

RESEARCH ARTICLE

Costs and benefits of vertical and horizontal transmission of dengue virus

Gloria Ruiz-Guzmán^{1,2}, José Ramos-Castañeda¹, Angélica Hernández-Quintero³ and Jorge Contreras-Garduño^{2,*}

ABSTRACT

Parasites can be transmitted vertically and/or horizontally, but the costs or benefits for the host of infection have only been tested after horizontal transmission. Here, we report for the first time, to our knowledge, the survival, reproduction and infection of *Aedes aegypti* during vertical and horizontal transmission of dengue virus 2 (DENV-2). Females infected horizontally produced more eggs, with a sex ratio skewed towards males, compared with uninfected controls. However, there was no significant difference in the number of emerging adults or in survival of mothers. In contrast, dengue-infected female offspring (vertical transmission) had a shorter lifespan but there were no significant differences in the number of eggs or sex ratio, compared with controls. Finally, the corroboration of infection revealed that virus infected about 11.5% and 8.8% of pools of mothers and of daughters, respectively. These results suggest that the mode of infection and the contact with the virus has no reproductive costs to female mosquitoes, which may explain why both types of transmission are evolutionarily maintained. In addition, we suggest that more attention should be paid to the male contribution to virus dissemination within and among populations and as reservoirs of the infection for human diseases.

KEY WORDS: Life-history traits, *Aedes aegypti*, Mutualism, Parasitism, Host–parasite relationship, Evolutionary parasitology

INTRODUCTION

The host–parasite relationship is one of the most fascinating and complex relationships within the field of biology. Whereas the parasite develops mechanisms to avoid the host's immune response, the host activates an efficient defense response against the parasite, generating resistance, tolerance and/or avoidance (Svensson and Råberg, 2010). The classical view of the host–parasite relationship is that hosts may pay a higher fitness cost (Moore, 2002; Combes, 2005; Moutailler et al., 2010; Poulin, 2011; Thomas et al., 2011; Sylvestre et al., 2013). However, host fitness may be affected by how parasites are transmitted, and this takes place in three different ways: (1) vertically (from mother to offspring), (2) horizontally (i.e.

through feeding or cannibalism) or (3) both vertically and horizontally (Vautrin and Vavre, 2008; Grunnill and Boots, 2016). It has been reported that parasites transmitted horizontally tend to have greater virulence than those transmitted vertically (Vautrin and Vavre, 2008; Magalon et al., 2010). Therefore, it is thought that parasites transmitted from mother to offspring should cause the least possible damage to the host, as an increase in the population of the latter provides a direct benefit to the former. Indeed, there is a tendency for the establishment of a mutual benefit in vertical transmission (Weeks et al., 2007). In contrast, in horizontal transmission, the host or parasite obtains a greater benefit while the other suffers a greater cost (Vautrin and Vavre, 2008; Engelstädter and Hurst, 2009).

If vertical and horizontal transmission occur in the same system, it has been proposed that the costs outweigh the benefits for the hosts or for the parasites (Magalon et al., 2010). Nevertheless, to our knowledge the costs and benefits in terms of host reproduction and survival have not yet been evaluated in a system where vertical and horizontal transmission occur simultaneously. Hence, we chose the relationship between the dengue virus 2 (DENV-2 virus) and the *Aedes aegypti* mosquito to analyze the above question. Although the existence of vertical transmission was debated, various recent studies have shown its existence, in both wild and laboratory conditions (Grunnill and Boots, 2016). Examination of how fitness traits are affected by the mode of parasite transmission may provide insights into the relative importance of vertical and horizontal transmission to virus dissemination in natural populations (Grunnill and Boots, 2016). The analysis of costs and benefits for female mosquitoes has previously been measured only in relation to horizontal transmission, ignoring the possible effects on the next generation of hosts and viruses (i.e. Maciel-de-Freitas et al., 2011; Sylvestre et al., 2013). Moreover, in the majority of previous studies on horizontal transmission, infection occurred by intrathoracic inoculation, which represents an unnatural mode of transmission and therefore could bias the results (Lambrechts and Scott, 2009).

