Parental and offspring larval diets interact to influence life-history traits and infection with dengue virus in *Aedes aegypti*

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The environmental conditions experienced by parents can influence offspring phenotype along with the conditions experienced by offspring. These parental effects are clear in organisms that display parental care and are less clear in other organisms. Here, we consider effects of parental and offspring larval nutrition on offspring development time, survivorship and infection with dengue virus in *Aedes aegypti*, the mosquito vector of dengue, chikungunya, yellow fever and Zika. Parents were raised on either high or low larval detritus inputs with subsequent offspring being divided into two groups, one receiving high nutrients and the other low. Low nutrient females from low nutrient parents (LL) developed significantly slower than those from high nutrient parents (HL). Females from all parent by offspring nutrient treatment groups were equally likely to become infected with dengue virus at 24 h, 3 days and 14 days. After 14 days, high nutrient females from low nutrient parents (LH) had 11 times higher viral titres and more disseminated infections than high nutrient females from high nutrient parents (HH). These results suggest that carry-over environmental stress from the parental generation can influence life histories and arbovirus infection in *Ae. aegypti* females. We found males to be robust to the life-history parameters measured, suggesting sex-specific differences which may relate to their lower nutrient requirements for metamorphosis.

1. Introduction

Parental effects occur when the environmental conditions experienced by parents influence offspring phenotypes. These
effects can be considered a type of inherited phenotypic plasticity [1] and their importance is clear in organisms displaying parental care [2]. However, parental effects influence the ecology and evolution of a wide range of taxa [2–4]. Adaptive parental effects occur when environmental conditions experienced by parents enhance offspring fitness [5]. Maladaptive parental effects occur when environmental conditions result in poorer quality offspring [6]. The conditions leading to parental effects vary across species and traits considered. In insects, parental nutrition has been linked to parental effects in several orders [7–11]. One offspring trait that has been linked to parental effects is immunity [9,11,12]. These studies demonstrate the importance of considering parental nutrition effects on immunity in other systems.

Container mosquitoes have received considerable attention in ecological studies partially because they vector arboviruses including dengue, chikungunya, yellow fever and Zika. Dengue alone is estimated to infect 390 million people per year, with 96 million cases with clinical manifestations [13]. The term ‘container’ refers to the natural and artificial larval habitats these mosquitoes rely upon. Allochthonous detritus inputs to these containers, including plant and animal detritus, serve as basal resources for microorganisms upon which developing larvae feed. The quantity and type of detritus in a container affect larval growth and development as does competition between conspecifics and heterospecifics [14]. Nutrient limitation is common in container systems [15].

In mosquitoes, larval nutrient limitation is associated with reduced survival to adulthood, longer development, compromised growth and reduced adult fitness (e.g. [11–17]). Nutritionally deprived females often lay fewer eggs [16] and have reduced fecundity [18]. Larval nutrition is positively associated with body size, a measure of net growth and metabolic reserves [18]. During nutritive stress, teneral protein, lipid and carbohydrate reserves acquired during larval development can be mobilized to promote survival [18,19]. Larger adult mosquitoes generally live longer [20,21], take larger blood meals [22] and have higher total fecundity [18] than smaller mosquitoes. However, after an initial blood meal, survival may no longer be associated with size [23]. Body size, adult longevity and blood-feeding behaviour are associated with vector potential [19,20,24]. *Aedes aegypti* and *Anopheles gambiae* often take multiple blood meals per gonotrophic event, a behaviour associated with the high transmission of pathogens by these species [24]. The ability of the vector to survive the extrinsic incubation period (EIP) of a pathogen (period of development in the vector) and to then engage in subsequent blood feeding is necessary for transmission.

Larval nutrition within a generation has been shown to influence vector competence of mosquitoes in complex ways. Vector competence refers to susceptibility to infection, replication and transmission of pathogens. Larval nutrient limitation is associated with increased vector competence in *Ae. albopictus* [25], *Ae. triseriatus* [26] and *Culex tritaeniorhynchus* [27]; both increased [17] and decreased vector competence in *Ae. aegypti* [28]; and no effect on vector competence in *C. tarsalis* [29]. The influence of larval nutrition on vector competence is associated with barriers to infection and innate immunity [26,30]. Larval nutrition may have epidemiological consequences because physical and physiological barriers to infection influence viral dissemination to secondary tissues, including mosquito saliva necessary for transmission. Given the importance of larval nutrition in influencing adult mosquito phenotypes and fecundity, a logical step is to consider how larval nutrition experienced by parents influences subsequent offspring.

