The Effect of Parasitism and Interpopulation Hybridization on *Aedes albopictus* (Diptera: Culicidae) Fitness

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Abstract

Recent research in mosquito population genetics suggests that interpopulation hybridization has likely contributed to the rapid spread of the container-breeding mosquitoes. Here, I used laboratory experiments to investigate whether interpopulation *Aedes* (Stegomyia) *albopictus* (Skuse) F1 and F2 hybrids exhibit higher fitness than parental populations, and whether hybrid mosquito performance is related to infection by the coevolved protozoan parasite *Ascogregarina taiwanensis* (Lien and Levine). Overall, there were significant differences in development time, wing length, and survival between the two parental mosquito populations, but no difference in per capita growth rate \( r \). Hybrid mosquitoes were generally intermediate in phenotype to the parentals, except that F2 females were significantly larger than the midparent average. In addition, *As. taiwanensis* parasites produced fewest oocysts when they were reared in hosts of hybrid origin. These data suggest that hybridization between previously isolated mosquito populations can result in slight increases in potential mosquito reproductive success, via increased hybrid body size, and via the temporary escape from coevolved parasites. These findings are significant because studies have shown that even slight hybrid vigor can have positive fitness consequences for population persistence. Although this was a laboratory experiment extending only to the F2 generation, many other invasive insects also carry coevolved parasites, and thus the patterns seen in this mosquito system may be broadly relevant.

Key words: *Aedes albopictus*, *Ascogregarina taiwanensis*, hybridization, parasite, population

Recent studies have shown rapid geographic divergence in *Ae. albopictus* life history traits in the United States (Armbruster and Conn 2006, O’Donnell and Armbruster 2009), as well as rapid adaptive evolution of the photoperiod response (Urbanski et al. 2012). Together these studies demonstrate the existence of considerable population structure in this species, and if host population structure contributes to parasite population structure, one would also expect some degree of geographic variation in parasite traits. In this study, I used laboratory experiments to address whether interpopulation *Ae. albopictus* hybrids have higher fitness than their parental populations, and whether hybrid mosquito performance is related to the ability of the coevolved protozoan parasite *Ascogregarina taiwanensis* (Apicomplexa: Lecudinidae) to successfully reproduce in hybrid mosquitoes.

*Aedes albopictus* is a competent vector of the dengue, chikungunya, and Japanese encephalitis viruses (Rai 1991), and is a potential vector of Zika virus (Chouin-Carneiro et al. 2016). Investigating how interpopulation hybridization affects *Ae. albopictus* life history traits in the presence and absence of parasites thus contributes to our overall understanding of the factors influencing population growth of these and other container-breeding mosquitoes.
Materials and Methods

Host Biology

*Aedes albopictus* are native to Asia but over past 30–40 yr have colonized every continent except Antarctica (Bonizzoni et al. 2013). Females are aggressive daytime biters and lay eggs in both natural and artificial containers. Mosquitoes hatch from desiccation- and cold-resistant eggs, and complete four larval stages and one pupal stage, before metamorphosing into an adult. Development time from egg to adult ranges from 10 d to over 4 wk, and is dependent on temperature, photoperiod, competition, and food availability (Alto and Juliano 2001; Tseng 2004, 2006; Costanzo et al. 2015; Noden et al. 2016). In nature, *Ae. albopictus* are commonly infected by the protozoan parasite *As. tawa nensis*, with the infection prevalence ranging from 21% to 100% (Garcia et al. 1994, Blackmore et al. 1995, Comiskey et al. 1999a, Reyes-Villanueva et al. 2003, Passos and Tadei 2008). Parasitism of *Ae. albopictus* by *As. taw a nensis* has been documented in Asia, the United States, Mexico, and Brazil (Blackmore et al. 1995, Chen 1999, Passos and Tadei 2008, Reyes-Villanueva et al. 2013).

Parasite Biology

The life cycle of the protozoan parasite *As. taw a nensis* has been well-characterized (Chen 1999). After ingestion by first-instar mosquito larvae, parasite oocysts release sporozoites. Sporozoites develop into trophozoites in host epithelial cells, which transform into macro- or microgametes in the larval mosquito Malpighian tubules. During mosquito pupation, parasite gametes fuse to form gametocyts, within which hundreds of oocysts form. Oocysts are released into the containers during metamorphosis of mosquitoes from pupa to adults, during oviposition, or by adults that happen to die in the container (Chen 1999, Tseng 2007). Transmission of the parasite can be horizontal or vertical, depending on whether oocysts released by females are ingested by her offspring. Infection of *Ae. albopictus* by *As. taw a nensis* can result in prolonged larval development, smaller adult size, and reduced female fecundity (Comiskey et al. 1999a; Aliabadi and Juliano 2002; Tseng 2004, 2006, 2007).