In order to analyze the costs and benefits of the DENV 2–*A. aegypti* relationship, a comparison was made of reproductive parameters between female mosquitoes that were or were not exposed to DENV-2. Females acquired the virus orally (through feeding) and transmitted it vertically to their offspring (Weaver and Barrett, 2004; Grunnill and Boots, 2016). By scrutinizing offspring, we tested the idea that with mixed-mode transmission there may be a tendency towards a mutual benefit in the host–parasite relationship or that virus populations could be reduced in the next generation. The parameters evaluated herein were female survival, and the number of eggs and adults according to treatment. Furthermore, as it is the females that feed from blood and transmit the disease to humans, attention was paid as to whether infected females produce more daughters than sons (Engelstädter and Hurst, 2009). Finally,

¹Departamento de Arbovirus, Centro de Investigación Sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública, Av. Universidad 655, Cuernavaca, Morelos C. P. 62508, México. ²Escuela Nacional de Estudios Superiores, Unidad Morelia, Universidad Nacional Autónoma de México, Antigua Carretera a Pátzcuaro No. 8701, Col. Ex-Hacienda San José de la Huerta, Morelia, Michoacán 58190, México. ³Departamento de Estadística, Universidad Autónoma de Aguascalientes, Av. Universidad No. 940, Ciudad Universitaria, Aguascalientes, Ags 20131, México.

*Author for correspondence (jcg@enesmorelia.unam.mx)

 J.C., 0000-0002-9231-0641

an assessment of the level of infection was made to confirm the virus transmission.

MATERIALS AND METHODS

Mosquitoes and viruses

Aedes aegypti (Linnaeus 1762) used in this work were obtained from the Instituto Nacional de Salud Pública (INSP) insectary. They were captured in Cuernavaca, Morelos, Mexico, and bred for approximately 3 years at a rate of 1 generation per month under controlled temperature (27°C), humidity (70%) and 12 h:12 h light: dark cycle. DENV-2 New Guinea C (NGC) strain was also obtained from the INSP DENV collection. The passage history of the stock used is: 2 C6\36, 3 Suckling Mouse Brain (SMB). The final stock is a 30% suspension of SMB infected with DENV-2 NGC in MEM 30% FBS and has a virus titer of 3×10^8 pfu ml⁻¹.

Experimental design

Young virgin *A. aegypti* females (4–5 days post-emerged) were mated and, after 24 h of fasting, they were split into two groups ($N=70$ individuals). The control group was fed once, for ~2 h, with a base suspension (BS) consisting of 25% (v/v) rabbit red blood cells, 50% SFB and 25% PBS (NaCl 137 mmol l⁻¹, KCl 2.7 mmol l⁻¹, Na₂HPO₄·2H₂O 10 mmol l⁻¹, KH₂PO₄ 2 mmol l⁻¹, pH 7.4, saccharose 40%, ATP 0.1 mmol l⁻¹). The DENV-2 group was fed once with the BS supplemented with DENV-2 NGC 3×10^8 pfu (~80 µl). All the procedures were carried out in a high-security insectary following the biosafety protocols of the INSP.

Feeding was carried out by means of artificial feeders as reported by Bennett et al. (2002). Mosquitoes with physical evidence of feeding (ingested blood) were separated and placed individually into 50 ml Falcon tubes, which were prepared with a sheet of filter paper to receive eggs, and from this time on, mosquitoes were fed using a cotton ball soaked in a 10% sucrose solution in distilled water. The number of eggs and mortality were recorded daily.

Oviposition took place within 4 days of feeding. On the fourth day, the filter paper was removed and placed in a plastic cup with water for adult development. After emergence, the number of individuals per mosquito and sexual proportion were determined. Individuals of the control and DENV-2 groups mated within the same group, and females were separated and fed with BS under similar conditions. The number of eggs, number of emerged adults, sexual proportion and mortality were recorded as described above. The advantages of this study over others are that we tested for differences in the mode of parasite transmission, rather than using resistant or susceptible strains, the strain utilized herein was heterogeneous to infection, resembling what happens in nature, and finally, unlike in some studies, which used intra-thoracic inoculation, in our case, the mosquitoes were fed by the oral route, which imitates the natural way of feeding.

