect on adult wing length.

RESULTS

From 384 larvae in four experimental runs, a total of 104 individuals emerged, 58 from the HH treatment (45% of treatment group), 38 from the LH treatment (31% of treatment group), and 8 from the LL treatment (6% of treatment group). Of the LL larvae that failed to emerge, 79% died and 15% were still fourth instars when the experiment was terminated after 20 days. Diet treatment affected both larval survival to emergence ($\chi^2_2 = 61.04$, $P < 0.0001$) and time to emergence ($\chi^2_2 = 96.45$, $P < 0.0001$). Larvae from the HH treatment had the highest survival rate and emerged significantly sooner than those from the LH and LL treatments (Tukey-Kramer HSD, $P < 0.0001$ for both; Fig. 1). The LH and LL larvae did not differ in their time taken to emerge as adults (Tukey-Kramer HSD, $P = 0.0578$; Fig. 1). Half of the emerged LL mosquitoes ($n = 4$, 3% of treatment group) came out early on day 11 and the other half came out late on days 14 and 15, while LH larva emergence dates ranged from day 12 to day 15.

Mean adult wing lengths at emergence in the three treatments were significantly different from each other ($\chi^2_2 = 82.07$, $P = 0.03$). In ascending order of size, LL individuals were the smallest (2.20 ± 0.12 mm, mean ± S.E.), LH individuals were intermediate in size (2.62 ± 0.03 mm), and HH individuals were the largest (2.85 ± 0.02 mm). A summary of our results is given on a plot of size (wing length) vs. age at emergence (day of emergence) (Fig. 1): HH individuals were substantially larger and younger than LL individuals, while LH individuals were roughly of the same age as the LL individuals but larger.

DISCUSSION

In this study, we assessed how a switch from low-growth to high-growth conditions very late in the larval phase affects the development of *A. gambiae* mosquitoes. Theory developed under the growth–mortality framework predicts that (1) larvae under poor conditions emerge late and small, and (2) larvae that are introduced to high-energy growth conditions late in development will achieve the adult size that they would have become if they had always enjoyed high-growth conditions (Day and Rowe, 2002; Phelan, 2012). Our results were only partially consistent with these predictions: few LL larvae emerged, and those that did were

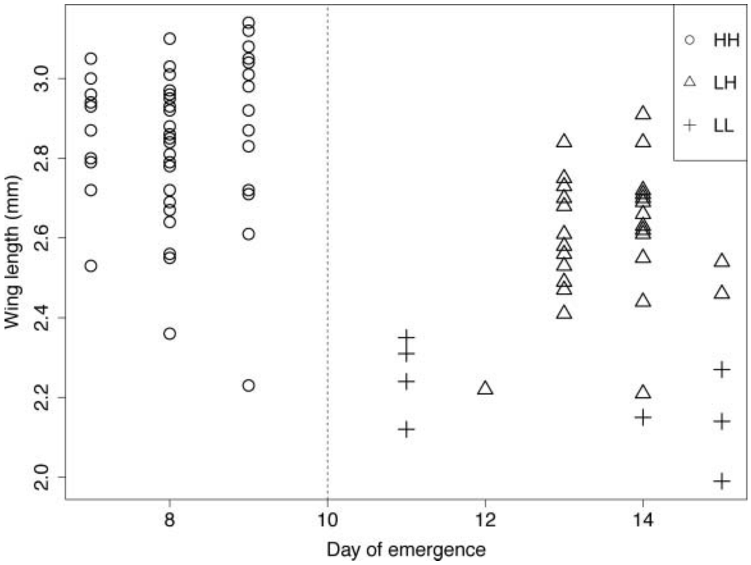

Fig. 1. Size vs. age plot of *Anopheles gambiae* larvae at emergence. The dashed line indicates the date of diet change for LH individuals (day 10).

smaller than the rest; however, the final size of our LH individuals was intermediate to that of the LL and HH individuals.

Timmermann and Briegel (1998) reared multiple species of mosquitoes, including *A. gambiae*, under a different experimental protocol: the food switch from low to high occurred at the start of the final instar and not at a pre-determined date when most larvae were already fourth instar for some time. They found that larvae were able to 'catch up' to emerge at a size equal to those reared under continuous high food, although emergence was delayed. This result differs from our own and is consistent with the predictions of the theory of optimal development.

Energetics offers one possible explanation for our observed deviation from theory prediction and Timmermann and Briegel's (1998) results. The model of Day and Rowe (2002) that was used to make our prediction does not explicitly consider metabolic energy. Rather, it considers growth conditions to be an instantaneous property of the environment such that growth within a given environment is a simple function of time. However, mosquito larvae and other organisms are capable of accumulating reserves over time that may later be allocated to growth, and are not limited in growth by constraints such as Dyar's law (Dyar, 1890). (*Note*: This rule does apply to exoskeletal structure such as the head capsule, but not to the soft-tissue thorax where reserves are stored in mosquito larvae.) Thus, fourth instar larvae in a HH setting have access to accumulated energy and material for growth that is unavailable to larvae from the LH environment. Perhaps in Timmermann and Briegel's (1998) treatments, the switch occurred early enough or food availability was high enough (under either the low or high treatment diet) that larvae could completely make up the energetic deficit experienced before the fourth instar period. Another possibility is that mosquito larvae that experience an increase in food availability late in their final instar may be constrained in their ability to continue to grow, and take advantage of good conditions during the fourth instar as they may have initiated a deterministic developmental programme by that point.

Our results suggest that models of optimal age and size at emergence could benefit from considering the role of metabolic energy within the broader growth–mortality framework.

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