Mosquito Parental Populations

Approximately 300–500 larvae of *Ae. albopictus* were collected from Arcadia, FL (27.2° N, 81.8° W; hereafter FLA), and from Bloomington, IN (39.2° N, 86.5° W; hereafter IND), United States. These two locations were chosen based on published data showing high genetic (Black et al. 1988, Kambhampati et al. 1991, Birungi and Munstermann 2002, Lourenço de Oliveira et al. 2003, Usmani-Brown et al. 2009, Medley et al. 2015) and phenotypic (Armbruster and Hutchinson 2002; Armbruster and Conn 2006, Leisnham et al. 2009, O’Donnell and Armbruster 2009) differentiation among geographically isolated populations. Additionally, previous surveys demonstrated a >70% *As. taw a nensis* infection prevalence in both FLA and IND *Ae. albopictus* (M.T., unpublished data). Infection of *Ae. albopictus* with *As. taw a nensis* was confirmed through larval dissections.

Larvae from the two locations were transported to Indiana University and reared in separate population cages. To simulate a “common garden” approach, colonies were maintained at 25 ± 2°C, 70% relative humidity, and a photoperiod of 14:10 (L:D) h. These conditions approximate the average summer environment of the two source locations combined. Adults were bloodfed using an artificial membrane system (Tseng 2003), and larvae were fed finely ground Nutriphase (Petsmart, Phoenix) rabbit food.

Generating Uninfected Mosquito Larvae

In laboratory cages, female *Ae. albopictus* lay their eggs on the interior surfaces of 200-ml plastic cups, above the water line. Containers are lined with standard laboratory paper towels, which allows for eggs to be collected after they are laid. Egg-impregnated paper towels are rinsed in chlorinated tap water to remove any attached parasite oocysts, and then soaked in dechlorinated water enriched with nutrient broth (1g/1000ml distilled H2O of 10g bactotryptone, 5 g yeast extract, 10 g NaCl) to deoxygenate the water and induce synchronous hatching. This method consistently produces uninfected *Ae. albopictus* larvae.

Isolating *As. taw a nensis* Parasites

To isolate *As. taw a nensis* parasites, individual mosquito pupae from each parental population were placed in 1.5-ml centrifuge tubes until metamorphosis to adulthood. Newly emerged adults were macerated in the emergence water and *As. taw a nensis* oocysts were quantified using a hemocytometer. Known quantities of oocysts were then added to the deoxygenated water used to synchronously hatch *Ae. albopictus* larvae.

Experimental Design

Prior to the start of the experiment, source populations were first reared in the common laboratory environment for two generations to minimize maternal environmental effects. Adult mosquitoes were then mass-mated (100 males × 100 females) to generate each of the two parental populations (FLA, IND) and two hybrid populations (F1, F2). The F1 population was created by mass-mating 100 FLA females to 100 IND males, and 100 IND females to 100 FLA males. To obtain an overall average effect of hybridization, the FLA female × IND male hybrid F1 population was combined with the FLA male × IND female hybrid F1 population to generate an “average” F1 mosquito population. F1 mosquito eggs were divided randomly into two groups. One group was hatchet out to create the F1 generation, which was then mated (100 F1 males × 100 F1 females) to create the F2 generation. The other batch of F1 eggs was used as the F2 population for the experiment. All four mosquito populations, FLA, IND, F1, and F2, were hatched from eggs simultaneously at the start of the experiment.

Each of the four host types (FLA, IND, F1, and F2) was infected with parasites from either parental population or kept uninfected (parasite type = FLA, IND, and uninfected) for a total of 12 host–parasite combinations.

To produce infected mosquito larvae, 200 uninfected first-instar larvae of each host type were simultaneously exposed to a dose of ~520,000 parasite oocysts per parasite type. One day after exposure to oocysts, each batch of 200 mosquito larvae was equally divided into 10 replicates (200-ml cups) for rearing purposes (Beier and Craig 1985; Tseng 2004, 2006). Preliminary studies have shown the infection prevalence at this oocyst dose to be consistently >95%. Mosquito eggs for the uninfected treatment were hatched in dechlorinated tap water. All rearing cups were given 0.2 ± 0.05 mg finely ground Nutriphase (Petsmart, Phoenix) rabbit chow daily until emergence.