The relationship between larval nutrition, immunity and parental effects may have consequences for mosquitoes that transmit pathogens. In the mosquito *An. stephensi*, parental food during the larval stage did not significantly affect offspring emergence time, size or survival but did influence offspring fecundity [8]. In *An. gambiae*, daughters of females reared in nutrient-deprived conditions as larvae were more likely to be infected with the malaria parasite, *Plasmodium berghei* [31]. However, maternal infection with a microsporidian, in *An. gambiae*, resulted in reduced susceptibility to malaria in daughters possibly due to immune priming [31]. These studies demonstrate that interactions between the parental environment and offspring phenotype are complex. Parental effects can influence host–parasite interactions in important vector species and consequently should be considered in research [32], especially for emerging arboviruses, which remain under-investigated.

In this study, we tested the hypothesis that parental and offspring larval nutrition affects larval and adult characteristics in offspring that have epidemiological consequences for the transmission of arboviruses. From this hypothesis, and based upon prior work on the effects of larval environment on mosquito vector biology, we predict that offspring from parents reared in low nutrients will have decreased fitness correlates and greater susceptibility to dengue infection relative to offspring from parents reared in high nutrient larval conditions. We also predict that high nutrient offspring will have increased fitness correlates and lower susceptibility to infection relative to low nutrient offspring
regardless of parental nutrition. We predict that interactive effects of parental and offspring nutrition will result in high food offspring from high food parents having increased fitness correlates and lower susceptibility to infection relative to other treatment combinations. Here, we investigate whether larval nutrition experienced by parents and offspring in *Ae. aegypti* influences offspring (i) life-history traits (growth, development, survival to adulthood) and (ii) susceptibility to dengue-1 virus infection. Though we focus on *Ae. aegypti* and dengue, due to the importance of this mosquito as a vector and the global burden of dengue, the concepts improve our general understanding of the potential role of parental effects on arbovirus mosquito vectors.

2. Material and methods

2.1. Source and rearing of parental mosquitoes

*Aedes aegypti* used were from an established colony of wild-caught mosquito larvae collected from Key West, FL, in 2012. We used *Ae. aegypti* from Key West as this population was responsible for dengue-1 virus transmission in 2009 and 2010 [33]. Insectary conditions were set to 28 ± 0.5°C and a 12L:12 D photoperiod and sustained throughout the experiment. Colony mosquito larvae were reared in enamelled pans (24 × 36 × 5 cm) in 1.51 of water in cohorts of approximately 200 mosquitoes on an equal mixture of brewer’s yeast and lactalbumin given in 0.2 g increments two to three times per week. Pupae were transferred to water-filled cups and placed into plastic cages (45.7 × 45.7 × 45.7 cm, BugDorm, MegaView Science Co. Ltd, Taichung, Taiwan) to emerge as adults. Adults were maintained on a 20% sucrose solution from cotton wicks and blood fed on live chickens approximately once per week. Chicken care followed the animal use and care policies of the University of Florida’s Institutional Animal Care and Use Committee (IACUC Protocol 201003892).

To reduce the possibility of parental effects generated in the wild, the F4 generation was used as the parental (P) generation for this study [34]. P larval nutrition treatments included natural sources of plant and invertebrate detritus and microorganisms. Ten 2.0 l cylindrical plastic containers (15.5 cm × 17.1 cm, height × diameter) for each treatment group were used to rear the P larvae. Each container received 2.0 l of tap water and 10 ml of tyre water inoculum (collected from tyres in at the Florida Medical Entomology Laboratory (FMEL) campus) with associated microorganisms. Each container received senescent live oak leaves (*Quercus virginiana*), a predominant host tree for container habitats in Florida and dead field crickets (*Gryllus* sp.) in amounts varying by treatment (low nutrition, 4 g oak leaves + 0.06 g crickets; high nutrition, 12 g oak leaves + 0.2 g crickets). The oak leaves were collected from the FMEL campus and dried for 48 h at 70°C. The crickets were obtained from the local pet supply store and dried for 48 h at 60°C using established methods [35].

Contents were incubated in containers for 4 days prior to use to allow microbial populations to establish. P generation eggs were hatched in containers with 1.0 l tap water and 0.2 g of an equal mixture of brewer’s yeast and lactalbumin. After 24 h, larvae were removed from nutrients, rinsed and 200 were added to each experimental container (0.1 larvae ml⁻¹). Larval density approximated the mean natural density in Florida containers occupied by *Ae. aegypti* and competitor *Ae. albopictus* (*N* = 790, mean ± s.e., 0.17 ± 0.02, range 0.00083–3.08 larvae ml⁻¹) [36]. Supplemental food consisting of half the initial amount was added every 7 days. P larvae were checked daily for pupation. Pupae were transferred to plastic vials containing water and sealed with cotton until emergence as adults. As adults, mosquitoes were sexed and placed in treatment-specific cages containing a water-filled cup lined with paper towel for an oviposition substrate. No more than 50 mosquitoes were added to each cylindrical cardboard cage (9.7 × 9.7 cm, height × diameter). Each cage contained approximately a 1 : 1 male to female ratio, and females within a 3-day age span.

