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Short communication

Growth and development of *Aedes aegypti* larvae at limiting food concentrations

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ABSTRACT

Mosquitoes have a complex life-cycle with dramatic changes in shape, function, and habitat. *Aedes aegypti* was studied by growing individual larvae at different concentrations of a defined rich food source. At higher food concentrations, rate of larval growth was faster, but the time required for 4th instar larvae to molt into the pupal stage was unexpectedly extended. These opposite tendencies resulted in constant times from hatching to pupation and up to adult eclosion at permissive food concentrations. The results demonstrate that nutritional conditions of 4th instar larvae impact initiation of the first metamorphic molt.

Transmission of pathogens by insects depends on the developmental stage of the vectors (Beerntsen et al., 2000). Mosquitoes transmit many tropical diseases including malaria, encephalitis and dengue (Ghosh et al., 2000; Peter et al., 2005). Understanding the life cycle of mosquitos (Huff, 1947) is therefore important for vector and disease control. Mosquito larvae feed on nutritious particles in their aqueous environment (Clements, 1963) and timing of pupating. Adult eclosion and size are correlated with the amount of food consumed by the developing larvae (Telang et al., 2007). The duration of the development from first instar larval stage to adult mosquito is faster when food is abundant (Tun-Lin et al., 2000). Temperature and larval density also influence growth and development (Lyimo et al., 1992).

The 4th molting stage, in which larvae become pupae, requires a drastic drop in Juvenile Hormone (JH) and increase in ecdysone for a successful molt (Lan and Grier, 2004; Margam et al., 2006). Limiting, *restrictive* amount of food during this last instar stage of *Aedes aegypti* prevents pupation (Telang et al., 2007), but the larvae tend to pupate even in the absence of food if sufficient resources have been accumulated in prior larval stages (Arrivillaga and Barrera, 2004). Consequently, 4th instar *A. aegypti* larvae respond to limiting but *permissive* food concentrations by premature pupation (Ben-Dov, 1990). Similarly, 3rd instar larvae of *Onthophagus taurus* (Coleoptera: Scarabaeidae), the last larval stage in this beetle, pupate earlier in response to food deprivation (Shafiei et al., 2001). The reported study therefore re-investigated the quantitative influence of limiting amount of defined food source on *A. aegypti* larval development.

1. Growth rate of *Aedes aegypti* larvae under limiting food conditions

A. aegypti larvae hatched in an autoclaved suspension (1 mg mL^{-1}) of Pharmamedia (Traders Protein, TX, USA) were washed twice in autoclaved tap water and transferred to small wells in covered 24-welled plates, each containing a single larva in 2 mL of sterile Pharmamedia at different concentrations (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.75, 1.0, 1.5 and 2 mg mL^{-1}), reared at 28 ± 2 °C. Two – 3 independent experiments were performed with 20–30 larvae exposed to each concentration. Larval developmental stages were scored 1–4, pupae as 5, adults as 6, and dead larvae as 0. The average scores were calculated for each food concentration during the whole period. The rates of larval development (Fig. 1; Table 1) and the frequency of dead larvae (Fig. 2) were correlated with the Pharmamedia concentrations.

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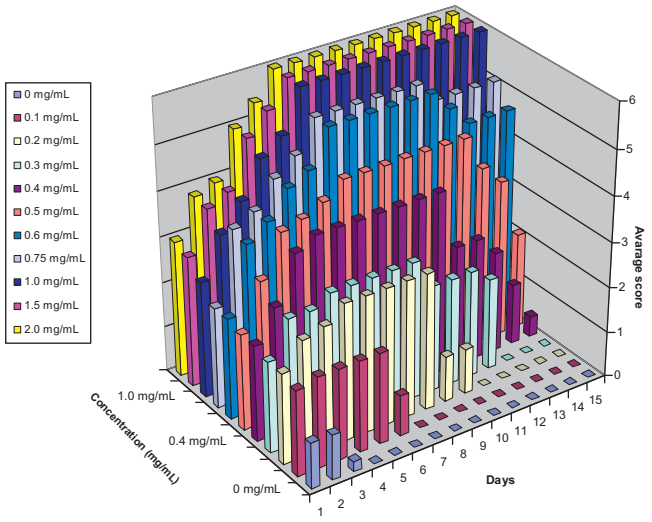

Fig. 1. Time dependence of average score (see text) of *A. aegypti* fed with different Pharmamedia concentrations during 15 days.