Data Collection

The following mosquito traits were measured—development time (number of days from hatching to pupation), adult wing length, and percent of larvae surviving to adulthood (per replicate). These traits are commonly used as indicators of overall fitness in this species (Comiskey et al. 1999a,b; Armbruster and Hutchinson 2002;
Reiskind and Zarrabi 2012). Wings were removed, scanned, and measured to the nearest 0.1 mm using the measuring tool in Adobe Acrobat Professional. Wing length was measured as the maximum length from the axillary incision to the apex of the wing, excluding the fringe (Pelizza et al. 2013).

Parasite oocyst number per adult mosquito was quantified by counting all oocysts released and retained from all adult mosquitoes in the rearing cup, and then dividing total oocyst number by the total number of adults per cup. Mosquito pupae from each rearing cup were placed in individual 1.5-mL centrifuge tubes. Oocysts shed during metamorphosis were contained in each tube. Adult mosquitoes then were macerated in a small volume of distilled water to release any retained oocysts. Parasite oocyst number is a proxy of parasite fitness because oocyst number is likely correlated with the number of subsequent infections in nature. Oocysts were quantified using a hemocytometer at 10×.

Data Analysis

Host Traits: Development Time, Wing Length, Larval Survival, per Capita Growth Rate r.

I examined the effect of “host type,” “parasite type,” and “host sex” on mosquito development time, and wing length using linear mixed effects models. “Host sex,” “host type,” and “parasite type” were modeled as fixed effects; “host sex” was nested within “rearing cup” (because each cup contained both male and female mosquitoes), and “rearing cup” was modeled as a random effect. Statistical models also included all possible interactions among “sex,” “host type,” and “parasite type.” “Sex” was included in the model because Aedes albopictus have both sex-specific life history traits and sex-specific responses to infection by A. taiwanensis (Tseng 2004, 2006). Lastly, to account for variation in wing length or development time that could have been owing to larval survival (i.e., rearing cups with fewer survivors could have given rise to larger mosquitoes if food was limiting), I included the “number of larvae surviving per replicate” as a covariate in the models.

I used an ANOVA to determine whether host- or parasite type explained significant variation in the percent of larvae per replicate surviving to adulthood. “Host sex” was not included as a factor in the ANOVA because although I could determine sex of the survivors, I could not nondestructively determine the sex of first-instar larvae at the beginning of the experiment. I also used ANOVA to analyze whether host- or parasite type explained variation in per capita growth rate, r. As advocated by Chmielewski et al. (2010), I used equation 7 from Livdahl and Sugihara (1984) to calculate r:

\[
r = \frac{\ln \frac{1}{N_0} \sum A f(w_x)}{D + \frac{1}{N_0} \sum x A f(w_x)}
\]

where \(A_x\) is the number of new adult females produced at time \(x\), \(N_0\) is the initial number of females (estimated at 10), \(f(w_{bar})\) is the function that links mosquito size to fecundity, and \(D\) is the time between emergence and reproduction. \(D\) is estimated at 14 d (Livdahl and Willey 1991, O’Donnell and Armbruster 2009), and the function linking wing length to egg number is—ln(egg number)=0.79 + 1.4*wing length (Armbruster and Hutchinson 2002).

Although it is unclear whether parasitized and unparasitized mosquitoes will show the same relationship between wing length and fecundity, I include this analysis as a first approximation of \(r\) in these populations, and to facilitate comparisons between this and previously published studies of Aedes albopictus population biology.

Fitness of Aedes albopictus interpopulation hybrids can also be assessed by comparing hybrid traits against the average trait value of the two parental populations (i.e., midparent value; O’Donnell and Armbruster 2009). Thus, for completeness, I also repeated the analyses for host development time, wing length, survival, and \(r\) using midparent means instead of the means for each parental population.

Parasite Trait: Oocyst Number

I used ANOVA to investigate whether host- or parasite type affected the number of oocysts produced by each infected host. This analysis was performed with rearing cup as the unit of replication.

All data were untransformed and met the assumptions of linear mixed-effects models and ANOVA. If the main effects were significant, I conducted Tukey HSD tests on replicate means to provide a conservative estimate of whether levels in each factor were significantly different from each other. All analyses were conducted in R version 3.3.0 (R Core Team 2016).