The mosquitoes were given 8–11 days to mate and were sustained on a 20% sucrose solution. After the mating period, sucrose was replaced with water for 2 days to increase propensity to blood feed. Mosquitoes were then offered a bovine blood meal warmed to 37°C using an artificial membrane feeding system (Hemotek®). Feeding rates were 73.7% for high food females and 64.0% for low food females. Fully engorged females were held in cages for 7 days during which gravid females laid eggs. Eggs were removed after 7 days and maintained in a humid environment until hatching (see Offspring rearing). Two high food and one low food parental treatment containers were lost due to water quality issues. This resulted in four fewer offspring containers from high food parents and two from low food parents than originally planned (see figure 1 for experimental design).
2.2. Offspring rearing

Four days prior to hatching the offspring (F₁ generation), experimental containers were set up for each F₁ treatment group. Hatching was staggered to ensure larvae were similar in age. Experimental containers were the same as those for the parental treatment groups. The F₁ generation eggs from each parental container were hatched and 200 of the resulting larvae were reared in low nutrients and 200 were reared in high nutrients using the same methods as the P generation resulting in four different P × F₁ larval nutrition treatment groups in a split plot design (figure 1). These treatment groups include: high food parents with high (HH) and low food offspring (HL) and low food parents with high (LH) and low food offspring (LL). The treatment manipulation allows for similar genetic diversity between the offspring treatment groups derived from each parental treatment group.

F₁ larvae were checked daily for pupation. Pupae were transferred to sealed vials until emergence as adults. Development was measured as the time in days from hatch to adult emergence. Survivorship to adulthood was recorded for each experimental replicate calculated from the initial cohort size.

Figure 1. Experimental design for parental and offspring nutrition treatments.
Upon emergence, adults were sexed and transferred into treatment-specific cages using a handheld battery-powered aspirator. Adults were maintained on a 20% sucrose solution for 8–10 days post-emergence. Forty-eight hours prior to blood feeding, the sucrose solution was replaced with water to improve feeding on infectious blood. Females were offered blood infected with dengue-1 virus isolated from Key West, FL, see GenBank: JQ675358.1, using the Hemotek® feeding system described by Alto & Bettinardi [37].

2.3. Mosquito infection with dengue virus

Dengue virus was propagated for blood meals following previously established methods [37,38]. Briefly, monolayers of African green monkey kidney (Vero) cells, in tissue culture flasks (175 cm²), were inoculated with 250 µl of dengue at a multiplicity of infection of 1.2 followed by 1h incubation at 37°C in a 5% CO₂ atmosphere. Post-incubation, 25 ml of media (199 media, 10% fetal bovine serum, 0.2% antimycotic and 0.2% penicillin–streptomycin) was added to each flask. After 7 days, the media (containing dengue) were combined with defibrinated bovine blood (Hemostat, Dixon, CA, USA) in a 1 : 1 ratio.

F1 females were offered dengue infectious blood for 1 h. Samples of the infectious blood meal were taken prior to blood feeding to determine dengue titres using quantitative reverse transcriptase–polymerase chain reaction (qRT–PCR). Post-blood-feeding females were cold anaesthetized and fully engorged females were separated and housed in cages. Fed F1 females were held individually for either 24 h, 3 or 14 days, where 14 days are the approximate EIP of dengue in Ae. aegypti at 28°C [39]. The 24 h, 3- and 14-day time points were specifically chosen because at these time points there is differential expression of Toll pathway-related genes in response to a dengue infectious blood meal [40]. The Toll pathway is one component of the innate immune response of mosquitoes to dengue virus [40,41] and as such can influence susceptibility to infection, viral dissemination and transmission potential. After the incubation period, females were individually stored in 2.0 ml centrifuge tubes at −80°C until assayed to determine infection status, body viral titre and (in 14-day mosquitoes) dissemination status. We did not test for disseminated infections in samples from the 24 h and 3-day time points because dissemination is unlikely at these time points [39].