Analysis of infection

To confirm the proportion of mosquitoes infected by feeding on DENV-2-infected blood and the proportion of offspring infected by vertical transmission, we chose to detect the virus genome in pools of females randomly chosen from each generation ($N=19$ pools of mothers and $N=9$ pools of daughters, with five females per pool) at the end of each assay. We decided to do this for three reasons: (1) because the RNA extraction procedure kills the mosquito, some variables that we measured in this work could not be determined or represented, such as survival and the offspring sex ratio; (2) although it was important to determine the association between the individual viral load and reproductive fitness, genetic differences in the colony of

mosquitoes used, such as vector competence, could give results that are hard to interpret (it would have been impractical to estimate the amount of blood ingested or susceptibility to infection for each mosquito); and (3) because of the distribution of the fitness parameters measured in this work shown by the non-infected mosquito population. Although the technique could have been optimized, there was no point in determining the individual viral load; our goal was to demonstrate the effect of vertical transmission of DENV-2 in the mosquito population fed with DENV-2 suspension, and in its offspring, and we analyzed the results from a population point of view.

The presence of DENV-2 in mosquitoes fed with DENV-2 suspension was determined by real-time RT-PCR (qRT-PCR). Briefly, 7–10 days after feeding, randomly selected mosquitoes of each group were taken and stored at –70°C until the end of the experiment. RNA of each group was extracted using the QIAamp Viral RNA Mini Kit® (Qiagen, USA); 4 µg of RNA was reverse transcribed and the cDNA amplified using the OneStep RT-PCR Kit® (Qiagen, USA) using DENV-2 primers (DENV qPCR+: CATATTGACGCTGGGAAAGAC; DENV qPCR–: TTCCATTTCTGGCGTTCTGTG) and a TaqMan probe (6FAM AGATCC-TGCTGTCTCCTCAG), in a 7500 Fast Real-Time PCR System (Life Technologies, USA) device, using the following conditions: 1 cycle: 40°C/30 min; 1 cycle: 92°C/15 min; 40 cycles: 95°C/15 s; 60°C/1 min.

Statistical methods

Survival after feeding was evaluated with the Kaplan–Meier estimator. To determine the difference in survival between groups, we first calculated the proportional risk of death (Grambsch and Therneau tests) and as data fitted this criterion we used the log-rank test.

As egg number was not normally distributed, it was analyzed with GAMLSS (generalized additive models for location, scale and shape). The Akaike criterion revealed that the binomial negative regression was the best model of GAMLSS to analyze our data; this modeled the heterogeneity (Rigby and Stasinopoulos, 2005). Longevity and treatment (control or DENV-2 infected) were used as covariates to analyze the number of eggs. Additionally, the oviposition rate was calculated to discard the possibility that the differences in egg number were due to survival time by using a Mann–Whitney *U*-test. To analyze the proportion of emerged adults, a beta binomial distribution was used and as covariates we used the type of food (infected or non-infected) and number of eggs. Finally, to analyze the proportion of emerged females, we used a negative binomial regression. All tests mentioned above were carried out in R (v3.3.1.) with survival and GAMLSS packages.

In addition, the minimum infection rate (MIR, i.e. the minimum percentage of infected individuals in pooled samples) was calculated as the number of positive pools per total number of specimens tested $\times 100$ individuals. The calculation of this rate is practical when infection rates are generally low. Two-sample analysis using Pooled Infection Rate v4 was carried out. This program was run in Microsoft® Office Excel® add-in. This add-in computes the MIR, used when all pools are the same size (Biggerstaff, 2008, 2009).

RESULTS

Survival

No differences in survival were observed between mothers in the control and DENV-2 groups (log-rank, $\chi^2=0.4$, $P=0.54$). However, a greater survival time was found with control daughters than with DENV-2 daughters (log-rank, $\chi^2=5.3$, $P=0.02$; Fig. 1).

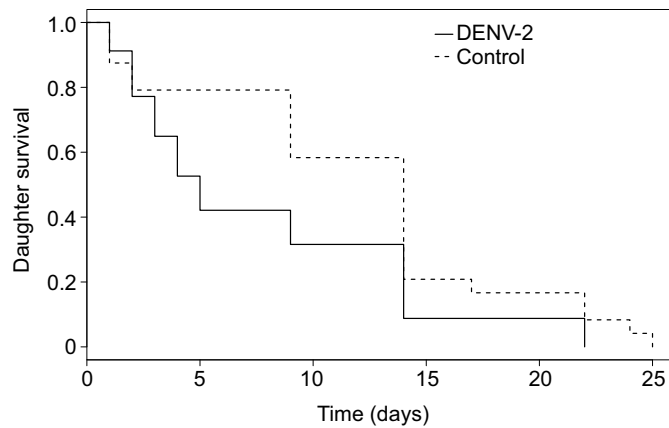


Fig. 1. Daughter survival according to food type. Daughters fed with rabbit blood infected with DENV-2 had a lower survival than those fed with non-infected blood (control). Survival was compared for 30 days under laboratory conditions (log-rank, $\chi^2=5.3$, $P=0.02$, $N=76$).