Results

Larval Mosquito Development Time

There was significant variation in development time among male and female mosquitoes (F1,1158 = 812, P < 0.001; Fig. 1a), and among host types (F3,167 = 7.742, P < 0.001; Fig. 1a). Female F2 hybrid mosquitoes developed faster than F1 or FLA mosquitoes (Tukey HSD: \(P = 0.02\) for both comparisons; Fig. 1a). Female F2 hybrid mosquitoes also developed faster than FLA or F1 mosquitoes (Tukey HSD: \(P = 0.02\) for both comparisons; Fig. 1a). Development time of F1 or F2 mosquitoes was not significantly different from the midparent average (Tukey HSD: midparent vs. F1, \(P = 0.44\); midparent vs. F2, \(P = 0.30\); Supp. Fig. 1a [online only]). Lastly, infection by As. taiwanensis delayed male, but not female development time (parasite × sex: F2,158 = 10.35, \(P = 0.0001\); Fig. 1b). Parasites from FLA and IND did not differ in their effect on mosquito development time, and there was no effect of larval survival per replicate on larval development time (F1,167 = 0.035, \(P = 0.8512\)).

Mosquito Wing Length

Adult female mosquitoes had longer wing lengths compared with those of adult males (F1,158 = 2.328, P < 0.001; Fig. 2a). Significant variation in wing length among host types was observed in female but not male mosquitoes (host × sex: F1,158 = 7.09, P < 0.001, Fig. 2). F2 females emerged at a larger size than FLA or F1 females (Tukey HSD: \(P = 0.006\), P < 0.001, respectively), and IND females emerged at a larger size than F1s (Tukey HSD: \(P = 0.014\)). Female F2s were also larger than the midparent average (Tukey HSD: \(P = 0.006\), Supp. Fig. 1b [online only]). There was no effect of parasite infection on male or female mosquito wing length (F2,158 = 0.24, \(P = 0.789\); Fig. 2b), and no effect of larval survival per replicate on adult wing length (F1,167 = 0.45, \(P = 0.50\)).

Larval Mosquito Survival

Both host- and parasite type affected larval mosquito survival (host: F3,168 = 4.41, \(P = 0.005\); parasite: F2,168 = 7.12, \(P = 0.001\); Fig. 3a and b). Survival from larva to adulthood was 64% for FLA and F1 mosquitoes, and 56% and 54% for IND and F2 mosquitoes, respectively (Tukey HSD: F1 vs. F2, \(P = 0.02\); FLA vs. F1, \(P = 0.07\); Supp. Fig. 3a). Larval survival of F1 or F2 mosquitoes was not significantly different from the midparent average (Tukey HSD: midparent vs. F1: \(P = 0.44\), midparent vs. F2: \(P = 0.33\); Supp. Fig. 1c [online only]). Lastly, a higher percentage of uninfected mosquito larvae survived compared with larvae infected with either parasite type (Tukey HSD: uninfected vs. FLA parasites, \(P = 0.003\); uninfected vs.
IND parasites, \( P = 0.013 \); Fig. 3b), and there was no difference in survival between mosquitoes infected with FLA or IND parasites (Tukey HSD: IND vs. FLA, \( P = 0.87 \); Fig. 3b).

**Per Capita Growth Rate, \( r \).** Parasite- but not host type explained variation in \( r \) (host: \( F_{3,168} = 7.74, P = 0.001 \); sex: \( F_{1,168} = 811.9, P < 0.001 \); parasite \( \times \) sex: \( F_{2,256} = 10.35, P = 0.001 \). Female mosquitoes are plotted above the dashed line; males are plotted below. Points that share a common letter are not significantly different from each other (Tukey HSD, \( P < 0.05 \)); colors help to distinguish different host types. Error bars are \( \pm SE \).

**Parasite Oocyst Number**

Across all host types, FLA parasites produced more oocysts per individual mosquito than did IND parasites (parasite type: \( F_{1,112} = 25.11, P < 0.001 \); Fig. 4), and oocyst number was dependent on the host–parasite combination (host \( \times \) parasite interaction: \( F_{2,112} = 5.83, P < 0.001 \)). FLA parasites produced significantly more oocysts when reared in FLA vs. F1 hosts (Tukey HSD: \( P = 0.02 \)), and IND parasites produced the least number of oocysts when reared in F2 hosts (Tukey HSD: \( F2 < F1 \) and \( F2 < \text{IND} \); \( P < 0.001 \)). When parasite oocyst number per individual is multiplied by the number of survivors per rearing cup to obtain the total number of oocysts produced per replicate population, the overall ANOVA results are very similar to those for oocysts produced per individual larva (parasite type: \( F_{1,112} = 14.4, P < 0.001 \); host \( \times \) parasite: \( F_{3,112} = 4.5, P < 0.001 \); Supp. Fig. 2 [online only]). At the replicate population level, the mean number of oocysts produced was significantly higher when FLA parasites were reared in FLA hosts compared with either F1 or F2 hosts (\( P = 0.015, P = 0.017 \), respectively). IND parasites produced the least number of oocysts when reared in F2 hosts (\( F2 < \text{IND} \); \( P = 0.003 \); \( F2 > F1 \); \( P = 0.001 \)). Overall, parasites appeared to produce the most number of oocysts when reared in sympatric hosts, and the fewest number of oocysts when reared in either F1 or F2 interpopulation hybrid hosts (Fig. 4, Supp. Fig. 1 [online only]).
Discussion

This study investigated the effects of interpopulation hybridization and parasitism on fitness correlates of *Ae. albopictus*. Hybridization has been suggested to be a possible mechanism behind population persistence in invasive mosquitoes (Fonseca et al. 2010, Kirkpatrick and Barrett 2015). The present study extends this literature by asking whether the putative success of hybrid mosquitoes is also linked to escape from parasitism.