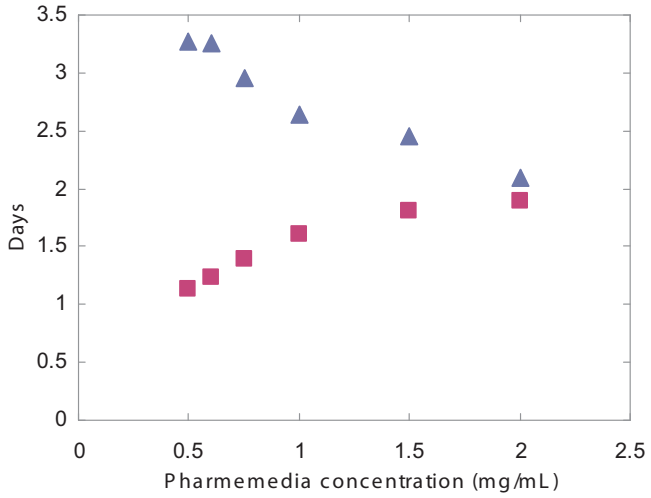

Fig. 3. Time (days) taken for neonate larvae to reach 4th instar stage (▲) and for the 4th instar larvae to reach the pupal stage (■)), in different Pharmamedia concentrations.

Feeding neonates with Pharmamedia postponed early larval death in water. Score of 3 or higher was obtained at concentrations of 0.4 mg mL^{-1}, in which some larvae were alive at 15 days. Score of 5 or higher was obtained at 0.6 mg mL^{-1} or higher concentrations. At concentration of 1.0 mg mL^{-1} or higher, mosquito developmental score was similar and the majority of the larvae eclosed as adults at day 6 (Fig. 1).

Table 1 records the average time (days) for 1st instar larvae to reach each developmental stage. Starved larvae did not reach 2nd instar stage. At all the Pharmamedia concentrations used, 1st instar larvae molted on average at 1.26 days. Concentrations of 0.2 and 0.3 mg mL^{-1} were sufficient to reach only the 3rd instar stage, and development was quicker in the higher concentration. Above 0.5 mg mL^{-1}, all stages of mosquito life cycle were observed (a single larva fed on 0.4 mg mL^{-1} that eclosed was excluded from the calculations).

2. Inverse correlation between times of larval development and of pupation

Shorter time to reach 4th instar was observed at higher food concentration (2.1 days at 2 mg mL^{-1}, 3.3 days at 0.5; Table 1;

Table 1
Average time (days) taken for newly hatched larvae to reach each developmental stage in different Pharmamedia concentrations.

Instar	Pharmamedia concentration (mg mL^{-1})										
	0	0.1	0.2	0.3	0.4	0.5	0.6	0.75	1	1.5	2
2	–	1.38	1.34	1.47	1.15	1.17	1.31	1.27	1.26	1.23	1
3	–	–	3.17	2.27	1.77	1.91	1.74	1.84	1.71	1.59	1.05
4	–	–	–	–	4.34	3.27	3.26	2.96	2.64	2.46	2.1
Pupa	–	–	–	–	–	4.4	4.5	4.35	4.25	4.27	4
Adult	–	–	–	–	–	5.8	6.14	5.95	6.03	5.81	5.58
4th instar → pupa	–	–	–	–	–	1.13	1.24	1.39	1.61	1.81	1.9
Pupa → adult	–	–	–	–	–	1.4	1.64	1.6	1.78	1.54	1.58