Number of eggs

Egg number differed significantly between control and DENV-2 groups for mothers but not for daughters. DENV-2 mothers laid more eggs than control mothers (estimator= -0.43 , $t=-2.31$, $P=0.02$; Fig. 2), although life span did not differ significantly between groups (log-rank, $\chi^2=0.4$, $P=0.54$); thus, the DENV-2 mothers had a higher oviposition rate than control mothers. No significant difference was found between daughters in the DENV-2 and control groups (estimator= 0.07 , $t=1.87$, $P=0.06$).

Proportion of adults that emerged

In both mothers and daughters, the number of eggs laid by each female negatively influenced the proportion of adults that emerged. That is, the higher the production of eggs, the lower the proportion of adults that emerged (for mothers, estimator= -0.02 , s.e.= 0.005 , $t=-4.18$, $P<0.005$; for daughters, estimator= -0.03 , s.e.= 0.003 , $t=-8.92$, $P=1.122 \times 10^{-11}$). However, the number of adults produced was not affected by the treatment (control or DENV-2).

Sex ratio

The proportion of females that emerged from a reproductive cycle was in general negatively influenced by the number of adults that emerged, meaning that the greater the number of adults, the lower the percentage of females. The DENV-2 mothers had a greater proportion of male offspring than the control mothers (estimator= -0.02 , s.e.= 0.008 , $t=-2.53$, $P=0.01$; Fig. 3). There was no significant difference between the two groups of daughters (estimator= -0.02 , s.e.= 0.01 , $t=-1.34$, $P=0.19$).

Proportion of females with an amplification of DENV-2

At the end of the experiment, DENV-2 infection was corroborated and MIRs were assessed, using qRT-PCR to determine the number of mothers (11 out of 19 pools; 57.89%) and daughters (4 out of 9 pools; 44.44%) in which the virus was present. Because we randomized the mosquitoes for qRT-PCR from the entire population of mothers and female offspring, the results represent the proportion of female mosquitoes infected with DENV-2. Overall, half of the female mosquitoes fed with DENV-2 suspension were productively infected; therefore, a large proportion of daughters were exposed to DENV-2 by vertical transmission, but probably also by horizontal sexual transmission (male offspring from DENV-2-infected mothers). We do not know the effective viral load needed to

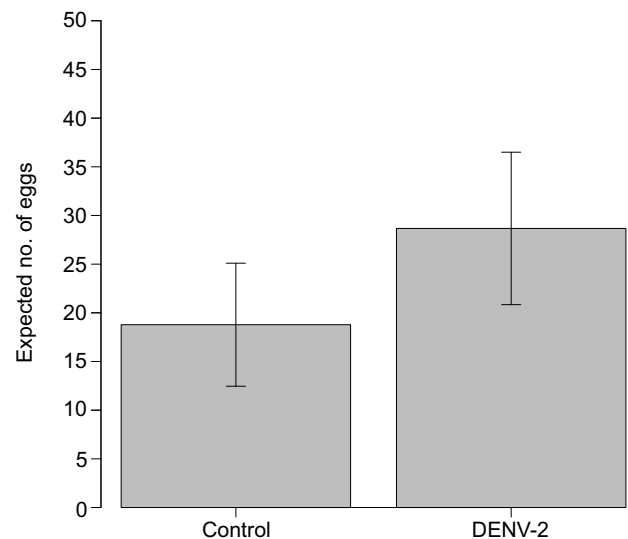


Fig. 2. Expected number of eggs per female according to food type.

Mothers fed with DENV-2-infected blood oviposited more eggs than those fed with non-infected blood (control). At the fourth post-feeding day, the number of eggs per female for each group was recorded. Mean \pm s.e.m. values are shown (estimator= -0.4271 , $t=-2.314$, $P=0.02$, $N=139$).

produce a qRT-PCR-positive mosquito, but regardless, it explains the relatively low MIR in daughters.

MIR

The MIR for mothers and daughters was 11.5 and 8.8, respectively. This means that in the F_0 generation (mothers), we should find an infection level of at least 11.5%, and in the F_1 generation (daughters) we should find an infection level of at least 8.8%. This assumption is very conservative because it assumes the presence of at least one infected mosquito per pool, although probably the infection rate of mothers could be underestimated.