Overall, there were significant differences in development time, wing length, and larval survival between the FLA and IND mosquito populations (Figs. 1a, 2a, and 3a), but no difference in per capita growth rate, \( r \). Interpopulation variation in life history traits corroborates previously published studies on geographic variation in *Ae. albopictus* phenotypes (Armbruster and Conn 2006, Leisnham et al. 2009, O’Donnell and Armbruster 2009) and complements population genetic data for this species (Black et al. 1988, Kambhampati et al. 1991, Birungi and Munstermann 2002, Lourenço de Oliveira et al. 2003, Usmani-Brown et al. 2009, Goubert et al. 2016).

Development time, wing length, survival, and \( r \) of F1 and F2 interpopulation mosquito hybrids were not strikingly different from those of the parental populations. Hybrid mosquitoes were generally intermediate in phenotype to the parental populations, with the only exception of F2 females attaining a larger size than both parental populations (Fig. 2a), and the midparent average (Supp. Fig. 1b [online only]). Fecundity is highly correlated with female body size in mosquitoes (Armbruster and Hutchinson 2002), and given the known benefit of even slight heterosis or hybrid vigor for population persistence (Drake et al. 2006), this increase in female F2 size could result in a reproductive advantage for hybrid mosquitoes. However, F2 mosquitoes also exhibited lower larval survival than FLA or F1 populations, so any reproductive advantage of a larger size could be discounted by decreased survival. Indeed, the preliminary analysis of \( r \) in these host types showed no fitness advantage for F2 hosts.

Interestingly, *As. taiwanensis* parasites produced fewest oocysts when they were reared in hosts of hybrid origin (Fig. 5, Supp. Fig. 2 [online only]). This reduced parasite reproductive success perhaps contributed to the larger size attained by F2 females (Fig. 2a), but it fails to explain the decreased survival of F2 mosquitoes, especially given that decreased parasite load has been shown to reduce...
mosquito morbidity or mortality from infection (Reyes-Villanueva et al. 2003). However, the reduction in oocyst production may still translate into a decrease in the number of oocysts available to be transmitted to the next generation of mosquitoes. Although these patterns of oocyst production are suggestive of some adaptation of parasites to local hosts (Kawecki and Ebert 2004), a stronger result is the geographic variation in parasite reproductive rates. FLA parasites produced almost 50% more oocysts (averaged across all host types) compared with IND parasites, and yet there was little difference in life history traits, larval survival, or r in hosts infected by FLA vs. IND parasites. Future studies that investigate a larger number of host–parasite population combinations could provide more insight into broader patterns of geographic variation in As. tawa-
nensis oocyst production.

In conclusion, a better understanding of the mechanisms or traits underlying the rapid spread and persistence of invasive mosquitoes may lead to more robust control measures. Here, data from a laboratory experiment using the highly invasive mosquito Ae. albopictus and its obligate coevolved parasite As. tawa-
nensis provide some support for the hypothesis that hybridization between previously isolated populations can increase population fitness, via slight increases in hybrid fitness (body size), and via the temporary escape from parasites (lower parasite reproductive success on hybrid mos-
quito populations). Although this was a laboratory experiment extending only to the F2 generation, the fact that many other problematic insects also carry coevolved parasites or microbes (Prenter et al. 2004, Taurum et al. 2013, Nguyen et al. 2016) suggests that the patterns seen in this mosquito–parasite system may be broadly relevant.

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Data Accessibility Statement

Raw data from this study are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.378bk (Tseng 2017).

Fig. 5. Effect of parasite type and host type on oocyst production by As. taiwa-
nensis. The number of oocysts produced by each parasite type depended on the host–parasite combination (host × parasite type: F2,112 = 5.83, P = 0.001). Parasites originating from FLA mosquitoes are plotted as circles; parasites originating from IND mosquitoes are plotted as triangles. The four host types: FLA, IND, F1, and F2, are plotted as light blue, dark blue, dark green, and light green, respectively. Error bars are ± SE.