2.4. Determination of infection status

F1 blood-fed females were dissected using sterilized forceps to separate the body, legs and wings. Wing length was measured, in millimetres from alula to wing tip, to approximate mosquito body size (reviewed in [42]). Bodies were homogenized in 1.0 ml of TRI Reagent® (Molecular Research Center, Inc., Cincinnati, OH, USA) with three glass beads at 25 Hz for 3 min using a Qiagen® Tissue Lyser. Total RNA was extracted following the TRI Reagent® protocol and stored at −80°C until qRT–PCR assays could be completed. RNA was then extracted from leg tissues of mosquitoes with dengue positive bodies as determined by qRT–PCR. Viral dissemination into the haemocoel was measured at 14 days as indicated by infection of body and leg tissues [43]. Disseminated infection is regarded as a state of advanced infection and a prerequisite for transmission. qRT–PCR for dengue was performed using the SuperScript® III Platinum® one-step qRT–PCR kit (Invitrogen Life Technologies, Carlsbad, CA, USA) and fluorogenic probes (TaqMan®, Applied Biosystems, Foster City, CA, USA). Dengue-1-specific primers and probes were designed by Callahan et al. [44] and can be found in electronic supplementary material, S1. The PCR reagent quantities and protocol used are described in [45]. Plaque assays, based on methods established in [38], were used to calculate dengue titre in a series of serially diluted standards. RNA from these standards was then extracted and qRT–PCR was used to develop a standard curve in plaque forming unit equivalents (PFUe) ml⁻¹. This standard curve was used to quantify viral titre in mosquito tissues.

2.5. Statistical analyses

Multi-variate analysis of variance (MANOVA) was used to determine the effect of parental nutrient environment, offspring nutrient environment and parental by offspring nutrient environment on high and low food offspring life-history traits: male development time, female development time and percent survivorship (PROC GLM, SAS v.9.3). To identify parental larval nutrient effects, the following treatments were compared: HL versus LL and HH versus LH. To consider offspring larval nutrient
effects, the following treatments were compared: HH versus HL and LH versus LL. Lastly, to identify parental and offspring combined larval nutrient effects, the following treatments were compared: HH versus LL and LH versus HL. Standardized canonical coefficients were used to measure the relative contribution of each life-history trait to significant treatment effects and their relationship to one another (positive or negative) [46]. In order to satisfy requirements for parametric testing, a natural log transformation was performed on viral titre data. The effect of nutrient treatment ($P \times F_1$), day and day by nutrient treatment on ln viral titre results were analysed using generalized linear mixed models (PROC GLIMMIX, SAS v. 9.3). Generalized linear mixed models were used because this procedure is more robust to departures from normality than the generalized linear model procedure. Significant effects were further analysed by all pairwise comparisons of nutrient treatment means and adjusted using the Bonferroni correction method [47]. The effect of parental larval nutrition on offspring mosquitoes infected or uninfected as well as absence or presence of disseminated infection was analysed using maximum-likelihood categorical analyses of contingency tables (PROC GENMOD, SAS v. 9.3). The absence or presence of dengue virus in abdomens and leg tissues was determined on an individual female basis with results from each offspring container being pooled together by $P \times F_1$ nutrient treatment group (HH, HL, LH, LL) due to poor feeding rates in order to meet testing requirements.

3. Results

3.1. Life-history traits

A total of 4336 offspring emerged to adulthood (2436 males and 1900 females). These adults were used in the life-history trait analysis for development time (males and females) and survivorship. Female size was only estimated for females with measurable wings that successfully fed on blood (511 females). See table 1 for descriptive statistics. MANOVA showed significant parental nutrient effects ($p = 0.0018$), offspring nutrient effects ($p < 0.0001$) and parental by offspring nutrient effects ($p < 0.0001$) on life-history traits (table 2). Multi-variate pairwise contrasts found significant parental effects on offspring when offspring were reared in low nutrients ($p < 0.0001$) but not when offspring were reared in high nutrients ($p = 0.17$). In low nutrient offspring, female development time contributed the most to the significant parental effect followed by survivorship (figure 2). Female size and male development time contributed little to the treatment effect. Low nutrient female offspring from low nutrient parents (LL) developed more slowly ($14.47 \pm 0.30$ days) than low nutrient female offspring from high nutrient parents (HL) ($13.06 \pm 0.21$ days).

Multi-variate pairwise contrasts showed significant offspring nutrient effects in offspring from both high nutrient parents ($p < 0.0001$) and low nutrient parents ($p < 0.0001$). In offspring from high nutrient parents, female development time contributed most to this effect with male development time, survivorship and female size contributing similarly. Specifically, in offspring from high nutrient parents, high food females (HH) took 36\% less time to develop than low nutrient females (HL). In offspring from low nutrient parents, female development time followed by male development time contributed most to this effect. Specifically, in offspring of low nutrient parents, high food females (LH) took 42.5\% less time to develop than low food females (LL), and high food males (LH) took 33.5\% less time to develop than low food males (LL).