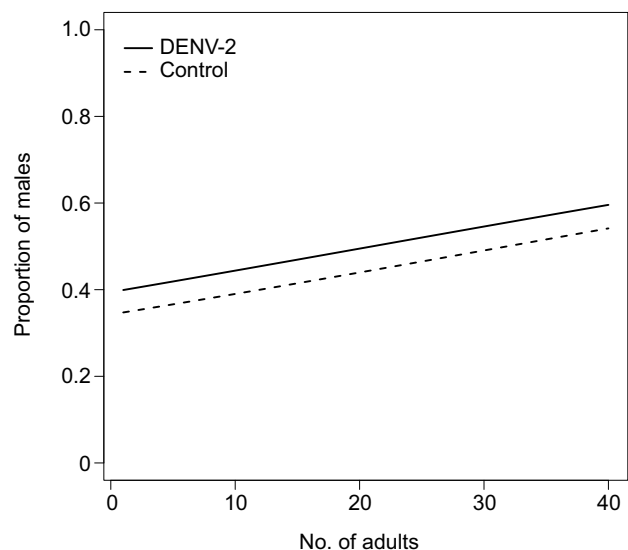


Fig. 3. Proportion of males produced by mothers following horizontal transmission. GAMLSS analysis shows a positive association between the number of adults and the proportion of males. Mothers fed with DENV-2-infected blood produced a sex ratio skewed to males compared with those fed with non-infected blood (control) (estimator= -0.02043 , s.e.= 0.008088 , $t=-2.526$, $P=0.01311$, $N=105$).

DISCUSSION

Most studies on parasitic transmission have focused on survival and egg number following horizontal transmission (Lambrechts and Scott, 2009; Grunnill and Boots, 2016), and this is the same scenario in mosquitoes infected with DENV-2 virus (Lambrechts and Scott, 2009). Here, costs or benefits of horizontal and vertical transmission were tested. The results showed that, compared with control mothers, DENV-2-infected mothers laid more eggs and the offspring sex ratio was skewed towards males. However, no significant differences were found in survival and the number of adults (males and females). In contrast, compared with control daughters, DENV-2 daughters had a shorter survival time, but there were no significant differences in the number of eggs and the offspring sex ratio. In addition, we confirmed the virus infection during vertical and horizontal transmission. Although here the MIR analysis showed a low level of infection, this concurred with previous results (Grunnill and Boots, 2016); hence, the results are discussed on the basis of a population of females exposed to virus and not in regard to individuals infected or not infected.

Mothers exposed to DENV-2 laid more eggs than those in the control group, and had the same survival rate. Although Maciel-de-Freitas et al. (2011) had a contrary finding in *A. aegypti*, their sample was skewed to small-sized females, and smaller organisms could be of lower quality than those of greater size, perhaps through having been exposed to a stressful environment during growth (Stearns, 1992). Moutailler et al. (2010) found that in both a susceptible (*A. aegypti*) and a resistant species (*Aedes formosus*), female mosquitoes infected with DENV-2 laid more eggs than those that were not infected. Hence, our results accord with those of Moutailler et al. (2010), suggesting that females exposed to DENV-2 by horizontal transmission laid more eggs compared with control. This may suggest a potential benefit for females of being infected by DENV-2. Nevertheless, at adulthood, there were no significant differences. This may be because in a stressful environment (i.e. with exposure to DENV-2 infection), females tend to bias their resources to egg number at the expense of quality, and consequently fewer insects reach adulthood (Stearns, 1992; Edward and Chapman, 2011) compared with control. Adult number is a more reliable predictor of an epidemiological risk than the number of eggs oviposited or hatched (the parameters usually reported), because emerged adults have a closer relationship to the vectorial density of mosquitoes (Wai et al., 2012). Indeed, we also found that a very small percentage of hatched larvae became adult mosquitoes. These results highlight the importance of quantifying not only egg production but also adult survival as a measure of reproductive success.