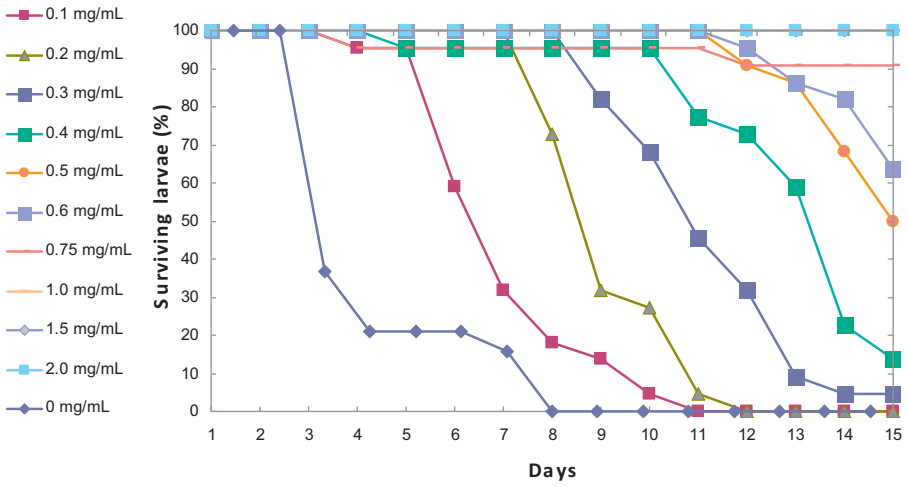

Fig. 2. Survival of larvae fed with different Pharmamedia concentrations.

Fig. 3). Unexpectedly, the time for 4th instar larvae to pupate *increased* from 1.1 to 1.9 days as food concentration *rose* from 0.5 to 2 mg mL^{-1} (Fig. 3). These parallel changes resulted in a relatively constant time (4.3 days) for a neonate to reach the pupal stage (Table 1). And since pupae do not feed, the time to eclose as adults is constant (1.6 days), and so is the time to complete the metamorphosis (5.9 days) at concentrations higher than 0.5 mg mL^{-1}. The same response to food deprivation occurs in larvae of the beetle *O. taurus* (Shafiei et al., 2001): well-fed larvae in the last (3rd) instar stage pupate after 17 days, whereas larvae which were starved from their 5th to 7th day pupate after 14 days. There too, no difference in the duration of the pupal stage between experimental and control animals was evident.

Synchronized 4th instar *A. aegypti* larvae pupate in their own, partially exhausted medium earlier than in fresh medium (Ben-Dov, 1990). Accumulation of compounds in used medium cannot be excluded as a factor influencing timing of pupation in addition to limiting food. Such factors were omitted in the study reported here: all larvae remain in the same medium throughout their life/growth cycle with different food concentrations. Larval nutrition may affect metamorphic capacity of *A. aegypti* through ecdysteroid titer (Telang et al., 2007). The results suggest that "in nature, larvae may use food depletion as a cue to time when to end the third [last] instar and when to initiate pupation" (Shafiei et al., 2001).

The average time for 1st instar larvae to reach 4th instar (3 days) at 0.75 mg mL^{-1} Pharmamedia (Table 1) was identical to that reported by Jenkins et al. (1992). The time to molt from 4th instar to pupa at this concentration was 1.4 days, similar to that reported by the same authors (1.5–2.2 days). This small difference is probably due to different rearing conditions (e.g., temperature, humidity). The minimal time required for a larva to develop from the 1st larval stage to pupal stage is not known. To successfully molt from 4th instar into a pupa, a larva must undergo a commitment period (Lan and Grier, 2004), in which the titer of JH rapidly disappears and 20-OH Ecdysone becomes predominant. Our results suggest that a correlation exists between these hormones and the amount of available food, a suggestion that needs to be further examined.

3. Concluding remarks

Development of *A. aegypti* in the presence of defined food source is reported. Larval growth rate is faster at higher food concentrations whereas molting from 4th instar to pupa is rather slower, hence total time for a newly hatched egg to reach adulthood is constant at unlimited food availability. The species maintains reproducing potential by initiating pupation after exhaustion of food provided the total resources in the medium are not restrictive (above a threshold). It is likely that at least *A. aegypti* larvae respond to starvation by raising ecdysteroid titers and hence affects the timing of pupation.

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