Multi-variate pairwise contrasts showed significant parental by offspring nutrient effects when parents and offspring were reared in similar environments (HH versus LL, $p < 0.0001$) and when parents and offspring were reared in dissimilar environments (HL versus LH, $p < 0.0001$). When comparing high food offspring from high food parents (HH) to low food offspring from low food parents (LL), female development time followed by survivorship contributed most to this effect. Specifically, high food females from high food parents (HH) took 42.5\% less time to develop than low food females from low food parents (LL). Additionally, high food offspring from high food parents (HH) had 5\% reduced survivorship relative to low food offspring from low food parents (LL). When comparing high food offspring from low food parents (LH) with low food offspring from high food parents (HL), female development time followed by male development time contributed most to this effect. Specifically, female high food offspring from low food parents (LH) took 36.3\% less time to develop than female low food offspring from high food parents (HL). Male high food offspring from low food parents (LH) took 28\% less time to develop than male low food offspring from high food parents (HL).
Figure 2. Bivariate plot of least square (LS) means (± s.e.) for offspring female development time and survivorship by treatment.

Table 1. Mean descriptive statistics (± s.e.) per container for offspring life-history traits, by treatment group.

| treatment | n (containers) | female development (days) | male development (days) | survivorship (%) | female wing length |
|-----------|----------------|---------------------------|-------------------------|------------------|--------------------|
| HH        | 8              | 8.32 ± 0.15               | 7.76 ± 0.16             | 60.86 ± 5.45     | 2.64 ± 0.02       |
| LH        | 9              | 8.32 ± 0.09               | 7.54 ± 0.06             | 72.72 ± 3.71     | 2.61 ± 0.01       |
| HL        | 8              | 13.06 ± 0.21              | 10.47 ± 0.17            | 63.31 ± 1.14     | 2.32 ± 0.03       |
| LL        | 9              | 14.47 ± 0.30              | 11.34 ± 0.24            | 64.06 ± 2.19     | 2.29 ± 0.05       |

Table 2. MANOVA for effects of parental nutrition, offspring nutrition, and parental by offspring nutrition interaction effects on offspring development (dev) time (females, males), survivorship to adulthood and female wing length with subsequent pairwise multi-variate contrasts.

| effect                              | num d.f./Den d.f. | Pillai's trace | p-value | standardized canonical coefficients |
|-------------------------------------|-------------------|----------------|---------|-----------------------------------|
|                                     |                   |                |         | female dev | male dev | survivorship | female wing length |
| parental nutrition                  | 4 / 26            | 0.47           | 0.0018  | 5.02       | -0.09    | 0.91         | -0.02              |
| HH versus LH                        | 4 / 11            | 0.41           | 0.17    | -1.10      | 0.57     | -0.89        | 0.61               |
| HL versus LL                        | 4 / 12            | 0.66           | <0.0001 | 0.85       | 0.74     | 0.09         | -0.24              |
| offspring nutrition                 | 4 / 26            | 0.97           | <0.0001 | 4.50       | 0.50     | 0.49         | -0.53              |
| HH versus HL                        | 4 / 10            | 0.97           | <0.0001 | 4.50       | 0.73     | 0.68         | -0.69              |
| LH versus LL                        | 4 / 13            | 0.97           | <0.0001 | 4.39       | 0.61     | 0.32         | -0.43              |
| parental × offspring nutrition      | 4 / 26            | 0.31           | 0.0403  | 3.01       | 1.29     | 0.01         | 0.45               |
| HH versus LL                        | 4 / 11            | 0.97           | <0.0001 | 4.68       | 0.66     | 0.76         | -0.32              |
| HL versus LL                        | 4 / 12            | 0.97           | <0.0001 | 3.72       | 1.35     | 0.05         | -0.64              |

3.2. Body infection with dengue virus

The mean dengue viral titre of the infectious blood meals was $7.2 ± 0.3 \log_{10} \text{PFUe ml}^{-1}$, which is within the range of viraemia in humans [48]. This titre is expected to result in approximately 75% of Ae. aegypti blood fed with dengue to have infected bodies [49]. Of the 1900 females, a total of 266 successfully fed on the dengue infectious blood meal and were tested for infection. Of these, 92 were assayed at 24 h, 95 at 3 days and 78 at 14 days. Of the 266 females, 195 (73%) were positive for dengue in the body. Descriptive statistics for per cent infected, least square means of body titre and per cent infected with disseminated infections can be found in table 3. Contingency table analysis showed that body infection
Table 3. Dengue virus infection and least square means for titre by treatment and day.