Within adults, in the case of horizontal transmission, more males than females were recorded. The importance of taking into account this variable is that when parasite transmission depends 100% on the efficiency of vertical transmission, the host sex ratio is skewed to females (Jiggins et al., 2000). However, the distortion of the host sex ratio sometimes occurs in infections in which parasites are horizontally transmitted (Jiggins et al., 2000). Given that DENV transmission is horizontal and vertical in *A. aegypti* (Adams and Boots, 2011), we expected a skew in the sex ratio towards female offspring in the DENV-2 group compared with the control group, but, surprisingly, the opposite was found, and no skew was observed in vertical transmission. As a negative correlation between egg number and adult number was found, it is possible that egg production is expensive and we suggest it could impact on the more costly sex to be produced: females. In several species, the larger sex is more costly to produce (Trivers and Willard, 1973) and in *A. aegypti* females are larger than males, so the least

expensive sex could be males. One possible reason for these differences in operational sex ratio is that it was skewed since the primary sex ratio (since hatching) and the literature suggests that the skewedness of sex ratio is dependent on the mother's condition (Trivers and Willard, 1973; Cameron, 2004). However, the negative relationship between egg number and adult number leads us to propose that males and females were produced equally at hatching, but a differential mortality existed, skewed towards females. Macke et al. (2012) suggest that the skew in sex ratio is related to egg size: females are derived from larger eggs than males; under this point of view, it is possible that the larger eggs and possibly larvae were of poorer quality than those of smaller size, and hence died earlier than smaller larvae (males). As far as we know, this is the first time that sex ratio has been reported in the DENV-2–*A. aegypti* system and more studies are needed to find out the mechanism by which the sex ratio is skewed, and how general this observation is in other hosts species whose parasites are horizontally and vertically transmitted.

Independently of the mechanism of sex ratio skewedness, the production of more males than females could affect virus transmission generation after generation. Male *Aedes albopictus* infected with DENV are capable of transmitting the virus in venereal infections to females, which in turn transmit the virus vertically to their offspring (Leon, 1978; Rosen, 1978). If males can pass the virus to females, this strategy could be favored by sexual selection because male *A. aegypti* pass seminal proteins in their ejaculate to females to avoid the females re-mating (Leon, 1978; Rosen, 1978). Hence, a female could produce all her offspring by a single mating event, and if she mates with an infected male, their offspring could also be infected. Besides ensuring that the offspring belong to the sexually active male, this mechanism could also facilitate the propagation of the virus among the mosquito populations. Hence, males could have an important role in virus propagation but as far as we know this idea has not been previously tested.

Another factor that must be taken into account is the life history theory, which suggests that there should be a trade-off between reproduction and survival because both are costly. It seems that the cost of infection was not evident in mothers, but it was in daughters in terms of survival. However, other parameters were similar between infected and control mosquitoes (the number of eggs, the number of emerged adults, and the proportion of males and females resulting from a reproductive cycle). Apparently, the investment in reproduction was costly for the infected daughters and compromised their survival. This could be due to the fact that infected daughters were exposed to infection during their entire lifetime, whereas infected mothers were exposed to infection only as adults. According to the hypothesis of terminal investment, organisms give priority to reproduction if they perceive that their survival is at risk (Krams et al., 2011, 2015), and, possibly, infected daughters prioritized reproduction at the expense of survival. It is also likely that the effect observed in the daughters reflected a prior investment in them by their mothers, as it is known that the investment in a certain behavior or physiological response is not limited within generations but the cost could be paid across generations (Clutton-Brock, 1984). In this sense, the mechanisms are not clear, but it is possible that mothers prioritized investment in the reproductive characteristics of their offspring at the cost of survival, and the mothers' strategy could be to lay eggs of lower quality, compared with those of the control group. Hence, daughters could pay the price for their mother's investment in terms of survival. However, despite this cost and its potential causes, it is very important to note that adult production was not affected by

horizontal and vertical transmission, and this is a more important variable at the adult stage than survival. So, if the mode of DENV-2 transmission does not impact negatively on its host's reproduction, both vertical and horizontal transmission of DENV could be maintained at the same time in *A. aegypti*.

Conclusions

Although DENV cannot maintain itself in populations when unfavorable conditions persist (Adams and Boots, 2011; Wai et al., 2012), a low level of vertical transmission in mosquitoes could maintain virus propagation in nature (Rohani et al., 2008; Adams and Boots, 2011). If the efficiency of the vertical transmission increases, the number of generations of mosquitoes with a significant effect on the transmission of DENV to humans will be greater (Adams and Boots, 2011). It has been suggested that larval cannibalism may maintain the presence of a virus within a population by vertical transmission (Grunnill and Boots, 2016), and here we suggest that more attention should be paid to males because they can also be infected, and they could transmit the infection to uninfected females (Leon, 1978; Rosen, 1978; Bustamante and Lord, 2010; Grunnill and Boots, 2016). Future studies should test the impact of males on vertical and horizontal transmission in laboratory and natural conditions to assess virus propagation and its maintenance in *A. aegypti* populations. In this sense, sexual selection studies are important to discover whether infected males, compared with non-infected males, are better able to induce a non-receptive state in females, and whether non-infected females may prefer as mating partners the virus-free males or the infected ones. As far as we know, the impact of sexual selection on DENV-2 transmission has not been tested. Although mosquitoes do not appear to receive benefits from DENV-2 infection, neither do they suffer a reproductive cost, and DENV-2 was present via both horizontal and vertical transmission. This apparent reproductive cost-free DENV-2 transmission could explain why this virus is transmitted vertically and horizontally in *A. aegypti*.

Acknowledgements

Jorge Canales provided valuable suggestions and help in the laboratory. Two anonymous reviewers substantially improved this paper.

Competing interests

The authors declare no competing or financial interests.

Author contributions

G.R.-G., J.R.-C. and J.C.-G. conceived and designed the experiments; G.R.-G. carried out the experiments; G.R.-G. and A.H.-Q. performed statistical analyses. All authors wrote the paper.

Funding

G.R.-G. was supported by a grant from Consejo Nacional de Ciencia y Tecnología (CONACyT 301800) to carry out her master's degree. This work was supported by grants from SEP-Consejo Nacional de Ciencia y Tecnología, Ciencia Básica to J.R.-C. (129497) and J.C.-G. (152666).

References

Adams, B. and Boots, M. (2011). How important is vertical transmission in mosquitoes for the persistence of dengue? Insights from a mathematical model. *Epidemics* **2**, 1–10.

Bennett, K. E., Olson, K. E., de Lourdes Muñoz, M., Fernandez-Salas, I., Farfan-Ale, J. A., Higgs, S., Black, W. C., IV and Beaty, B. J. (2002). Variation in vector competence for dengue 2 virus among 24 collections of *Aedes aegypti* from Mexico and the United States. *Am. J. Trop. Med. Hyg.* **67**, 85–92.

Biggerstaff, B. J. (2008). Confidence intervals for the difference of two proportions estimated from pooled samples. *J. Agric. Biol. Environ. Stat.* **13**, 478–496.

Biggerstaff, B. J. (2009). *Version 4.0: a Microsoft® Office Excel® Add-In to Compute Prevalence Estimates from Pooled Samples*. Fort Collins, CO, USA: Centers for Disease Control and Prevention.

Bustamante, D. M. and Lord, C. C. (2010). Sources of error in the estimation of mosquito infection rates used to assess risk of arbovirus transmission. *Am. J. Trop. Med. Hyg.* **82**, 1172–1184.

Cameron, E. Z. (2004). Facultative adjustment of mammalian sex ratios in support of the Trivers-Willard hypothesis: evidence for a mechanism. *Proc. R. Soc. B Biol. Sci.* **271**, 1723–1728.

Clutton-Brock, T. H. (1984). Reproductive effort and terminal investment in iteroparous animals. *Am. Nat.* **123**, 212–229.

Combes, C. (2005). *The Art of Being a Parasite*. Chicago: The University of Chicago Press.

Edward, D. A. and Chapman, T. (2011). *Mechanisms Underlying Reproductive Trade-Offs: Costs of Reproduction. Mechanisms of Life History Evolution. The Genetics and Physiology of Life-History Trade-Offs*. Oxford: Oxford University Press.

Engelstädter, J. and Hurst, G. D. (2009). The ecology and evolution of microbes that manipulate host reproduction. *Annu. Rev. Ecol. Evol. Syst.* **40**, 127–149.

Grunnill, M. and Boots, M. (2016). How important is vertical transmission of dengue viruses by mosquitoes (Diptera: Culicidae)? *J. Med. Entomol.* **53**, 1–19.

Jiggins, F. M., Hurst, G. D. and Majerus, M. E. N. (2000). Sex-ratio-distorting *Wolbachia* causes sex-role reversal in its butterfly host. *Proc. R. Soc. B Biol. Sci.* **267**, 69–73.

Krams, I., Daukste, J., Kivleniece, I., Krama, T., Rantala, M. J., Ramey, G. and Šauša, L. (2011). Female choice reveals terminal investment in male mealworm beetles, *Tenebrio molitor*, after a repeated activation of the immune system. *J. Insect Sci.* **11**, 56.