| day | trt | no. females | % viral RNA | titre (PFUe ml⁻¹) | % disseminated |
|-----|-----|-------------|-------------|-------------------|---------------|
| 1   | HH  | 29          | 100 (29/29) | 8975 ± 1095 (n = 7) | —             |
|     | LH  | 23          | 95.5 (22/23) | 5585 ± 719 (n = 7) | —             |
|     | HL  | 28          | 96.4 (27/28) | 7575 ± 1254 (n = 7) | —             |
|     | LL  | 11          | 100 (11/11)  | 7670 ± 2520 (n = 6) | —             |
| 3   | HH  | 26          | 30.8 (8/26)  | 5511 ± 882 (n = 5)  | —             |
|     | LH  | 24          | 75.0 (18/24) | 12 874 ± 6938 (n = 8) | —             |
|     | HL  | 23          | 39.1 (9/23)  | 8194 ± 3879 (n = 8)  | —             |
|     | LL  | 14          | 50.0 (7/7)   | 2310 ± 924 (n = 5)   | —             |
| 14  | HH  | 22          | 22.7 (5/22)  | 42 312 ± 12 369 (n = 5) | 18.2 (4/22)  |
|     | LH  | 24          | 45.8 (11/24) | 544 676 ± 211 083 (n = 7) | 33.3 (8/24)  |
|     | HL  | 22          | 45.5 (10/22) | 62 192 ± 29 455 (n = 4) | 36.4 (8/22)  |
|     | LL  | 10          | 30.0 (3/10)  | 98 900 ± 39 448 (n = 3) | 30.0 (3/10)  |

Table 4. Body infection by parental nutrition, parental by day treatment effects and day effect.

| effect | offspring comparison | χ² | p-value | d.f. |
|--------|-----------------------|----|---------|-----|
| high food F₁ | parental treatment HH versus LH | 0.07 | 0.7869 | 1   |
|         | day × parental treatment HH versus LH | 4.49 | 0.106 | 2   |
|         | day HH versus LH | 56.88 | <0.0001 | 2   |
| low food F₁ | parental treatment HL versus LL | 0.43 | 0.514 | 1   |
|         | day × parental treatment HL versus LL | 1.70 | 0.428 | 2   |
|         | day HL versus LL | 36.99 | <0.0001 | 2   |

did not depend on larval nutrient treatment (P × F₁) (p = 0.81, χ² = 0.98, d.f. = 3) or day by larval nutrient treatment (P × F₁) interaction effect (p = 0.27, χ² = 7.55, d.f. = 6) for any of the treatment groups (table 4). Body infection did depend upon day of assay post-infection (p < 0.0001, χ² = 89.25, d.f. = 2).

3.3. Viral titre and dissemination

Mosquito viral titre was influenced by treatment (P × F₁) (p = 0.0455, F = 2.84, d.f. = 3), day (p < 0.0001, F = 44.30, d.f. = 2) and treatment by day interaction (p = 0.0148, F = 2.92, d.f. = 6). Subsequent pairwise contrasts (electronic supplementary material, S2) found that at 24 h and 3 days, viral titres were not significantly different for treatment groups. At 14 days when offspring were reared in high nutrients, those from low nutrient parents (LH) had nearly 13 times higher viral titres (544 676 PFUe ml⁻¹) compared to those with high nutrient parents (HH) (42 312 PFUe ml⁻¹) (figure 3). At 14 days, offspring reared in high nutrients from low nutrient parents (LH) had 8.7 times higher viral titres (544 676 PFUe ml⁻¹) compared with offspring reared in low nutrients from high nutrient parents (HL) (62 192 PFUe ml⁻¹). Contingency table analysis found that larval nutrition (P × F₁) did not significantly affect virus dissemination in offspring (p = 0.453, χ² = 2.63, d.f. = 3).

4. Discussion

4.1. Life-history traits

We investigated whether parental and offspring nutrition influences offspring life-history traits (development time, survivorship and size) and susceptibility to infection with dengue. While other
Figure 3. Means (± s.e.) of body dengue-1 virus titre (plaque forming unit equivalents ml⁻¹) of infected offspring (14 days post-infectious blood meal) by parental by offspring nutrition treatment groups.

studies have been conducted on parental effects in mosquitoes, we are unaware of any studies on parental larval nutrition as it relates to offspring life histories and arboviral infection. Allocation of resources to life histories is influenced by environmental stress, including parental environmental stress, and underlying plasticity and constraints on these traits [50]. According to our results, offspring reared in high nutrients did not display significant differences in the life-history traits measured (male development time, female development time, female size and survivorship) regardless of parental nutrition. As holometabolous insects, mosquito larvae must allocate maternal- and larval-derived nutrients to maintenance, growth and storage [50]. When larval-derived nutrients are high, the importance of maternally derived nutrients may be lower than when larval-derived nutrients are low, especially if offspring were able to achieve the maximum growth rate for the population under the given temperature and food sources.