Krams, I. A., Krama, T., Moore, F. R., Rantala, M. J., Mänd, R., Mierauskas, P. and Mänd, M. (2015). Resource availability as a proxy for terminal investment in a beetle. *Oecologia* **178**, 339–345.

Lambrechts, L. and Scott, T. W. (2009). Mode of transmission and the evolution of arbovirus virulence in mosquito vectors. *Proc. R. Soc. B Biol. Sci.* **276**, 1369–1378.

Leon, R. (1978). Sexual transmission of dengue viruses by *Aedes albopictus*. *Am. J. Trop. Med. Hyg.* **37**, 398–402.

Maciel-de-Freitas, R., Koellam, J. C. and Lourenço-de-Oliveira, R. (2011). Lower survival rate, longevity and fecundity of *Aedes aegypti* (Diptera: Culicidae) females orally challenged with dengue virus serotype 2. *Trans. R. Soc. Trop. Med. Hyg.* **105**, 452–458.

Macke, E., Magalhães, S., Bach, F. and Olivieri, I. (2012). Sex-ratio adjustment in response to local mate competition is achieved through an alteration of egg size in a haplodiploid spider mite. *Proc. R. Soc. B Biol. Sci.* **279**, 4634–4642.

Magalon, H., Nidelet, T., Martin, G. and Kaltz, O. (2010). Host growth conditions influence experimental evolution of life history and virulence of a parasite with vertical and horizontal transmission. *Evolution* **64**, 2126–2138.

Moore, J. (2002). *Parasites and the Behavior of Animals*. New York: Oxford University Press.

Moutailler, S., Guichoux, E., Vazeille, M. and Failloux, A. B. (2010). Differential mortalities of dengue-infected *Aedes aegypti*: preliminary results. *Ann. Soc. Entomol. Fr.* **46**, 359–366.

Poulin, R. (2011). *Evolutionary Ecology of Parasites*. Princeton, NJ: Princeton University Press.

Rigby, R. A. and Stasinopoulos, D. M. (2005). Generalized additive models for location, scale and shape. *J. R. Stat. Soc. Ser. C. Appl. Stat.* **54**, 507–554.

Rohani, A., Zamree, I., Joseph, R. T. and Lee, H. L. (2008). Persistency of transovarial dengue virus in *Aedes aegypti* (Linn.) Southeast Asian. *J. Trop. Med. Public Health* **39**, 813–816.

Rosen, L. (1978). Sexual transmission of dengue viruses by *Aedes albopictus*. *Am. J. Trop. Med. Hyg.* **38**, 398–402.

Stearns, S. (1992). *The Evolution of Life Histories*. Oxford: Oxford University Press.

Svensson, E. I. and Råberg, L. (2010). Resistance and tolerance in animal enemy-victim coevolution. *Trends Ecol. Evol.* **25**, 267–274.

Sylvestre, G., Gandini, M. and Maciel-de-Freitas, R. (2013). Age-dependent effects of oral infection with Dengue virus on *Aedes aegypti* (Diptera: Culicidae) feeding behavior, survival, oviposition success and fecundity. *PLoS ONE* **8**, e59933.

Thomas, F., Brodeur, J., Maure, F., Franceschi, N., Blanchet, S. and Rigaud, T. (2011). Intraspecific variability in host manipulation by parasites. *Infect. Genet. Evol.* **11**, 262–269.

Trivers, R. L. and Willard, D. E. (1973). Natural selection of parental ability to vary the sex ratio of offspring. *Science* **179**, 90–92.

Vautrin, E. and Vavre, F. (2008). Interactions between vertically transmitted symbionts: cooperation or conflict? *Trends Microbiol.* **17**, 95–99.

Wai, K. T., Arunachalam, N., Tana, S., Espino, F. and Kittayapong, P. (2012). Estimating dengue vector abundance in the wet and dry season: implications for targeted vector control in urban and peri-urban Asia. *Pathog. Glob. Health* **106**, 436–445.

Weaver, S. C. and Barrett, A. D. T. (2004). Transmission cycles, host range, evolution and emergence of arboviral disease. *Nat. Rev.* **2**, 789–801.

Weeks, A. R., Turelli, M., Harcombe, W. R., Reynolds, K. T. and Hoffmann, A. A. (2007). From parasite to mutualist: rapid evolution of *Wolbachia* in natural populations of *Drosophila*. *PLoS Biol.* **5**, e114.