Offspring reared in low nutrients did display significant differences in life-history traits attributed to parental nutrition with female development time contributing the most to this effect. Specifically, females reared in low nutrients, from low nutrient parents (LL) extended the juvenile developmental period by 10% compared with females from high nutrient parents (HL). A minimum amount of nutrition is required for mosquito larvae to pupate and fully mature [51]. The differences in development time of LL females versus HL females may reflect differences in maternal egg resource allocation as a product of parent larval nutrition or reflect epigenetic imprinting. Mothers from greater nutrient larval environments may have been able to allocate more nutrients to eggs, resulting in offspring with more resources that required less from the environment to reach the threshold to develop.

Aedes aegypti larvae display significant development time plasticity and can develop very quickly or resist starvation and develop very slowly [52]. The difference in development time due to parental larval nutrition may reflect some of this underlying plasticity. Epigenetic marking is another potential mechanism for generating parental effects in insects [9,53–55]. While survivorship contributed to the significant effect of parental larval nutrition in offspring reared in low nutrients (HL versus LL), it did so minimally and was not significantly different between these two groups. Males in low nutrients were robust to parental nutrient effects.

Aedes aegypti larval nutrient metabolism varies significantly by sex [51] with females requiring greater resources to pupate. This may be the reason significant differences were seen in low nutrient female offspring but not male offspring. The lower nutrient requirements of the male mosquito probably allowed males to develop more quickly regardless of parental nutrition. A study conducted on the collembolan Orchesella cincta showed that parental nutrition did not influence male development time; however, it did influence male weight at maturity and the production of spermatophores [56]. When considering parental effects on Ae. aegypti males in the future, it would be worthwhile to measure alternative life-history parameters like sperm quality.

While parental nutrition did not lead to significant differences in measured life-history traits of high nutrient offspring, survivorship did vary between offspring from low food parents (LH) and those from
high food parents (HH). Offspring reared in high food, from parents reared in low food (LH) had the highest survivorship out of all four treatments. When compared with HH offspring, LH offspring had over 11% greater survivorship. The low nutrient status of LH parents may have resulted in fewer, higher quality eggs being produced allowing for greater survivorship. These results are consistent with studies on the grasshopper *Chorthippus biguttulus*, whose high nutrient parents produced superior offspring which developed faster than those from low nutrient parents [57]. Similarly, an epigenetic trade-off may exist between development and survivorship for traits that were not considered in this study.

When considering the effect of offspring nutrition, significant effects were found regardless of whether parents were reared in high or low nutrients. These effects were also primarily seen on female offspring development time with those that were reared in high nutrients developing more quickly than those reared in low nutrients, regardless of parental nutrition. High food females took 42.5% less time to develop than low food females when from high food parents and 36.4% less time to develop when from low food parents. The larger effect size from offspring nutrition relative to parental nutrition on offspring life-history traits suggests that offspring nutrition is probably more important to development than parental nutrition in this species.

4.2. Infection with dengue-1 virus

Feeding rates of female offspring varied by treatment and this may have biased dengue virus infection, titre and dissemination results. Body infection with dengue did not depend upon parental nutrition, day or day by parental nutrition interaction effect for any treatment groups. Offspring were equally likely to be infected with dengue regardless of treatment group. These results suggest that susceptibility to arboviral infection was not influenced by parental larval nutrition. Several studies on parental effects in arthropods have shown that parental nutrient deprivation leads to heightened resistance to pathogens [58–60], but this is inconsistent with our results. Other studies demonstrate no parental effects on offspring disease resistance [10] or parental stress leading to lower pathogen resistance [11,61]. Variation in resistance to bacterial pathogens associated with parental nutrient deprivation has been shown to vary significantly between different host genotypes in the crustacean *Daphnia magna* [59].

The mosquitoes used in this study were the F4 generation of a 2-year-old colony and were probably genetically similar contributing to similar infection rates across treatment groups. The proportion of mosquitoes infected was lower than some studies using *Ae. aegypti* and dengue and higher than others [45,49,62,63]. This was expected, given the importance of viral strain within serotypes [63,64], viral dose [49] and mosquito population [65] in determining susceptibility to infection. The same viral strain, viral dose and mosquito population were used in [45], yet the proportion of individuals infected in this study was much lower and may be related to differences in temperature and food between the two studies.

4.3. Viral titre and dissemination

Offspring treatment and offspring by day treatment significantly affected viral titres, but only after 14 days. Infection at 24 h marks the initial ingestion of the virus and its early entry into the midgut. Differences in susceptibility to infection at this point are typically seen when mosquitoes imbibe different amounts of virus [66]. As our mosquitoes were offered the same amount of virus, little to no difference in titre was expected at 24 h and this is consistent with our results. At 3 days, new virions are released from the midgut of infected mosquitoes and start infecting additional tissues [40]. During this time, Toll pathway defences against infection are active and can influence dengue titres [40]. Consequently, 3 days provide a snapshot of early infection. We did not observe any treatment differences in virus titre at 3 days, suggesting that the early immune response was not affected by parental larval nutrition.

The EIP for dengue virus in *Ae. aegypti* is generally accepted to be at 5–15 days between 25 and 28°C [39]. We used 14 days to test viral titres and to test for dissemination because it is close to the longest expected EIP and would increase our ability to detect differences among treatment groups. At 14 days, LH offspring had 13 times higher viral titres than HH offspring. The low nutrient condition of parents may have resulted in higher viral titres despite offspring being reared in high nutrients. Other studies on insects have found that offspring of low nutrient parents demonstrate reductions in the expression of innate immune markers [11,61]. Reductions in the innate immune system may explain our results; however, this is outside the scope of the present study. Life-history theory demonstrates that resources are limited and trade-offs occur in terms of how organisms invest these resources in growth, maintenance, storage and reproduction. Immune responses carry physiological costs, and trade-offs
exist when allocating resources to immune function [67]. It is possible that when food conditions are limited in the parental environment, offspring have reduced immune investment as an adaptation to have additional resources for other life-history traits. It is interesting that differences in viral titres were not seen at 24 h or 3 days, but were at 14 days for HH versus LH offspring, suggesting differences late in the infection cycle. In *Ae. aegypti*, infection with dengue virus is modulated by the Toll and JAK-STAT pathways [40,41], and future studies in this system should consider these pathways and other barriers to infection.

Dissemination of the virus into leg tissues indicates the virus has escaped the midgut barrier in the mosquito. Of offspring infected at 14 days, dissemination rates were between 30 and 37% in LH, HL and LL offspring. HH offspring demonstrated dissemination rates of 18.2% of those infected. This is marginally significantly different from rates seen in LH offspring. It is possible that when offspring were reared in high nutrients, those from high nutrient parents (HH) were in better condition resulting in fewer disseminated infections relative to those from low nutrient parents (LH). It is possible that high nutrients experienced by parents and subsequent offspring may improve the efficacy of the midgut escape barrier leading to fewer disseminated infections relative to other treatment combinations. Larval nutrient deprivation has been shown to increase viral susceptibility in *Ae. aegypti* [17] as well as other vector mosquitoes [26]. Our observations marginally support the hypothesis that high-quality larval nutrition for parents and offspring led to reductions in dissemination. Viral dissemination is not a reliable indicator of transmission potential due to additional barriers to infection. However, dissemination to secondary tissue does demonstrate that the virus has bypassed the midgut escape barrier [68]. Future studies should consider parental effects on transmission.

5. Conclusion

Nutritional quality behaves as predicted for a stressor in which it is expected that good condition parents will produce high-quality offspring and poor condition parents will produce lower quality offspring [2,69,70]. These results suggest that parental larval nutrition is important to *Ae. aegypti* female offspring. Parental diet effects were seen on development time, infection with dengue later during the infection process, and dissemination. Carry-over effects of parental nutrition are evident in this system, and the ones measured do not appear to confer any advantages to offspring in low nutrient environments for the traits considered. Future studies should consider manipulating the types of nutrients provided, measuring offspring immune response to pathogens at earlier time points, and considering additional male traits. Additional studies are needed to assess whether parental and offspring larval diets interact to influence other parameters of vectorial capacity, an index of risk of local transmission of pathogens.

Ethics. Chicken care and use was reviewed and approved by the Institutional Animal Care and Use Committee (Protocol VB-17) of the University of Florida.

Data accessibility. Our data are deposited at Dryad: http://dx.doi.org/10.5061/dryad.6c78sn1 [71].

Competing interests. We declare we have no competing interests.

Authors’ contributions. K.Z. conceived of and designed the project. K.Z. and B.E. carried out mosquito husbandry, viral propagation, molecular work and collected project data. K.Z. analysed and interpreted the data with B.W.A. K.Z. wrote the manuscript. B.W.A. and B.E. edited the manuscript. All authors gave final approval for manuscript publication.

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